Supporting Information

An Isaindigotone-Based NIR Fluorogenic Probe for Visualizing Mitochondrial DNA G-Quadruplexes in Living Cells

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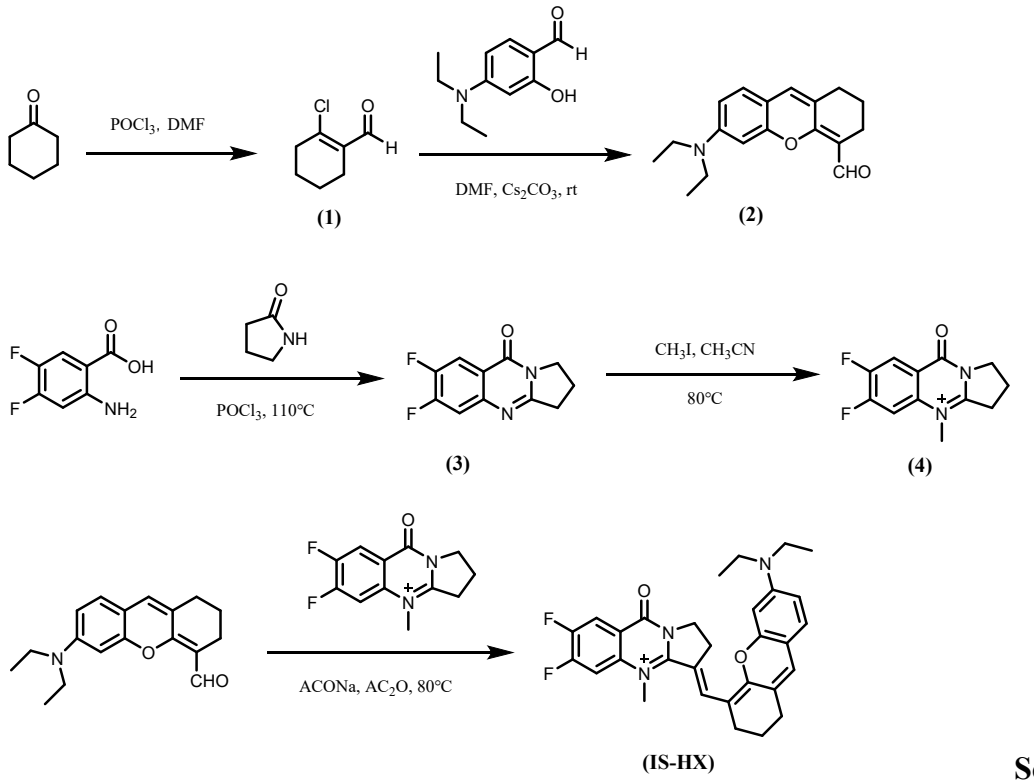
1. Experimental Procedures

1.1 Materials and instruments

Organic solvents were of analytical grade and obtained from Titan Scientific. (Shanghai, China). All the organic solvents were dried over 4Å molecular sieves before use. All other reagents of analytical grade were purchased from J&K Chemical, Tokyo Chemical Industry, Leyan or Heowns and used without further purification unless otherwise indicated. Cell counting Kit-8 (CCK-8) was obtained from Topscience (Shanghai, China). HepG 2 cells (human hepatoma cell line) was obtained from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Beijing, China). Ultrapure water with an electric resistance >18.2 MΩ was obtained from a Millipore Milli-Q water purification system (Billerica, MA). DMEM high glucose medium, penicillin, streptomycin and 10% heat-inactivated fetal bovine serum were purchased from Thermo Fisher (MA, USA).

Thin-layer chromatography (TLC) was performed on silica gel aluminum sheets with an F-254 indicator. Column chromatography was conducted using 200-300 mesh SiO2 (Qingdao Ocean Chemical Products). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-III 400 instrument (Bruker) using tetramethylsilane as an internal standard. Mass spectroscopy (MS) analysis was performed on LCQ advantage ion trap mass spectrometry (Thermo Fisher Scientific, Bremen, Germany). UV-vis absorption spectra were recorded with a Shimadzu UV-2450 spectrophotometer with an interval of 2 nm. Fluorescence spectra were obtained with an FS5 spectrofluorometer (Edinburgh, UK) with excitation and emission slits of 5.0 nm. Circular dichroism (CD) spectra were recorded on a MOS-450 spectrometer (Bio-Logic, France). Fluorescence imaging of cells was performed on a Nikon A1+ confocal microscope (Japan) with 60× objective lens. Flow cytometry was carried out using a FACSCelsta Flow cytometer (BD Biosciences, USA).

1.2 Synthetic procedures



Scheme S1. Synthetic routes for the probe **IS-HX.**

Synthesis of 2-chlorocyclohex-2-ene-1-carbaldehyde (Compound 1): Place DMF (4 mL, 50 mmol) in a round-bottom flask. Under a nitrogen atmosphere and ice bath, slowly add $POCl_3$ (4 mL, 44 mmol), and stir at room temperature for 2 hours. Subsequently, add cyclohexanone (2.5 g, 25 mmol) and react at room temperature for 3 hours. Slowly pour the reaction mixture into crushed ice, adjust the pH to neutral using NaOH, and extract with EA. Concentrate the organic phase to obtain a viscous reddish-brown liquid. Since **compound 1** is unstable, it is directly used in the subsequent reaction without purification.

Synthesis of 6-(diethylamino)-2,3-dihydro-1H-xanthene-4-carbaldehyde (Compound 2)： Compound 1 (5 g, 51 mmol), cesium carbonate (33 g, 102 mmol), and 2-hydroxy-4-diethylaminobenzaldehyde (9.8 g, 51 mmol) were uniformly mixed. Add 20 mL of anhydrous DMF, and react at room temperature overnight under a nitrogen atmosphere. Filter the mixture; after concentrating the filtrate, purify the residue by silica gel column chromatography (PE/DCM, 1:1–0:1, V/V) to obtain **compound 2** as an orange-yellow solid with a yield of 67%. ^{1}H NMR (400 MHz,

Chloroform-d) δ 10.26 (s, 1H), 6.98 (d, J = 8.6 Hz, 1H), 6.61 (s, 1H), 6.40 (d, J = 8.7 Hz, 1H), 6.34 (s, 1H), 3.37 (q, J = 7.2 Hz, 4H), 2.52 (t, J = 6.2 Hz, 2H), 2.43 (t, J = 6.1 Hz, 2H), 1.69 (q, J = 6.1 Hz, 2H), 1.18 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.17, 162.05, 154.18, 149.58, 128.16, 127.61, 123.10, 111.32, 110.25, 107.75, 97.14, 44.61, 29.84, 21.62, 20.67, 12.60. MS (ESI) m/z: calcd for compound 2 ($C_{18}H_{21}NO_2$, $[M+1]^+$), 283.16; found: 284.42.

Synthesis of 6,7-difluoro-2,3-dihydropyrrolo[2,1-b]quinazolin-9(1H)-one (Compound 3): Place 4, 5-difluoroanthranilic acid (2 g, 11.6 mmol) and 2-pyrrolidone (1.97 g, 23.2 mmol) in a flask. Under ice bath conditions, slowly add $POCl_3$ (25 mL, 270 mol). After the complete addition of $POCl_3$, transfer the flask to a 110 °C environment and react for 12 h. Once the temperature decreases, pour the reaction mixture into crushed ice, and adjust the pH to 7 using NaOH. Subsequently, extract with EA; after concentrating the organic phase, purify by column chromatography (PE/EA, 10:1–4:1, V/V) to obtain **compound 3** as a white solid with a yield of 61%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (t, J = 9.3 Hz, 1H), 7.40 (dd, J = 10.9, 7.1 Hz, 1H), 4.19 (t, J = 7.3 Hz, 2H), 3.16 (t, J = 8.0 Hz, 2H), 2.30 (p, J = 7.8 Hz, 2H).^{13}C NMR (101 MHz, $CDCl_3$) δ 160.35, 160.33, 159.60, 156.05, 155.90, 153.50, 153.35, 150.59, 150.45, 148.09, 147.95, 146.93, 146.82, 146.80, 117.40, 117.35, 117.33, 114.68, 114.50, 113.81, 113.79, 113.62, 113.60, 46.67, 32.50, 19.46.^{19}F NMR (376 MHz, $CDCl_3$) δ -126.77, -126.83, -137.05, -137.11.

Synthesis of 6,7-difluoro-4-methyl-9-oxo-1,2,3,9-tetrahydropyrrolo[2,1-b]quinazolin-4-ium (Compound 4): Place compound 3 (3 g, 13.5 mmol) and iodomethane (3.8 g, 27 mmol) in a pressure tube. Add 4 mL of CH_3CN, and react at 80 °C for 36 h. Once the temperature decreases, filter the mixture; after drying, **compound 4** was obtained as a pale yellow solid with a yield of 59%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (t, J = 9.0 Hz, 2H), 4.31 (t, J = 7.8 Hz, 2H), 3.99 (s, 3H), 3.73 (t, J = 8.0 Hz, 2H), 2.35 (p, J = 7.7 Hz, 2H).^{13}C NMR (101 MHz, DMSO) δ 156.52, 156.49, 156.29, 156.15, 153.73, 153.59, 151.25, 151.12, 148.74, 148.60, 137.75, 137.65, 117.58, 117.55, 117.51, 117.48, 116.60, 116.57, 116.40, 116.37, 109.52, 109.28, 51.07, 34.79, 18.47.^{19}F NMR (376 MHz, DMSO) δ -122.35, -122.41, -133.79, -133.85.

