Supporting Information

Synthesis, Photochromic Properties, and Bioactivity of Spiropyrans with Electron Donating/Withdrawing Substituents on Indoline and [2H]-Chromene rings

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1.General Information

All reagents and solvents were purchased from the commercial market (Spectrochem, SRL, Alfa Aesar, TCI, and Sigma Aldrich) and used as such unless otherwise mentioned. Column chromatography was carried out with silica gel (200-300 mesh). Analytical Thin Layer Chromatography (TLC) was performed using silica gel 60 F254 precoated (0.25mm) and products were visualized under a UV chamber (254 nm and 365 nm). ^{1}H-NMR, ^{13}C-NMRand ^{19}F-NMR spectra were acquired on a Bruker Avance spectrometer operating at 400 MHz and 100 MHz, and 400 MHz respectively, with $CDCl_3$ as the solvent. Chemical shifts (δ) were referenced to residual solvent peaks ($CHCl_3$: δ = 7.28 ppm for ^{1}H-NMR; δ = 77.0 ppm for ^{13}C-NMR). Signal multiplicities are denoted as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). The top spin 3.6.2 package was used for processing all the NMR. The High-Resolution Mass Spectra (HRMS) were measured on Water Xevo G2-XS Q-TOF equipped with Electrospray Ionization (ESI) using Time of Flight mass spectrometry. UV-vis absorption spectra were obtained on a Shimadzu UV-Vis-NIR spectrophotometer (Japan Analytical Instruments), operating in medium scan mode with 1.0 nm slit widths. Matched quartz cells were used for all measurements. UV and visible photoirradiation studies employed wavelengths of 370 nm and 440 nm respectively. All absorption scans were saved as ACS II files and processed using OriginLab software to generate the presented graphs.

2. Synthetic Scheme of different Spiropyran:

The spiropyran derivatives (**1a**, **2a**, **3a**, and **4a**) were synthesized through a multi-step process involving condensation and functionalization reactions. Each spiropyran derivative was purified using column chromatography to achieve high purity. Structural confirmation was performed using ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR and mass spectrometry.