Synthesis of IS-HX： Place compound 2 (0.2g, 0.7mmol), compound 4 (0.2 g, 0.85 mmol), acetic anhydride (2 mL), and sodium acetate (70 mg, 0.85 mmol) in a round-bottom flask. Evacuate the air inside the flask, and react at 80 °C for 5 h. Once the temperature decreases, extract with DCM; after concentrating the organic phase, purify by column chromatography (DCM/MeOH, 1:0–20:1,V/V) to obtain **IS-HX** as a black-green solid with a yield of 45%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (q, J = 13.8, 11.4 Hz, 3H), 7.29 (d, J = 8.7 Hz, 1H), 7.22 (s, 1H), 6.68 (d, J = 8.4 Hz, 1H), 6.64 (s, 1H), 4.15 (s, 5H), 3.45 (d, J = 7.1 Hz, 4H), 3.32–3.27 (m, 2H), 2.76 (d, J = 6.0 Hz, 2H), 2.62 (t, J = 6.0 Hz, 2H), 1.76 (s, 2H), 1.14 (t, J = 7.0 Hz, 6H).^{13}C NMR (101 MHz, DMSO) δ 160.02, 158.61, 156.64, 155.42, 150.89, 141.38, 140.24, 140.07, 132.80, 129.07, 123.14, 115.79, 115.59, 114.65, 111.55, 111.49, 110.45, 109.25, 109.02, 96.72, 46.61, 44.54, 42.39, 28.86, 27.09, 26.98, 21.71, 13.00.^{19}F NMR (376 MHz, DMSO) δ -124.65, -137.59. MS (ESI) m/z: calcd for **IS-HX** ($C_{14}H_{15}F_2N_3O_2$, $[M]^+$), 502.23; found: 502.55.

1.3 In vitro assays

Circular dichroism spectroscopy

Circular dichroism (CD) spectra were measured on a MOS-500 spectrophotometer (BioLogic, France) with a temperature-controlled circulator. Before measurement, oligonucleotides were mixed with reaction buffer and annealed. CD spectra for G4s (3 μM) with or without **IS-HX** (3 μM) were recorded in the range of 230 – 320 nm with a bandwith of 5 nm, a step size of 0.5 nm and 0.5 s per point, and all samples were measured three times. The quartz cuvette of 10 mm path length was used in all experiments.

UV-Vis spectroscopy

For absorption measurements, **IS-HX** (3.0 μM) with or without c-Myc DNA G4 (5.0 μM) were mixed in PBS buffer (10 mM, pH = 7.4, 50 mM K^+) and absorbance spectra were recorded in the range of 550 nm – 850 nm.

Fluorescence Studies

Fluorescence spectra of **IS-HX** (3.0 μM) with or without c-Myc DNA G4 (5.0 μM) in PBS buffer (10 mM, pH = 7.4, 50 mM K^+) were recorded in the range of 740 – 850 nm with an excitation wavelength of 720 nm.

For selectivity studies, **IS-HX** (3.0 μM) were incubated with varying nucleic acid structures including ssDNA, dsDNA, RNA/DNA G4s (Table S1) and fluorescence spectra were recorded. **IS-HX** (3 μM) was incubated with various testing substances including BSA (2.0 μg/mL), glycine (100 μM), GSH (5 mM), L-leucine (100 μM), L-proline (100 μM), L-tryptophan (100 μM), L-arginine (100 μM), HClO (100 μM), Cu^{2+} (100 μM), Zn^{2+}(100 μM), Mg^{2+} (100 μM), Fluorescence spectra were recorded in the range were recorded in the range of 740 – 850 nm with an excitation wavelength of 720 nm.

For anti-interference performance assay, **IS-HX** (3 μM) with c-Myc DNA G4 (5.0 μM) was incubated with various testing substances including BSA (2.0 μg/mL), glycine (100 μM), GSH (5 mM), L-leucine (100 μM), L-proline (100 μM), L-tryptophan (100 μM), L-arginine (100 μM), HClO (100 μM), Cu^{2+} (100 μM), Zn^{2+}(100 μM), Mg^{2+} (100 μM), Fluorescence spectra were recorded in the range were recorded in the range of 740 – 850 nm with an excitation wavelength of 720 nm.

For fluorescence titration, **IS-HX** (3 μM) were incubated with different concentrations of c-Myc DNA G4 (0 – 5.0 μM) and fluorescence spectra were

obtained with an excitation and emission slit widths of 5 nm. The association constant (K_a) of **IS-HX** to c-Myc DNA G4 was calculated with Benesi – Hildebrand method[S1].

$$\frac{1}{F-F0}=\frac{1}{Ka(Fmax-F0)[DNA]}+\frac{1}{Fmax-F0}$$

F_0 is the fluorescence intensity of **IS-HX** without c-Myc DNA G4, F is the fluorescence intensity measured with c-Myc DNA G4, F_{max} is the intensity of **IS-HX** in the presence of excessive c-Myc DNA G4. The values of 1/ ($F-F_0$) were plotted against those for 1/[G4s] and K_a is determined to be intercept/slope.

For Job's plot, fluorescence spectra for **IS-HX** with different molar fractions of c-Myc DNA G4 in PBS buffer (10 mM, pH = 7.4, 50 mM K^+) were acquired. The total concentrations of c-Myc DNA G4s and **IS-HX** were fixed to 10 μM. Fluorescence intensities at 758 nm were plotted versus the molar fractions of c-Myc DNA G4.

For photostability studies, **IS-HX** (5.0 μM) incubated with c-Myc DNA G4 (5.0 μM) in PBS buffer (10 mM, pH 7.4, 50 mM KCl) were irradiated on an FS5 for 1 h and fluorescence intensities at 758 nm were recorded in real time. For reference, cyanine 5 (Cy5) was also irradiated under the same conditions and fluorescence intensities at 660 nm were recorded.

Molecular Docking Experiment.

Structure of c-Myc DNA G4 was obtained from the RCSB protein data bank (PDB ID: 2L7V[S2]). **IS-HX** was geometrically optimized by Gaussian 16 under SMD (water) implicit solvation model with density functional theory B3LYP-GD3BJ/def-TZVP level. The likely binding positions of molecules on G4 were explored by AutoDock Vina 1.2.3 and global docking was performed by setting the grid size that covers the entire G4. Top 1 docking model was used for further docking with more G4 by ZDOCK. All docking results were visualized by UCSF ChimeraX.

1.4 Cellular Experiments

Cell Culture

HepG2 cells were cultured in Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 U/mL). HepG2 cells were incubated at 37 °C in a humidified incubator containing 5% CO_2 (v/v). The cell density was determined using a TC20™ automated cell counter (BIO-RAD, USA).

Confocal Imaging

HepG2 cells were seeded in confocal imaging chambers at a density of 1×10^5 cells. After 12 h, the cells were incubated with 3.0 μM of **IS-HX** in serum-free cell culture medium at 37 °C for designated time intervals. Then the live cells were washed three times with 1×PBS and imaged by confocal imaging (Nikon, Japan).

Cytotoxicity Study.

The cytotoxicity of **IS-HX** against living cells was evaluated by a standard CCK-8 assay. HepG2 cells were seeded at 1×10^4 cells per wells. All the cells were first cultured at 37 °C for 24 h. Then the **IS-HX** probe (0, 1, 3, 5, 7, 10, 30, 50, 100 μM) in 200 μL fresh culture medium was added and incubated for another 24 h. Afterwards, the culture medium was removed and cells were washed with 1×PBS (200 μL). Then, CCK-8 reagent (10 μL, 5 mg/mL) was added and incubated at 37 °C for 4 h. The absorbance at 450 nm was obtained on an ELx800™ microplate reader.

Flow Cytometry Assay

HepG2 cells cells were cultured in 6-well plates at a density of 1×10^4 cells/well for 24 h and then incubated with **IS-HX** as indicated. The cells were washed twice with PBS (pH = 7.4), treated with 0.25% trypsin and centrifuged at 1500 rpm for 3 min at room temperature. The cell pellet was washed and suspended in PBS for flow cytometry analysis on a FACSVerseTM flow cytometer (BD Biosciences, USA). Data was analyzed with Flow Jo software.

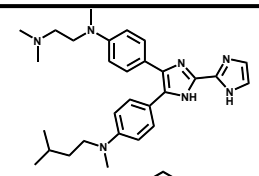
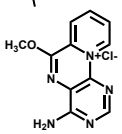
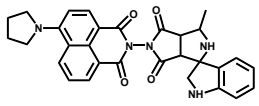
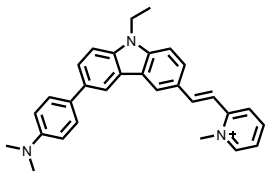
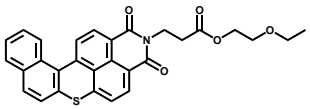
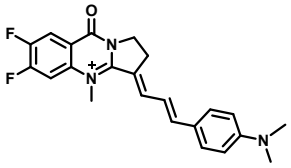
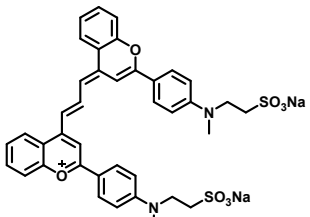
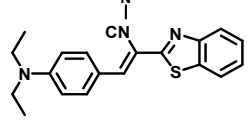
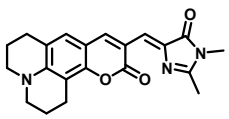
Imaging DNA G4 Under Oxidative Stress

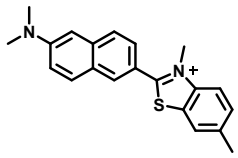
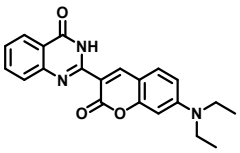
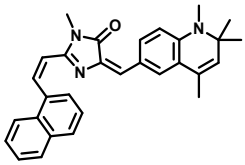
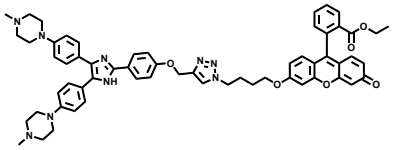
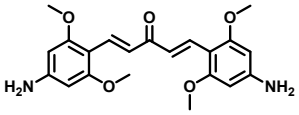
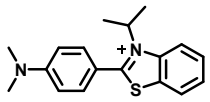
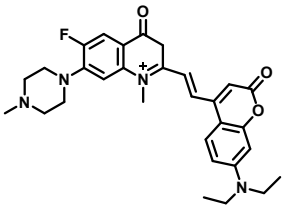
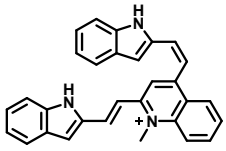
To study glycolysis-induced formation of mtDNA G4, HepG2 cells were cultured under hypoxic conditions (Cells were placed in a sealed modular incubator chamber which was flushed with a humidified gas mixture of 5% CO_2, 1% O_2, and 94% N_2 at a flow rate of 20 L/min for 4 minutes. The chamber was then sealed and incubated at 37 °C for 6 hours to establish hypoxic conditions.) for 6 h and then stained with **IS-HX** (1 μM) for 1 h. To inhibit glycolysis, HepG2 cells were treated with 2-MeOE2 (0.1 μM) or KC7F2(10 μM) for 24 h and then incubated with **IS-HX** (1 μM) for 1h. The cells were washed with 1 × PBS three times and imagedwith a Nikon.