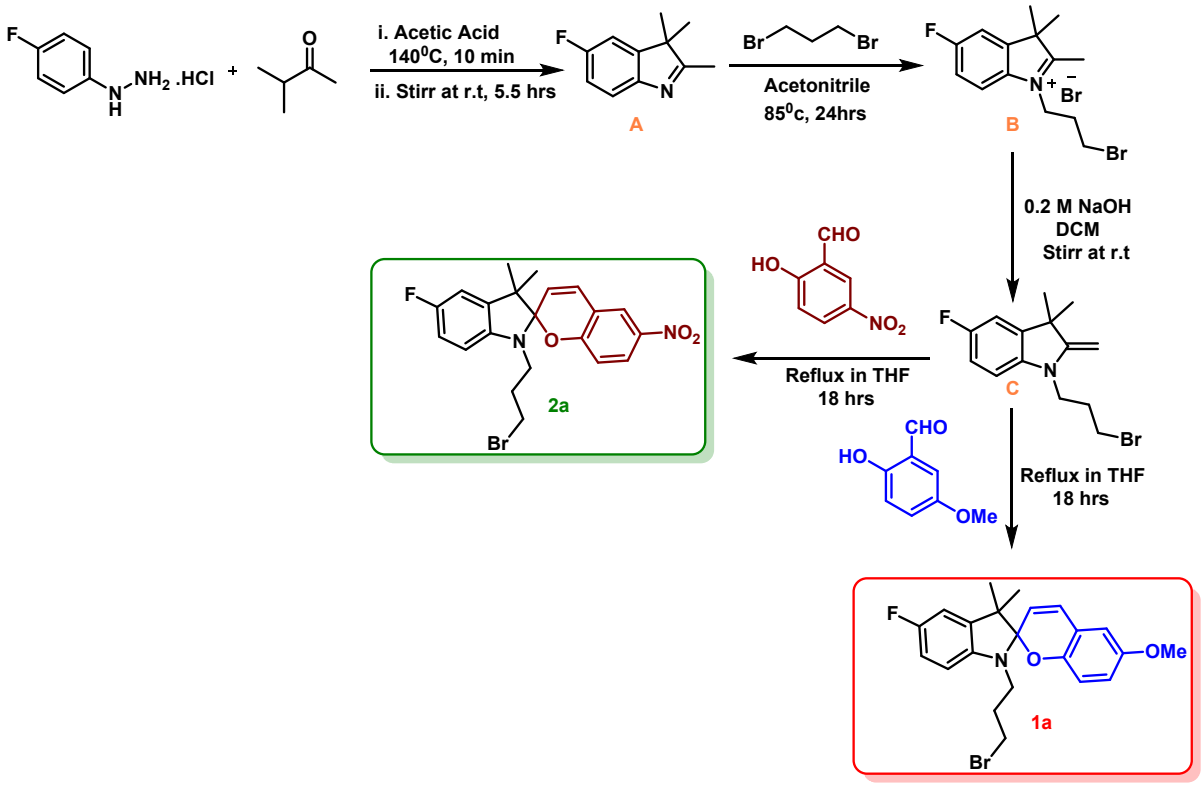


Figure S1: Synthesis of different intermediates and the targeted spiropyran (**1a** and **2a**).

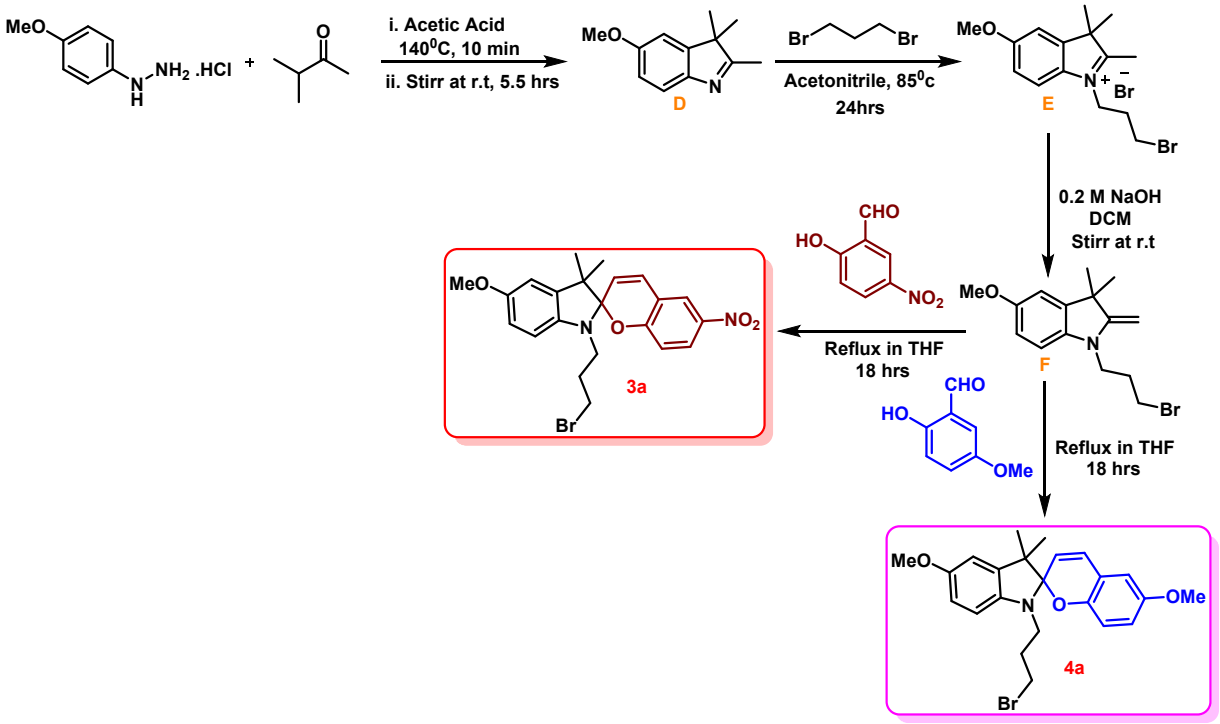
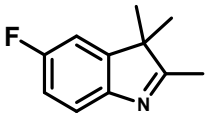


Figure S2: Synthesis of different intermediates and the targeted spiropyran (**3a** and **4a**).