Table S1. List of oligonucleotides studied in this study

Name	Sequence（5’ to 3’）
ds26	CAATCGGATCGAATTCGATCCGATT
A30	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
22AG	AGGGTTAGGGTTAGGGTTAGGG
KRAS	AGGGGGGTGTGGAAGAGGGAAGAGGGGGAGG
Te126	AAAGGGTTAGGGTTAGGGTTAGGGAA
WtTe126	TTAGGGTTAGGGTTAGGGTTAGGGTT
VEGF	GGGAGGGTTGGGGTGGG
bc12	GGGCGGGCGCGGGAGGAAGGGGGCGGG
mtDNA1050	GGGCTTGATGTGGGGAGGGGTGTTTAAGGG
mtDNA16250	GAAGCGGGGGAGGGGGGGTTTGGTGGAAAT
mtDNA8095	GGGAGGTAGGTGG
mtDNA8095(antisense)	CCACCTACTCCC
c-Myc	TGAGGGTGGGTAGGGTGGGTAA
C-KIT-1	AGGGAGGGCGCTGGGAGGAGGG
5′-AF488-c-Myc	5′-AF488-TGAGGGTGGGTAGGGTGGGTAA

Table S2. The K_d value of various fluorescent probes of G4s.

Compound Structure	Target	K_d (μM)	Ref.
	c-Myc	0.75	BBA-Revcancer, 2025, 1880, 189391.
	Bcl-2	18.3	J. Mol. Struct., 2025, 1348, 143531.
	c-Myc	4.02	ACS Applied Bio. Materials. 2025, 8, 3728-3747.
	c-Myc	0.09	Dye. Pigments. 2025, 242, 112963.
	Pu22	3.7	Nanoscale Horiz., 2025, 10, 1660.
	c-Myc	1.09	Anal. Chem. 2024, 96, 17329−17336.
	Pu22	1.71 ± 1.13	J. Am. Chem. Soc. 2024, 146, 11669-11678.
	c-Myc	1.68 ± 0.12	Angew. Chem. Int. Ed. 2023, 62, e202215049.
	NG16	1.37 ± 0.18	Chem. Sci. 2023, 14, 4538.

	c-Myc	1.16	Anal. Chem. 2022, 94, 10283-10290.
	c-Myc	11.10	Anal. Chem. 2021, 93, 12, 5267-5276.
	c-Myc	1.06 ± 0.05	J. Am. Chem. Soc. 2021, 143, 46, 19317-19329.
	Pu18	0.53	Anal. Chem. 2019, 91, 2480-2487.
	VEGF	3.03	ACS Omega. 2018, 3, 10487-10492.
	c-Myc	6.25	Nucleic Acids Research, 2018, 46, 7522-7532.
	Terra	0.57	Angew. Chem. Int. Ed. 2018, 57, 4702-4706.
	Telo26	9.80	Dye. Pigments. 2017, 143, 331-341.

References

[S1] Jung, H. S.; Lee, J-H.; Kim, K.; Koo, S.; Verwilst, P.; Sessler, J. L.; Kang, C.; Kim, J. S. A mitochondria-targeted cryptocyanine-based photothermogenic photosensitizer. J. Am. Chem. Soc. 2017, 139, 9972-9978.

[S2] Dai, J.; Carver, M.; Hurley, L. H.; Yang, D. Solution structure of a 2: 1 quindoline–c-Myc G-quadruplex: insights into G-quadruplex-interactive small molecule drug design. J. Am. Chem. Soc. 2011, 133, 17673-17680.

Additional Figures

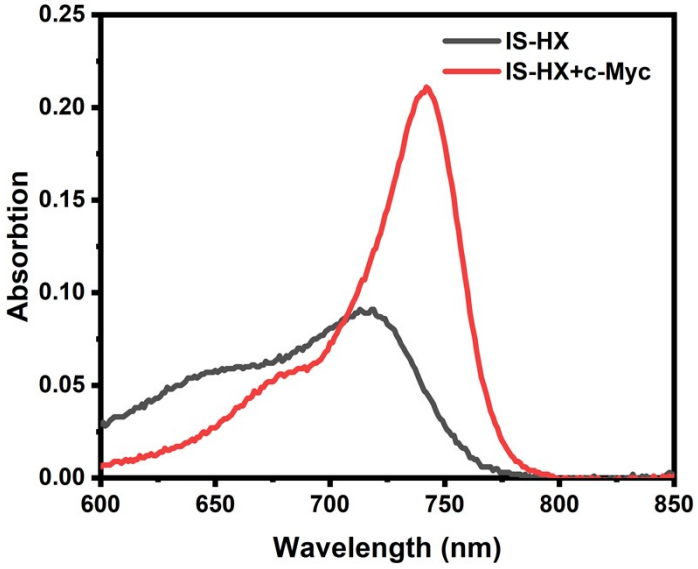


Figure S1. Absorbance spectra for **IS-HX** (3.0 μM) in the absence or presence of c-Myc (5.0 μM) in PBS buffer (10 mM, pH = 7.4, 50 mM K^+).

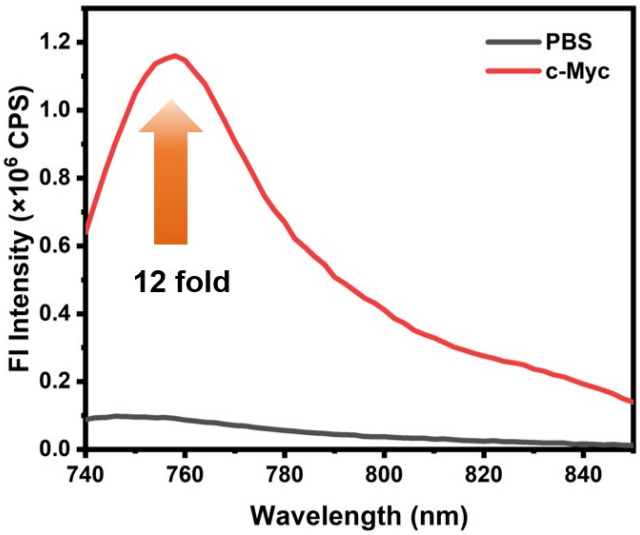


Figure S2. Fluorescence spectra for **IS-HX** (3.0 μM) in the absence or presence of c-Myc (5.0 μM) in PBS buffer (10 mM, pH = 7.4, 50 mM K^+).

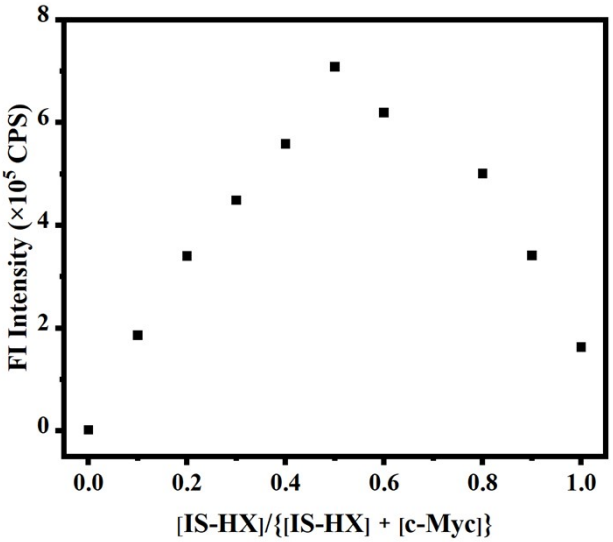


Figure S3. Job's plot for **IS-HX** and c-Myc DNA G4. The total concentrations for **IS-HX** and G4 were fixed to 10 μM. Job's plots were obtained by plotting fluorescence intensities for **IS-HX** at 758 nm versus the molar fractions of G4.

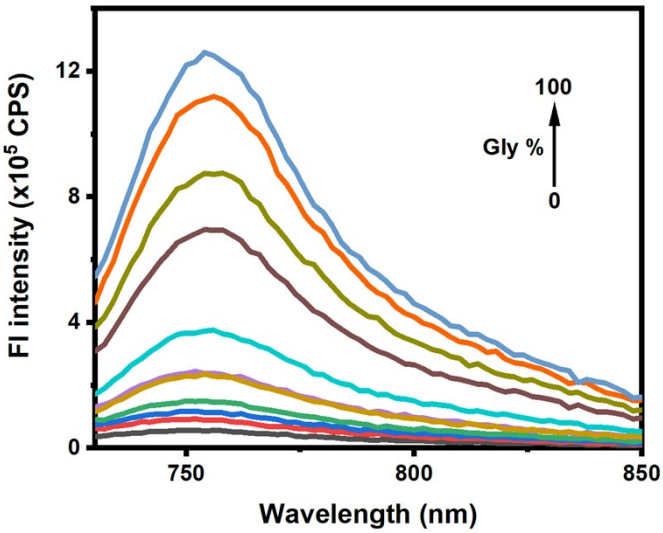


Figure S4. Fluorescence spectra of **IS-HX** (3μM) in mixtures of PBS and glycerol with varying volume ratios (glycerol content ranging from 0 to 100 %).

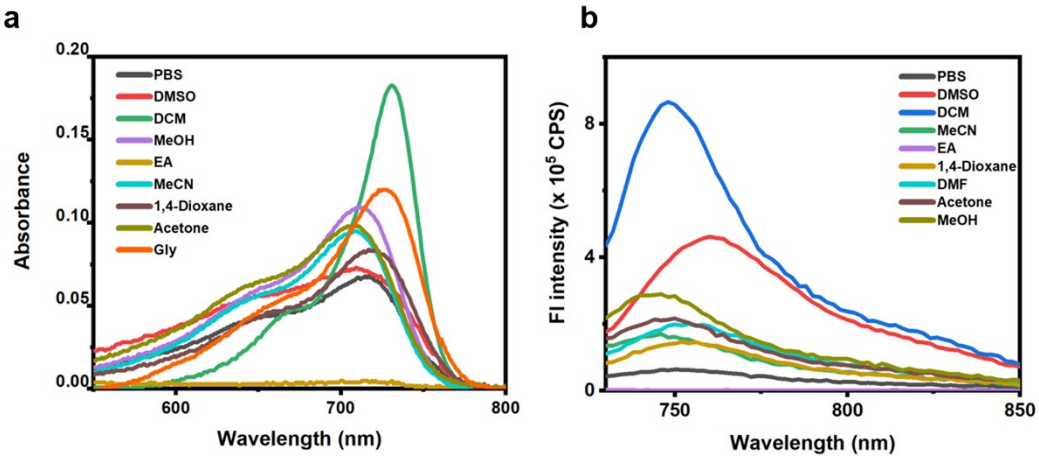


Figure S5. (a) Absorption spectra of **IS-HX** in different solvents. (b) Fluorescence spectra of **IS-HX** in different solvent.

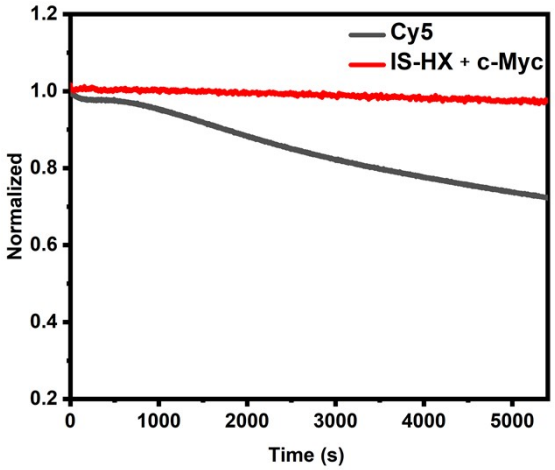


Figure S6. Photostability of **IS-HX** (5.0 μM) in the presence of c-Myc DNA G4 (5.0 μM) using Cy5 (5.0 μM) as a reference.

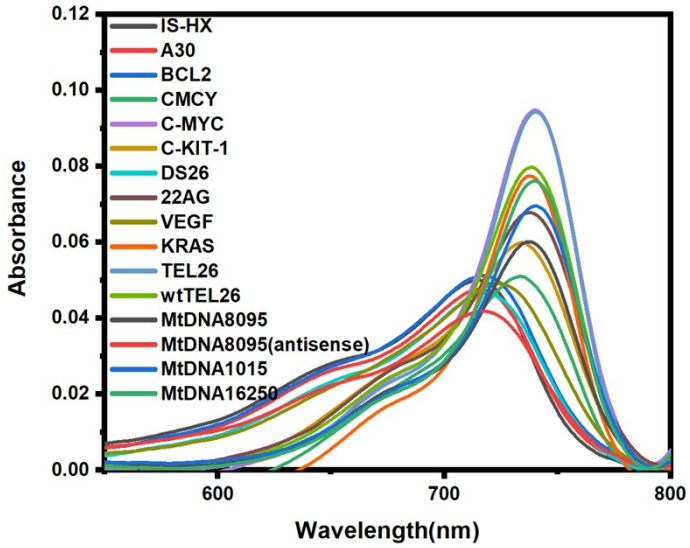


Figure S7. Absorption spectra for **IS-HX** (5.0 μM) in the presence of ssDNA, dsDNA RNA/DNA G4 in PBS buffer (10 mM, pH = 7.4, 50 mM K^+).

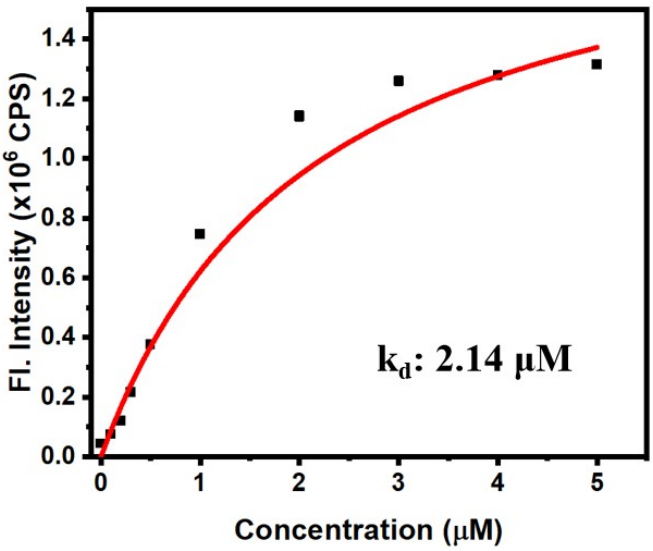


Figure S8. Dissociation constants of **IS-HX** (3 μM) toward c-Myc DNA G4 (10 mM, pH 7.4, 50 mM K^+, λem = 758 nm).

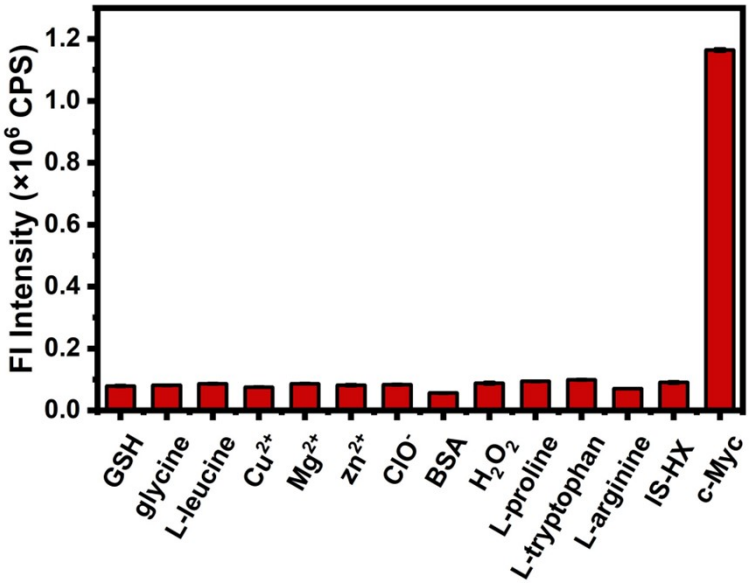


Figure S9. Selectivity of **IS-HX** (3 μM) towards various substances based on fluorescence analysis.

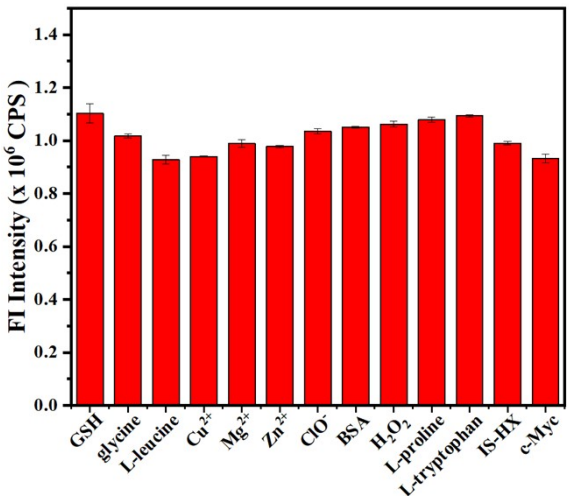


Figure S10 The anti-interference performance of IS-HX (3.0 μM) towards various substances based on fluorescence analysis.

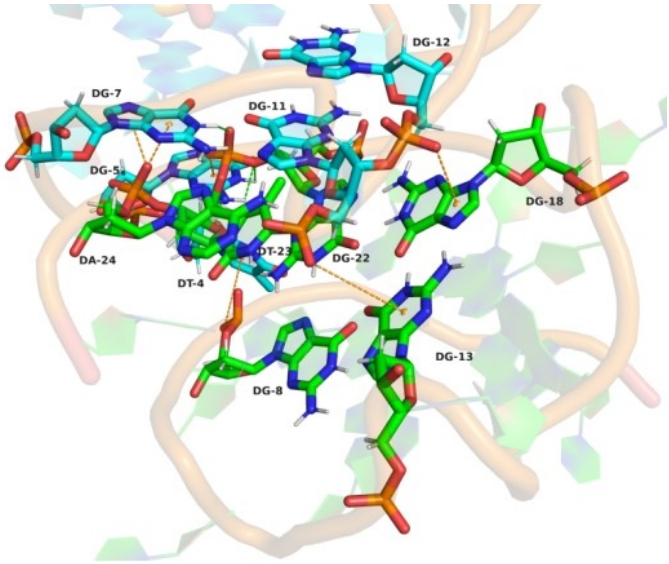


Figure S11. The backbone of G4 structure was rendered in tube and colored in cyan; Compound **IS-HX** is rendering by green; Hydrogen bonds are depicted as green dashed lines; Anion-π interactions are depicted as orange dashed lines. (The D in DG, DT, and DA refers to DNA G4, G, T, and A are the corresponding base types)

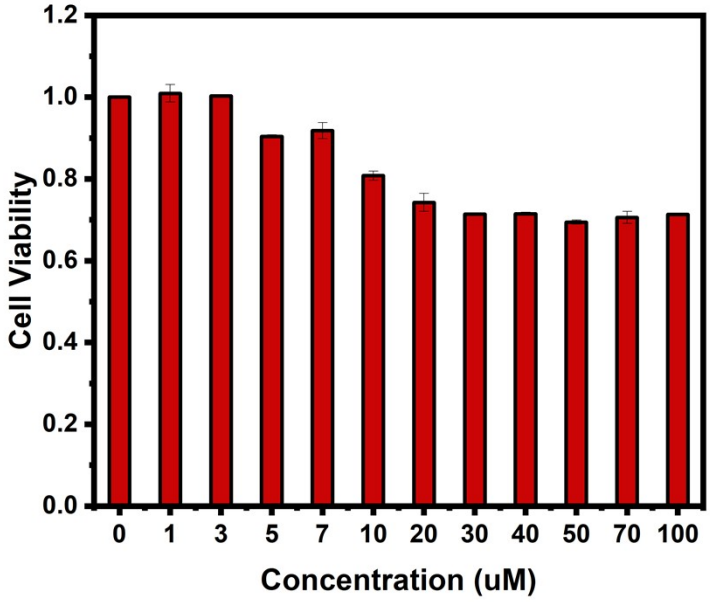


Figure S12. The effect of **IS-HX** (0-100 μM) on the viability of HepG2 cells. The viability of the cells without **IS-HX** is defined as 1. The error bars represent the standard deviation from three independent measurements.