3. Characterization Data:

5-fluro-2,3,3-trimethyl-3H-indole (A)

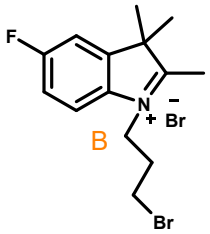


A

To a solution of 3-methyl-2-butanone (5.90 mL, 55.2 mmol, 2 equiv.) in glacial acetic acid (92 mL), 4-fluorophenyl hydrazine hydrochloride (4.49 g, 27.6 mmol, 1 equiv.) was added. The resulting suspension was heated to 140°C for 10 minutes to dissolve the starting materials completely. The reaction mixture was then stirred at room temperature for 5.5 hours. After completion, the mixture was evaporated to remove the solvent and extracted with diethyl ether. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using a 1:11.5 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **A** (isolated yield: 93%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.35, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.51- 7.48 (m, 1H), 6.99 – 6.95 (m, 2H), 2.27 (s, 3H), 1.29 (s, 6H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 162.41, 159.98, 149.08, 147.50, 147.41, 120.42, 120.33, 114.20, 113.97, 109.16, 108.92, 54.07, 22.86, 15.11.
- **HRMS (ESI): calcd.** for $C_{11}H_{13}NF$ +[M+H] + 178.1032; found: 178.1039.

1-(3-bromopropyl)-5-fluro-2,3,3-trimethyl-3H-indol-1-ium (B)

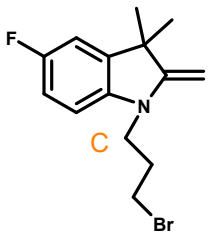


B

A solution of compound **A** (5.8 g, 32.8 mmol) and 1,3-dibromopropane (9.2 mL, 90 mmol) in acetonitrile (20 mL) was refluxed for 24 hours. After completion of the reaction and cooling to room temperature, the acetonitrile was removed under reduced pressure. A solvent mixture of acetone and diethyl ether (1:1) was added to the resulting oily residue, leading to the precipitation of a solid. The obtained solid was dried under vacuum and used directly in the next step without further purification.

- **HRMS (ESI): calcd.** for $C_{14}H_{18}BrFN$+[M+]+ 298.0601; found: 298.0602.

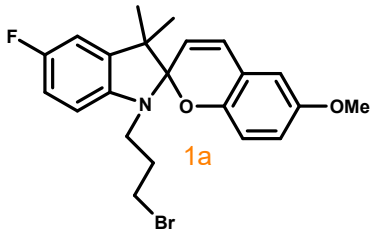
1-(3-bromopropyl)-5-fluoro-3,3-dimethyl-2-methyleneindoline (C)



Compound **B** (894 mg, 3 mmol) was dissolved in dichloromethane (DCM) (20 mL), and a 0.2 M aqueous NaOH solution (20 mL) was added. The mixture was stirred at room temperature for 15 minutes. The organic layer was separated, and the aqueous layer was extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford compound **C**. The crude product was used directly in the next step without further purification.

- **HRMS (ESI): calcd.** for $C_{14}H_{18}BrFN+$ [M+H] + 298.0606; found: 298.0604.

1'-(3-bromopropyl)-5'-fluoro-6-methoxy-3',3'-dimethylspiro[chromene-2,2'-indoline (1a)

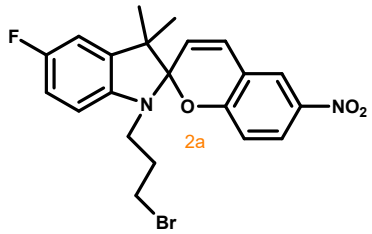


A solution of compound **C** (0.637 g, 2.14 mmol) and 2-hydroxy-5-methoxybenzaldehyde (350 mg, 2.3 mmol) in tetrahydrofuran (THF) (30 mL) was refluxed for 18 hours under an argon atmosphere. After cooling the reaction mixture to room temperature, the THF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography using a 5:95 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **1a** (isolated yield: 70%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.85, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 6.87- 6.82 (m, 3H), 6.72 – 6.70 (m, 1H), 6.66 – 6.64 (m, 2H), 6.51- 6.48 (m, 1H), 5.73- 5.70 (d, J = 10.16 Hz, 1H), 3.78 (, 3H), 3.45- 3.38 (m, 3H), 3.25- 3.18 (m, 1H), 2.28- 2.21 (m, 1H), 2.12- 2.05 (m, 1H), 1.29 (s, 3H), 1.18 (s, 3H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 158.53, 156.18 (coupling due to F atom), 153.29, 148.07, 143.50, 138.57, 129.70, 120.08, 118.80, 115.58, 115.43 (coupling due to F atom), 113.15, 112.92 (coupling due to F atom), 111.61, 109.93, 109.70 (coupling due to F atom), 106.47, 106.39, 104.65, 55.79, 52.08, 42.29, 31.92, 31.39, 25.68, 20.01.

- **HRMS (ESI): calcd.** for $C_{22}H_{24}BrFNO_2$+[M+H]+ 432.0974; found: 432.0972.

1'-(3-bromopropyl)-5'-fluoro-3',3'-dimethyl-6-nitrospiro[chromene-2,2'-indoline (**2a**)

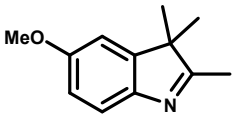


2a

A solution of compound **C** (0.777 g, 2.66 mmol) and 2-hydroxy-5-methoxybenzaldehyde (434.2 mg, 2.66 mmol) in tetrahydrofuran (THF) (30 mL) was refluxed for 18 hours under an argon atmosphere. After cooling the reaction mixture to room temperature, the THF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography using a 4:96 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **2a** (isolated yield: 60%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.88, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.06- 8.03 (m, 2H), 6.97 – 6.94 (d, J = 10.26 Hz, 1H), 6.90 – 6.83 (m, 2H), 6.79- 6.76 (d, J = 8.68 Hz, 1H), 6.56- 6.53 (m, 1H), 5.89- 5.87 (d, J= 10.33Hz, 1H), 3.47- 3.36 (m, 3H), 3.29- 3.22 (m, 1H), 2.29- 2.20 (m, 1H), 2.12- 2.05 (m, 1H), 1.28 (s, 3H), 1.21 (s, 3H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 159.27, 158.89, 156.54, 142.92, 141.14 (coupling due to F atom), 137.88, 137.81(coupling due to F atom), 128.64, 126.01, 122.84, 121.39 (coupling due to F atom), 118.44, 115.53, 113.54, 113.31, 110.02, 109.78, 107.06, 106.98, 106.90 (coupling due to F atom), 52.68, 42.22, 31.62, 30.95, 25.71, 19.72.
- **HRMS (ESI): calcd.** for $C_{21}H_{21}BrFN_2O_3$+ [M+H] + 447.0720; found: 447.0724.

5-methoxy-2,3,3-trimethyl-3H-indole (D)

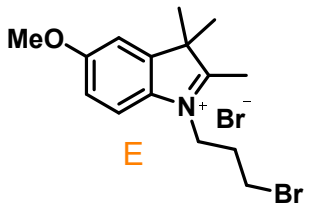


D

To a solution of 3-methyl-2-butanone (5.90 mL, 55.2 mmol, 2 equiv.) in glacial acetic acid (92 mL), 4-methoxyphenyl hydrazine hydrochloride (4.82 g, 27.6 mmol, 1 equiv.) was added. The resulting suspension was heated to 140°C for 10 minutes to dissolve the starting materials completely. The reaction mixture was then stirred at room temperature for 5.5 hours. After completion, the mixture was evaporated to remove the solvent and extracted with diethyl ether. The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography using a 1:11.5 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **A** (isolated yield: 94%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.29, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 7.38- 7.36 (d, J = 8.35 Hz, 1H), 6.77 – 6.74 (m, 2H), 3.74 (s, 3H), 2.17 (s, 3H), 1.21 (s,6H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 185.74, 173.79, 157.87, 147.18, 119.96, 111.98, 108.04, 55.56, 53.67, 23.14, 15.18.
- **HRMS (ESI): calcd.** for $C_{12}H_{16}NO^+$ [M+H] + 190.1232; found: 190.1235.