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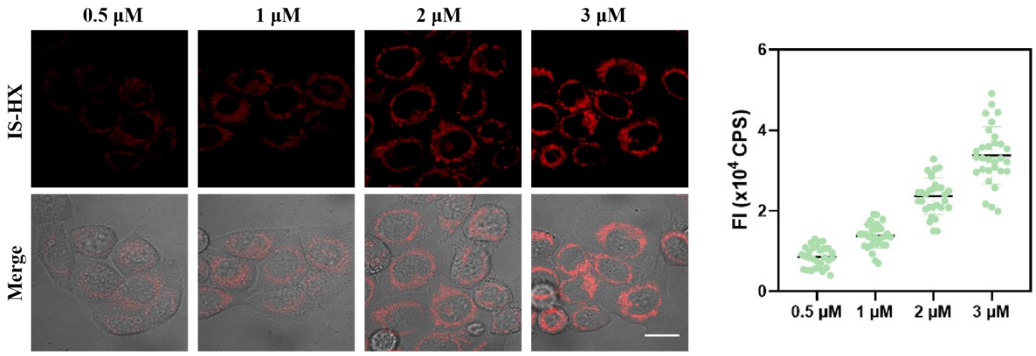

Figure S13. (a) CLSM imaging for HepG2 cells treated with varying concentrations of **IS-HX**. (b) Average fluorescence intensity in 30 interest regions for each cell group in Figure a; Scale bar: 30 μm.

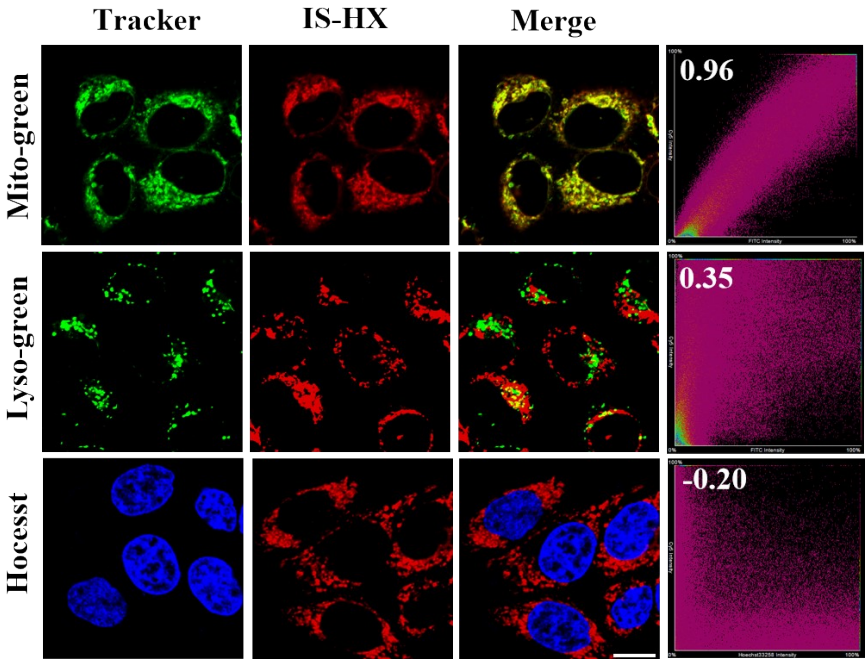

Figure S14. CLSM imaging for HepG2 cells treated with **IS-HX** and costained with Mito-tracker Green, Lyso-tracker Green, or Hoechst 33342. Scale bar: 30 μm.

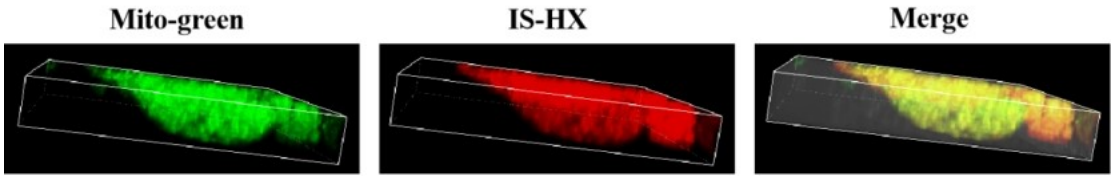

Figure S15. Fluorescence imaging through the z-axis of HepG2 cells incubated with **IS-HX** (1μM) for 1 h and followed by 100 nM Mito-Tracker Green for 30 min. (Scale bar = 10 μm.).

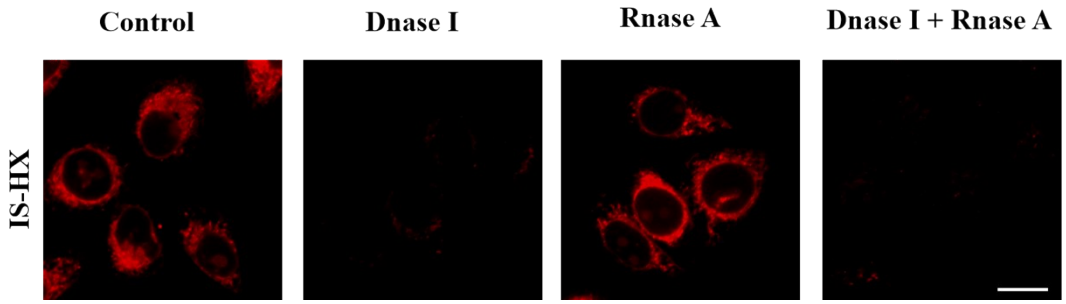

Figure S16. CLSM imaging for HepG2 cells stained with **IS-HX** and treated with DNase I, RNase A, or RNase A, followed by DNase I. Scale bar: 30 μm.

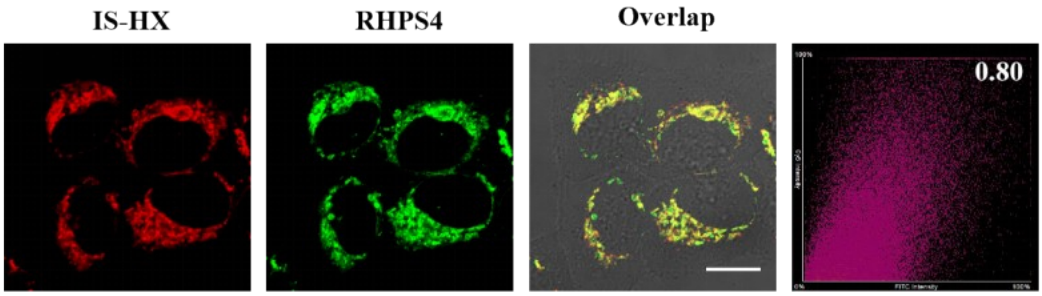

Figure S17. CLSM imaging for HepG2 cells treated with RHPS4 and co-stained with **IS-HX.** Scale bar: 30 μm.

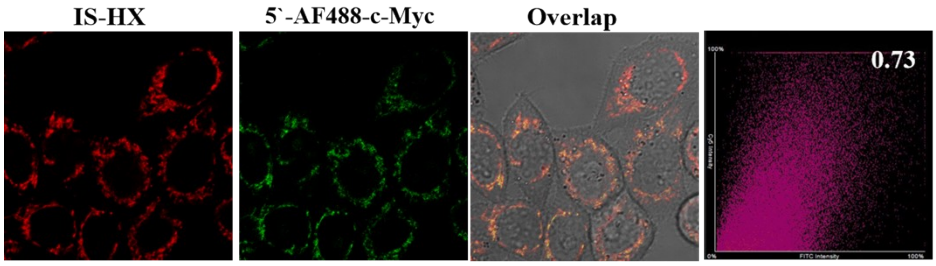

Figure S18. CLSM imaging for live HepG2 cells transfected with 40 pmol c-Myc DNA G4 forming oligonucleotide labelled with 5′ - AF488 using lipofectamine 3000 and then incubated with IS-HX (3.0 μM) for 1 h successively. Scale bar: 30 μm.

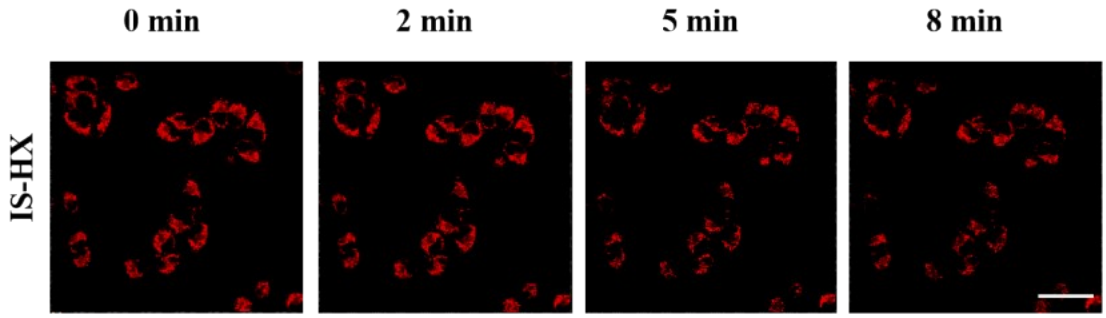

Figure S19. Photostability of **IS-HX** in live cells. HepG2 cells were incubated with **IS-HX** and fluorescence images were obtained at different intervals after continuous irradiation. Scalebars=30 μm.

NMR and MS spectra

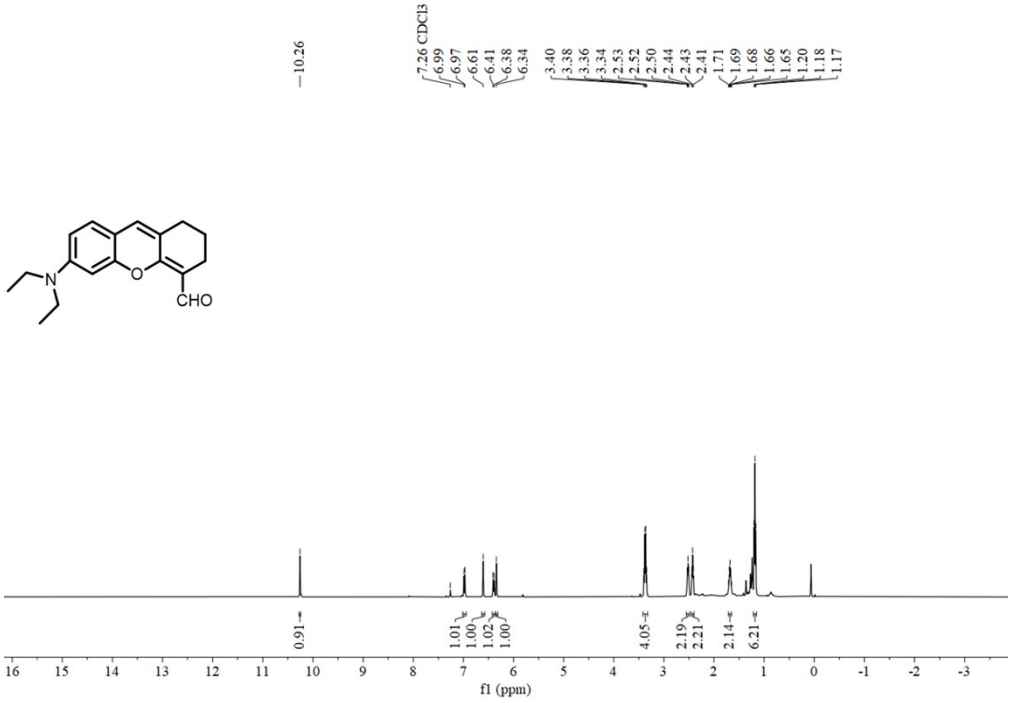

Figure S20. ^{1}H NMR spectrum of **compound 2** in $CDCl_3$.

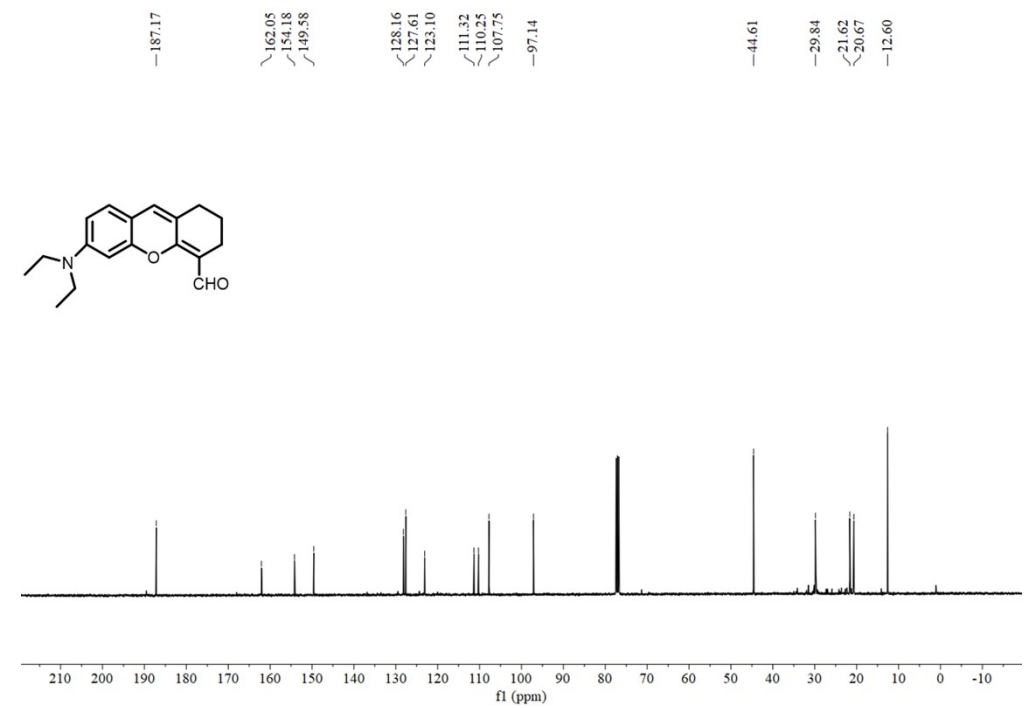

Figure S21. ^{13}C NMR spectrum of **compound 2** in $CDCl_3$.

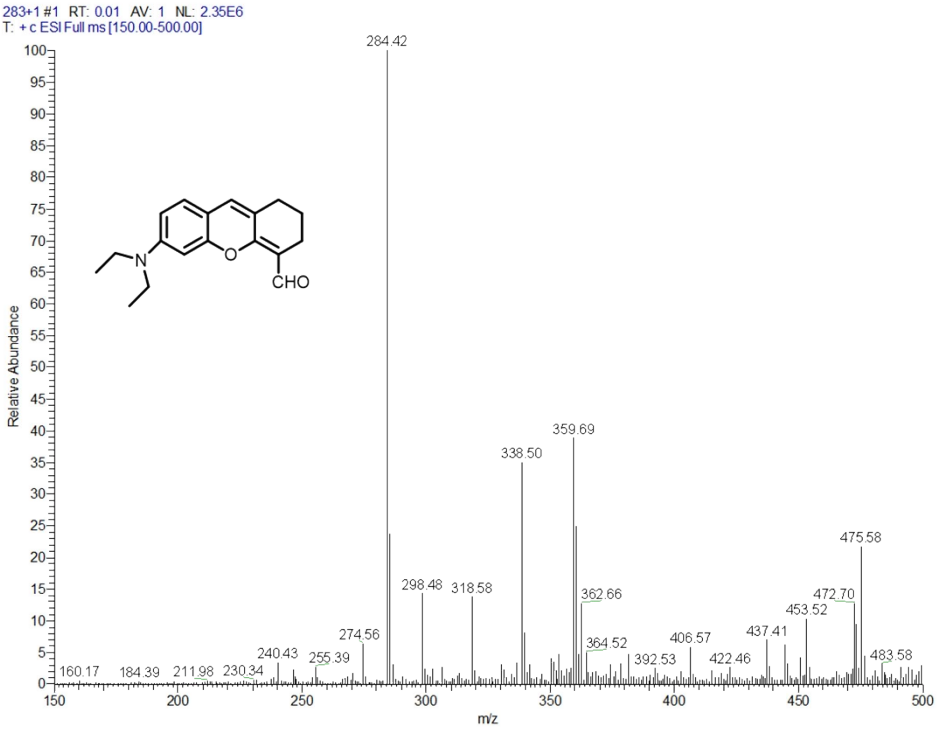

Figure S22. ESI-MS spectrum of **compound 2.**

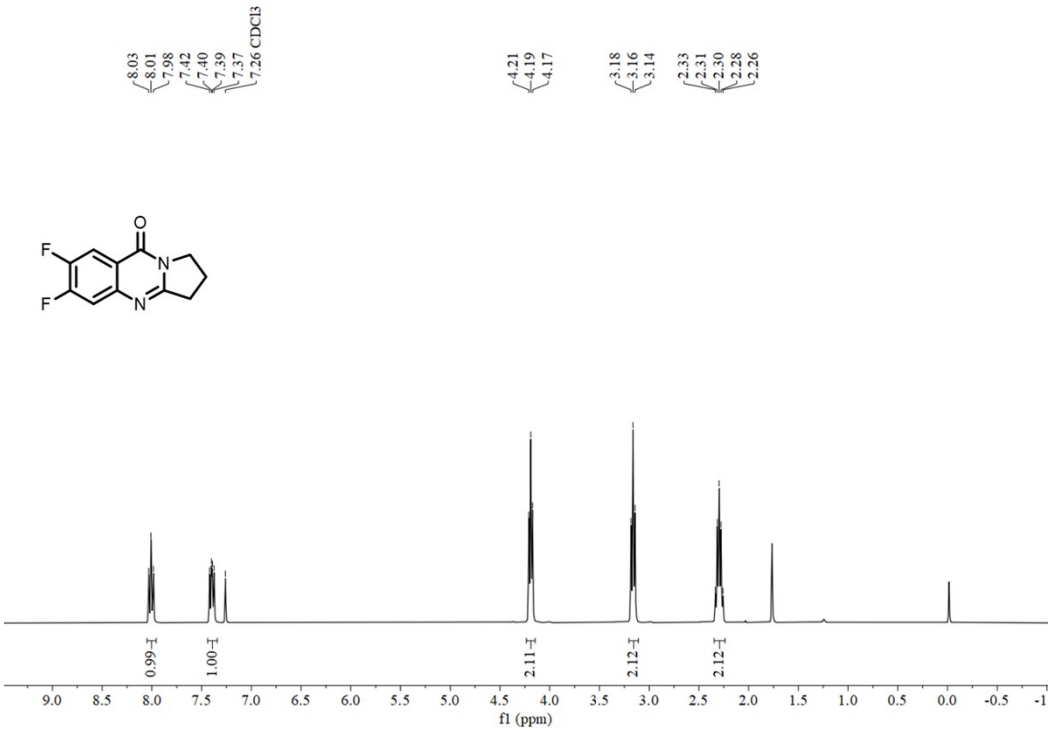

Figure S23. ^{1}H-NMR spectrum of **Compound 3** in $CDCl_3$.