1-(3-bromopropyl)-5-methoxy-2,3,3-trimethyl-3H-indol-1-ium (E)

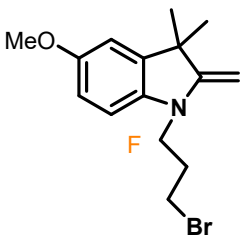


E

A solution of compound **D** (5.67 g, 30 mmol) and 1,3-dibromopropane (9.2 mL, 90 mmol) in acetonitrile (25 mL) was refluxed for 24 hours. After completion of the reaction and cooling to room temperature, the acetonitrile was removed under reduced pressure. A solvent mixture of acetone and diethyl ether (1:1) was added to the resulting oily residue, leading to the precipitation of a solid. The obtained solid was dried under a vacuum and used directly in the next step without further purification.

- **HRMS (ESI): calcd.** for $C_{15}H_{21}BrNO^+$ [M+] + 310.0807; found: 310.0808.

1-(3-bromopropyl)-5-methoxy-3,3-dimethyl-2-methyleneindoline (F)



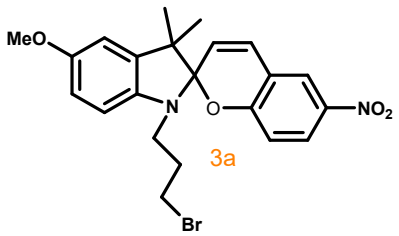
F

Compound **E** (927.21 mg, 3 mmol) was dissolved in dichloromethane (DCM) (20 mL), and a 0.2 M aqueous NaOH solution (20 mL) was added. The mixture was stirred at room temperature for 15 minutes. The organic layer was separated, and the aqueous layer was

extracted with DCM (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to afford compound **F**. The crude product was used directly in the next step without further purification.

- **HRMS (ESI):** calcd. for $C_{15}H_{21}BrNO^+$ [M+H] + 310.0807; found: 310.0807.

1'-(3-bromopropyl)-5'-methoxy-3',3'-dimethyl-6-nitrospiro [chromene-2,2'-indoline (3a)

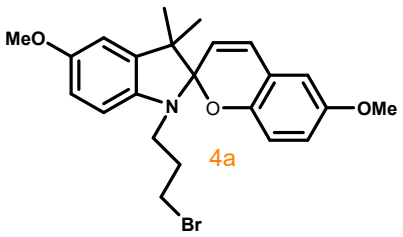


3a

A solution of compound **F** (3.97 g, 12.84 mmol) and 2-hydroxy-5-nitrobenzaldehyde (2.17 gm, 13 mmol) in tetrahydrofuran (THF) (50 mL) was refluxed for 18 hours under an argon atmosphere. After cooling the reaction mixture to room temperature, the THF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography using a 5:95 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **3a** (isolated yield: 60%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.9, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 8.05- 8.02 (m, 2H), 6.95 – 6.93 (d, J = 10.40 Hz, 1H), 6.78 – 6.73 (m, 3H), 6.57- 6.55 (d, J = 8.00 Hz, 1H), 5.90- 5.87 (d, J = 10.35 Hz, 1H), 3.81 (s, 3H), 3.47- 3.35 (m, 1H), 3.27- 3.20 (m, 1H), 2.31- 2.22 (m, 1H), 2.11- 2.05 (m, 1H), 1.28 (s, 3H), 1.21 (s, 3H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 159.54, 154.18, 141.01, 137.71, 128.43, 125.94, 122.79, 121.75, 118.53, 115.53, 111.35, 109.84, 107.21, 106.93, 55.94, 52.79, 42.27, 31.77, 31.11, 25.86, 19.80.
- **HRMS (ESI): calcd.** for $C_{22}H_{24}BrN_2O_4^+$ [M+H]+ 459.0919; found: 459.0917.

1'-(3-bromopropyl)-5',6-dimethoxy-3',3'-dimethylspiro[chromene-2,2'-indoline (4a)



4a

A solution of compound **F** (1 gm, 3.23 mmol) and 2-hydroxy-5-nitrobenzaldehyde (0.52 gm, 3.4 mmol) in tetrahydrofuran (THF) (25 mL) was refluxed for 18 hours under an argon atmosphere. After cooling the reaction mixture to room temperature, the THF was evaporated under reduced pressure. The residue was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography using a 5:95 mixture of ethyl acetate and hexane as the mobile phase, yielding pure product **4a** (isolated yield: 70%).