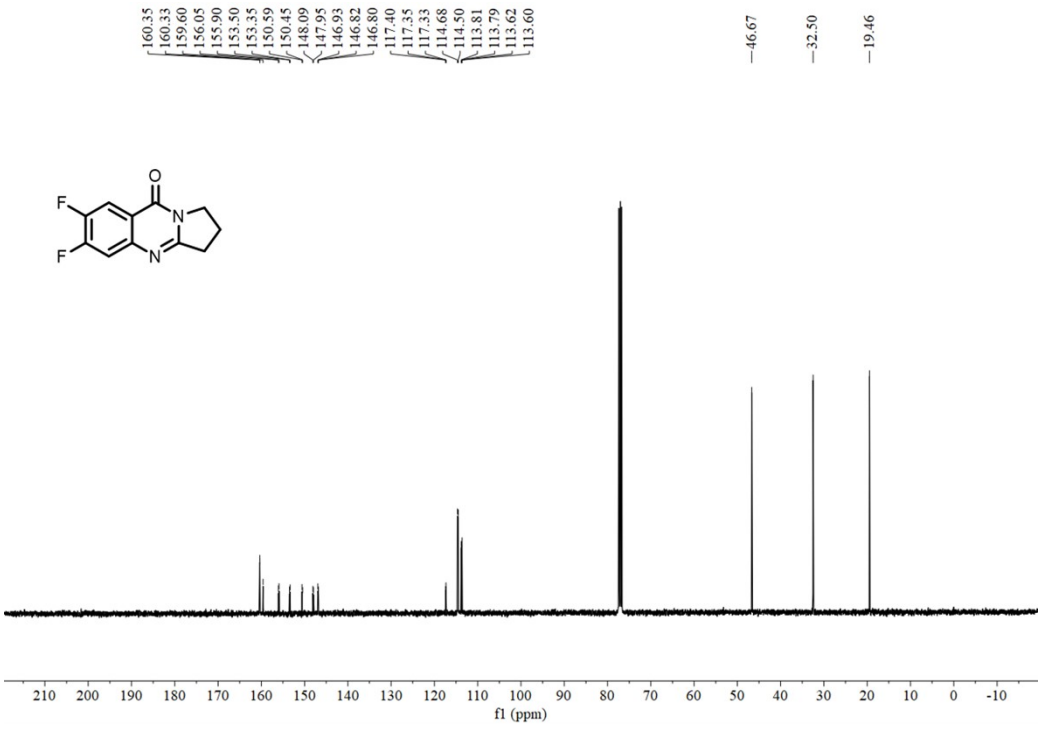

Figure S24. ^{13}C NMR spectrum of **Compound 3** in $CDCl_3$.

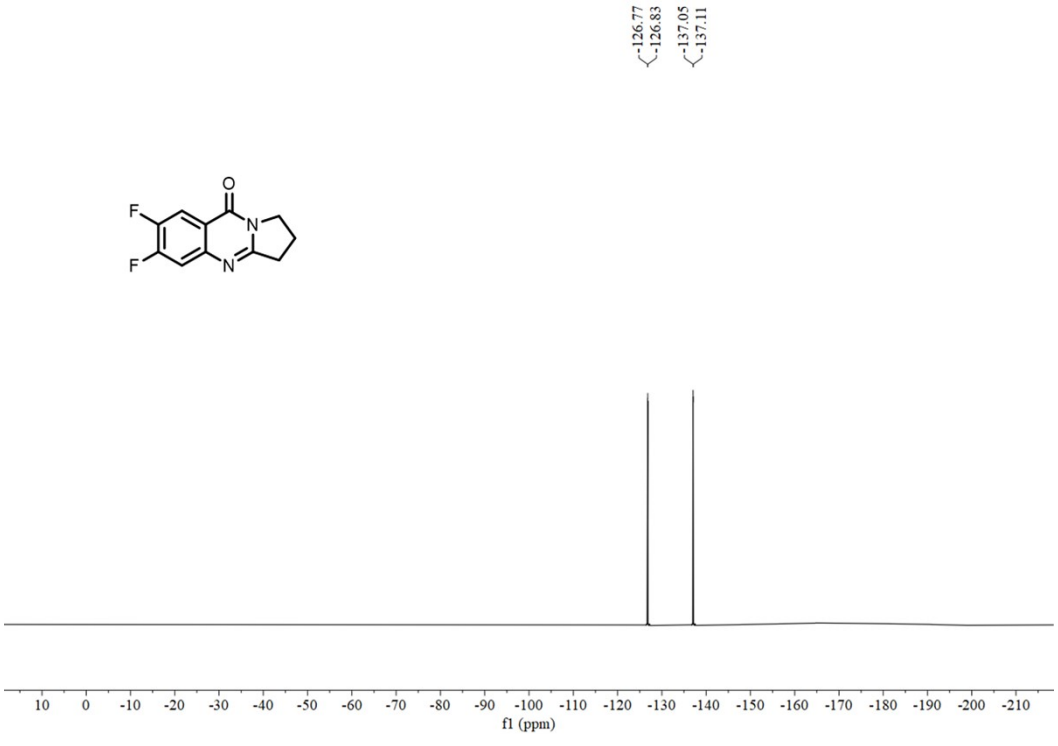

Figure S25. ^{19}F-NMR spectrum of **Compound 3** in $CDCl_3$.

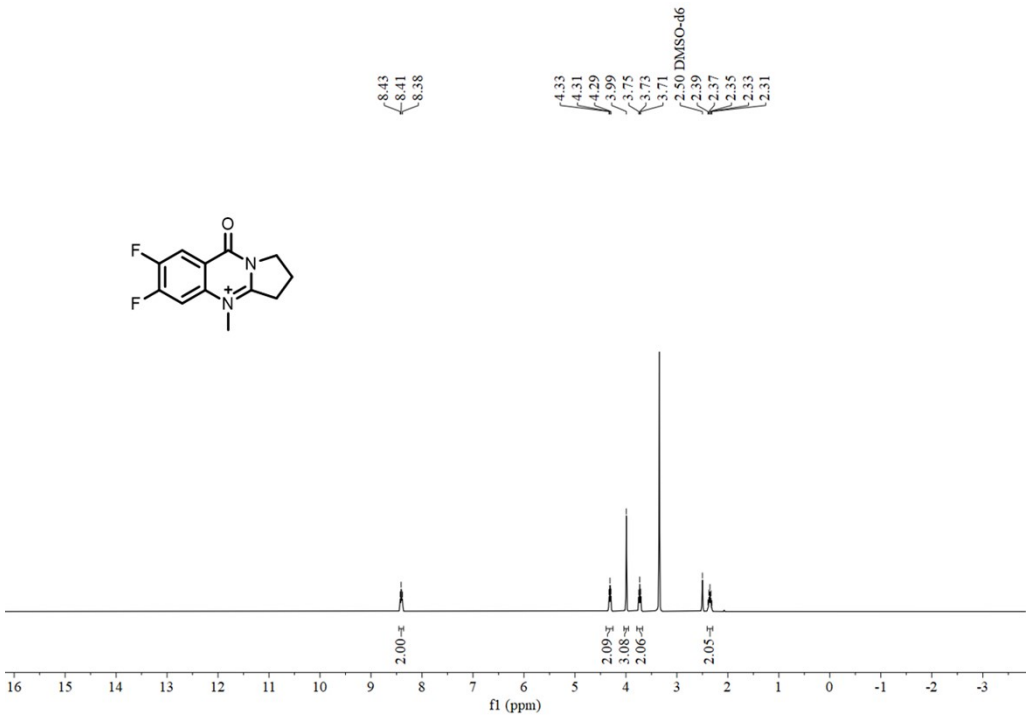

Figure S26. [1]H-NMR spectrum of **Compound 4** in DMSO-d_6

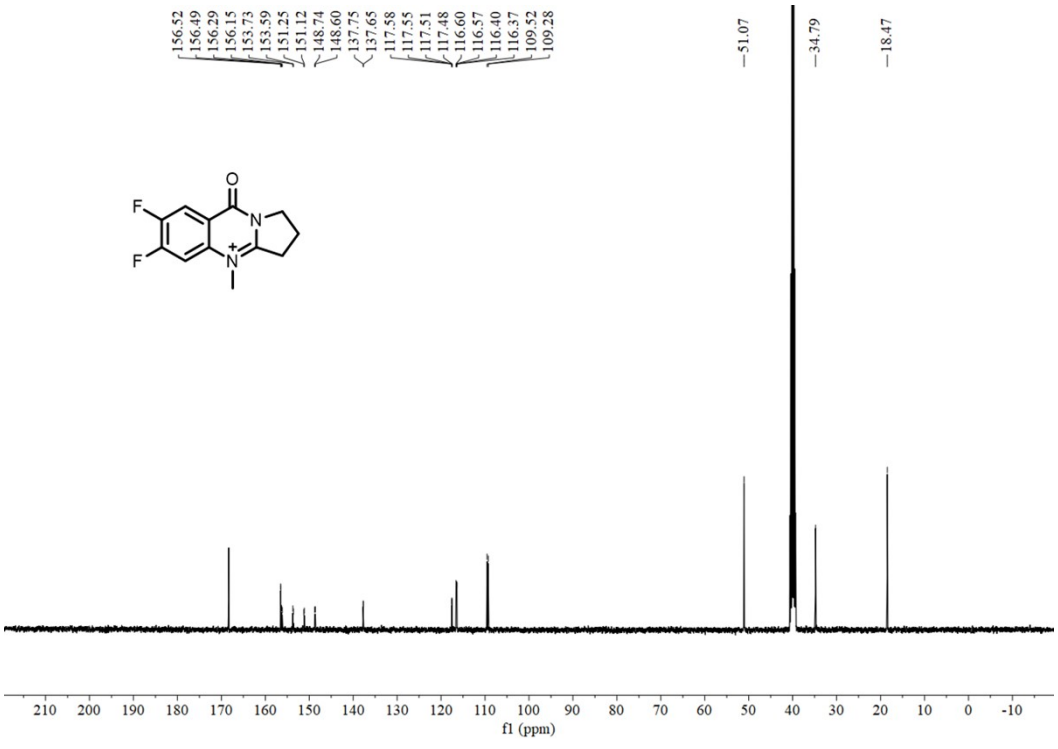

Figure S27. [13]C NMR spectrum of **Compound 4** in DMSO-d_6.

Figure S28. ^{19}F-NMR spectrum of **Compound 4** in DMSO-d_6.