- **TLC (EtOAc: Hexane, 3:7 v/v):** R_f = 0.8, $KMnO_4$.
- **^{1}H NMR (400 MHz, $CDCl_3$):** δ 6.84- 6.81 (d, J= 10.21 Hz, 1H), 6.75 – 6.64 (m, 5H), 6.54 – 6.52 (d, J = 8.32 Hz, 1H), 5.74- 5.72 (d, J= 10.22Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.46- 3.37 (m, 3H), 3.23- 3.17 (m, 1H), 2.30- 2.23 (m, 1H), 2.13- 2.06 (m, 3H), 1.31 (s, 3H), 1.19 (s, 3H).
- **^{13}C NMR (101 MHz, $CDCl_3$):** δ 153.69, 153.20, 148.29, 141.69, 138.42, 129.45, 120.46, 118.90, 115.58, 111.57, 111.11, 109.88, 106.46, 104.67, 55.95, 55.79, 52.17, 42.39, 32.10, 31.51, 29.72, 25.81, 20.11.
- **HRMS (ESI): calcd.** for $C_{23}H_{26}BrNO_3$+ [M+H] + 444.1174; found: 444.1174.

4. Kinetics spectra of all the spiropyran

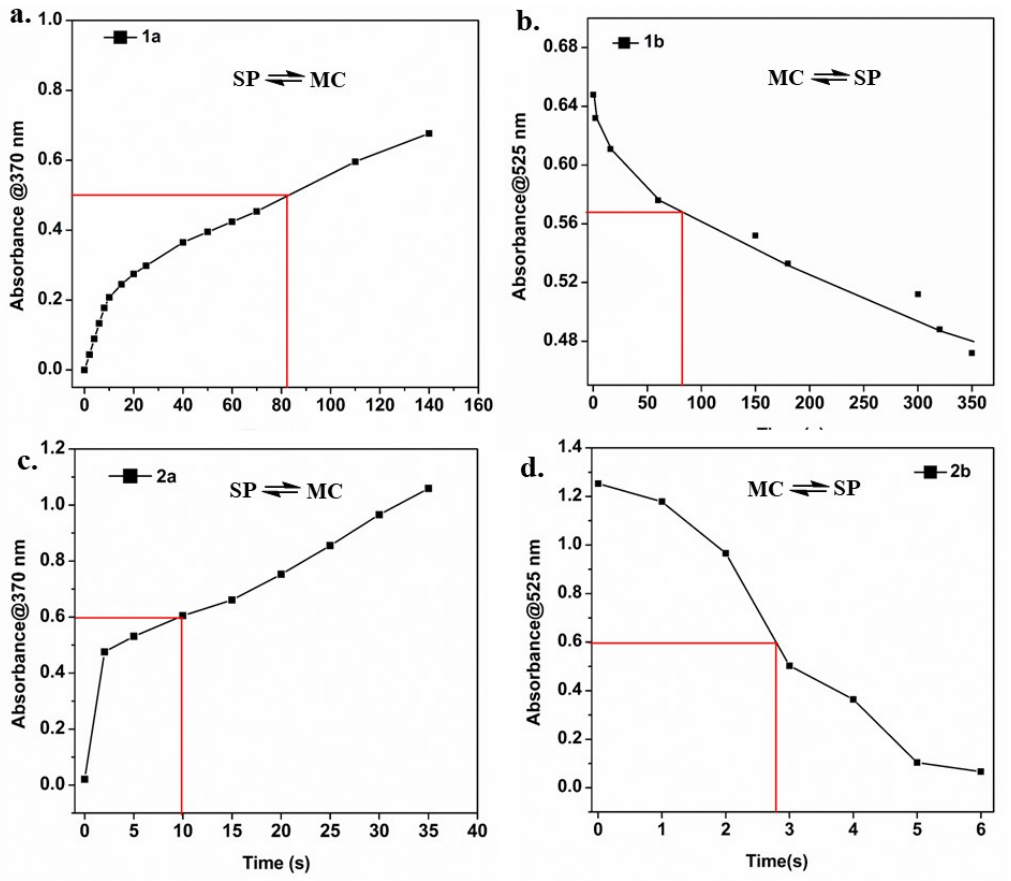


Fig S3. Time-dependent UV-Vis absorption spectral changes (a) and (b) for compounds **1a** and **1b**; (c) and (d) for compounds **2a** and **2b**.

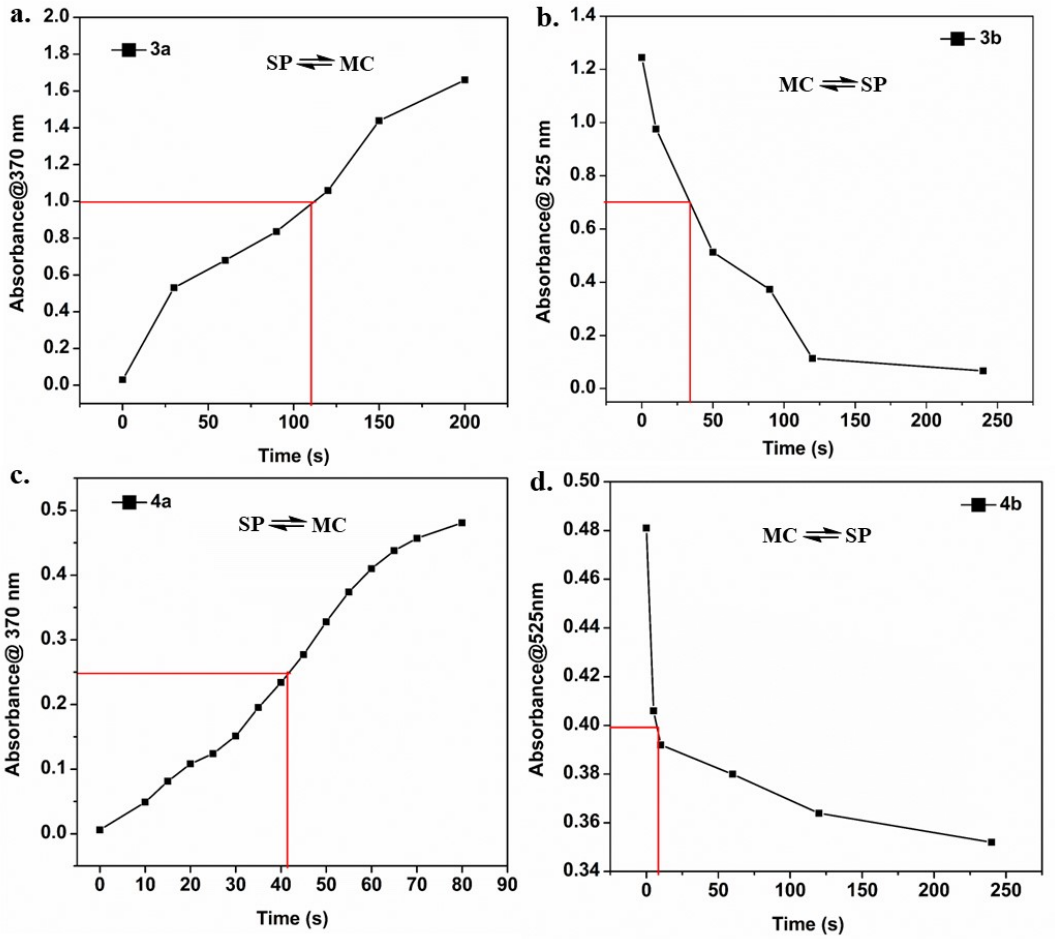
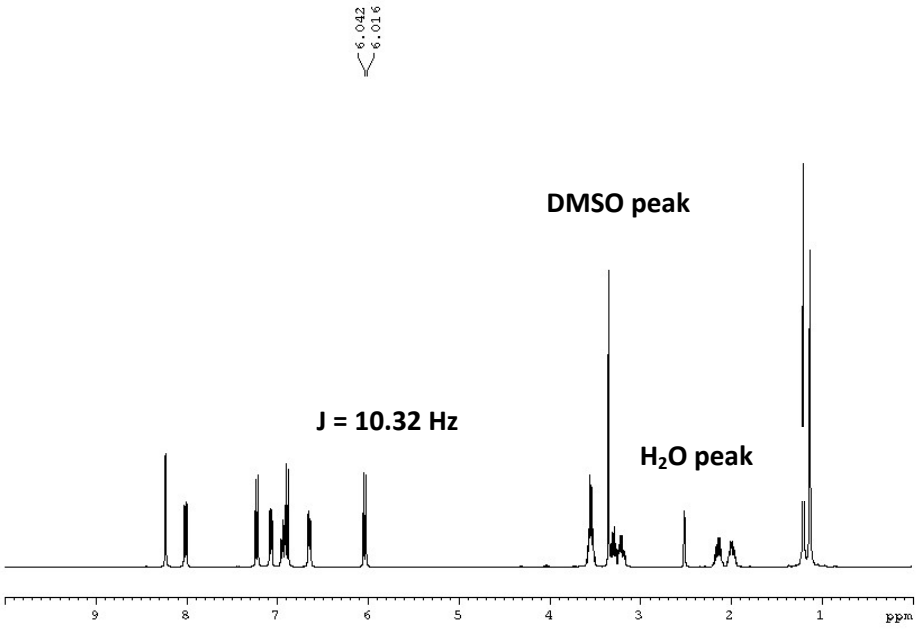