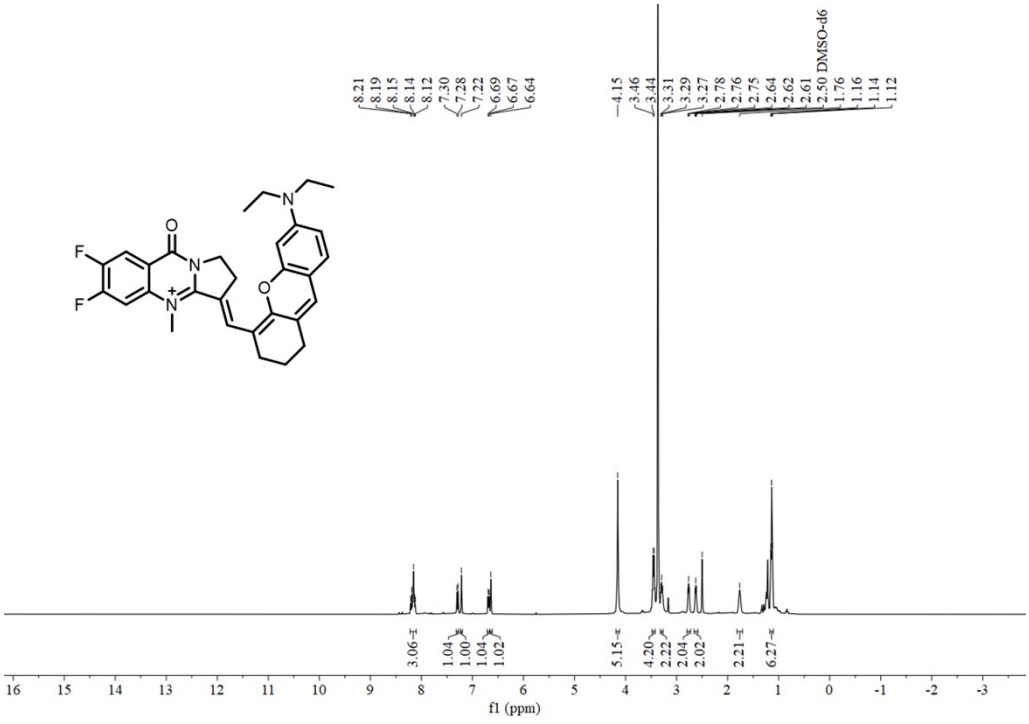

Figure S29. ^{1}H NMR spectrum of **IS-HX** in DMSO-d_6.

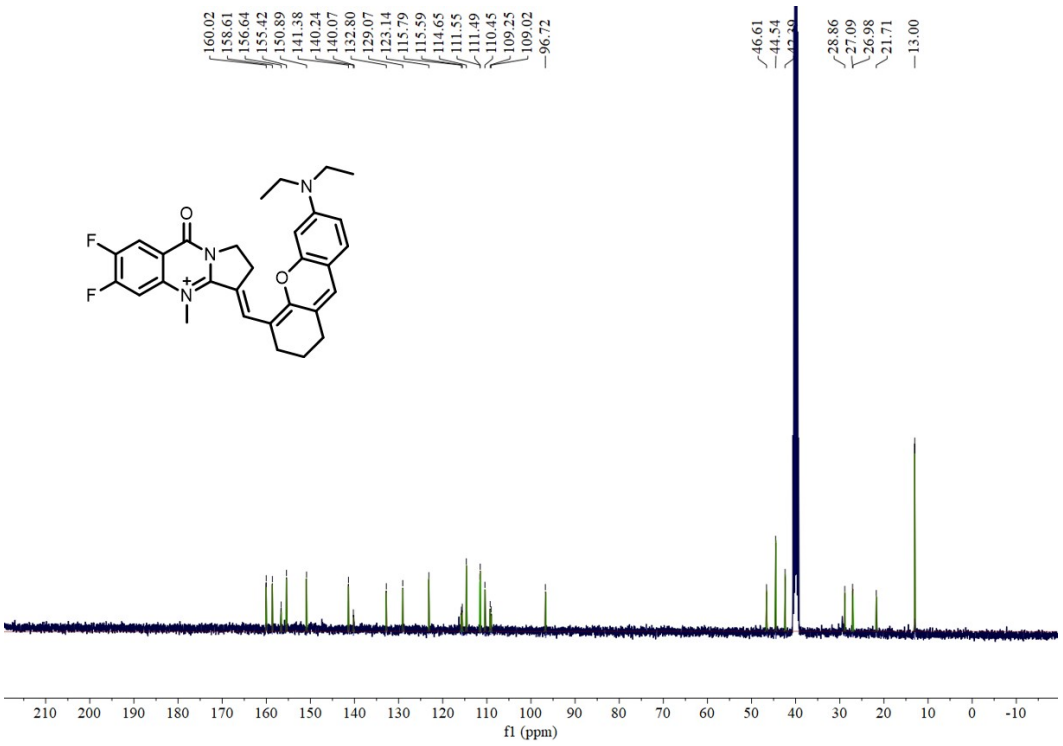

Figure S30. ^{13}C NMR spectrum of **IS-HX** in DMSO-d_6.

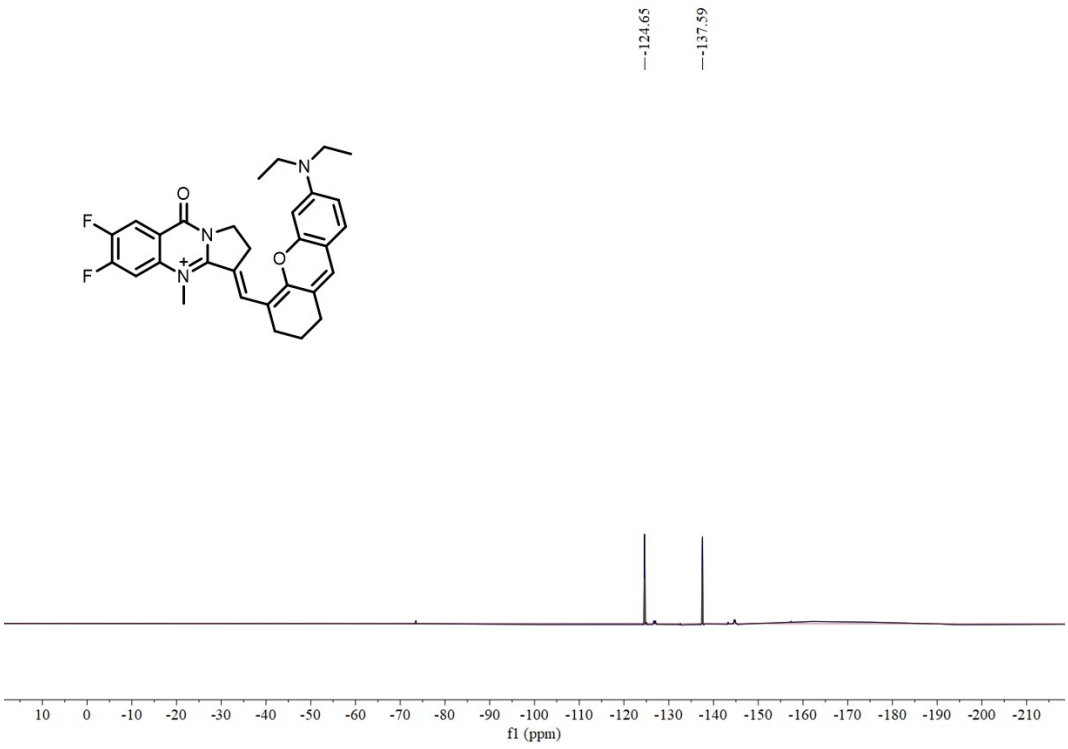

Figure S31. ^{19}F NMR spectrum of **IS-HX** in DMSO-d_6.

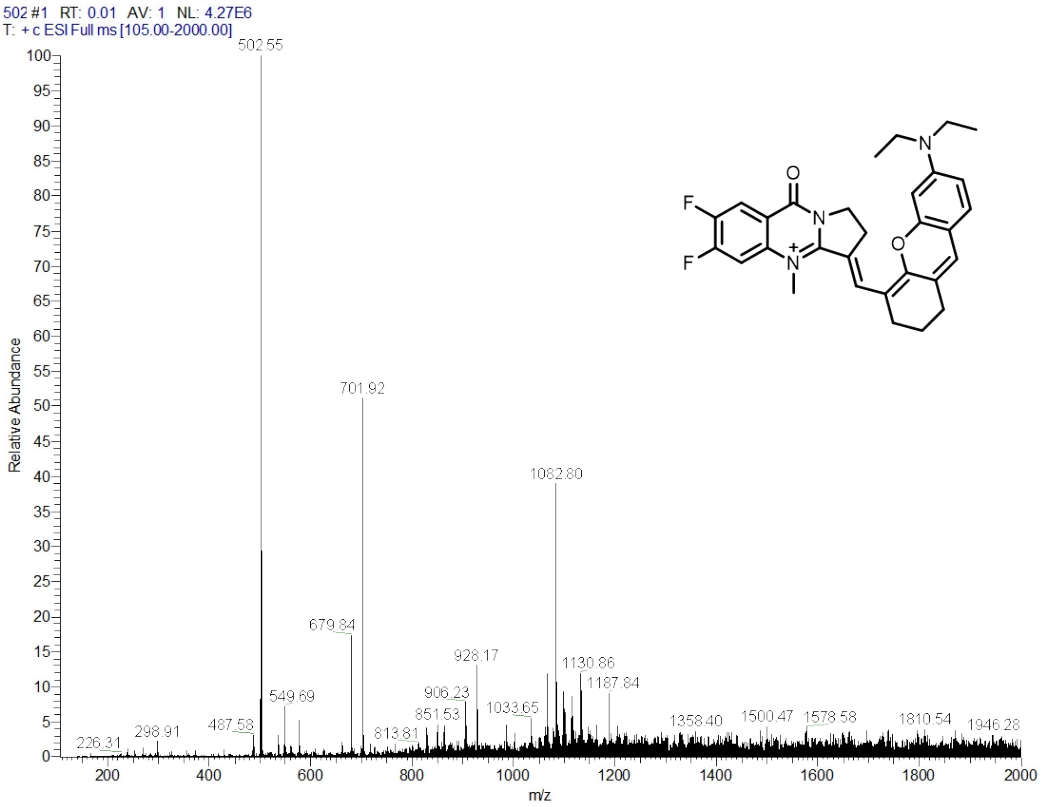

Figure S32. ESI-MS spectrum of **IS-HX**.