Fig S4. Time-dependent UV-Vis absorption spectral changes (a) and (b) for compounds **3a** and **3b**; (c) and (d) for compounds **4a** and **4b**.

5. ^{1}H NMR spectra of 2a before and after UV (370 nm) irradiation

Procedure: The solution of the photochromic compound is irradiated in a thin quartz cuvette (l=2 mm, V=0.6 ml), and the irradiated solution is then poured into an NMR tube and the ^{1}H NMR spectra are recorded. DMSO-d_6 is taken as a solvent. The irradiation time is 5 min and 20 min.



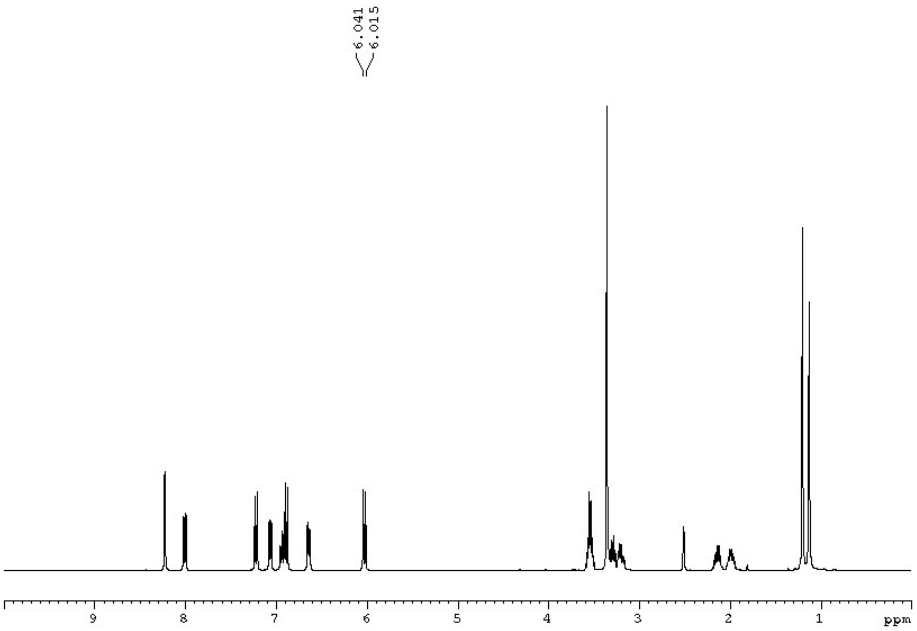
Before UV irradiation

After 5 min UV irradiation (370 nm)

DMSO peak

J = 10.46 Hz

H_2O peak



After 20 min UV irradiation (370 nm)

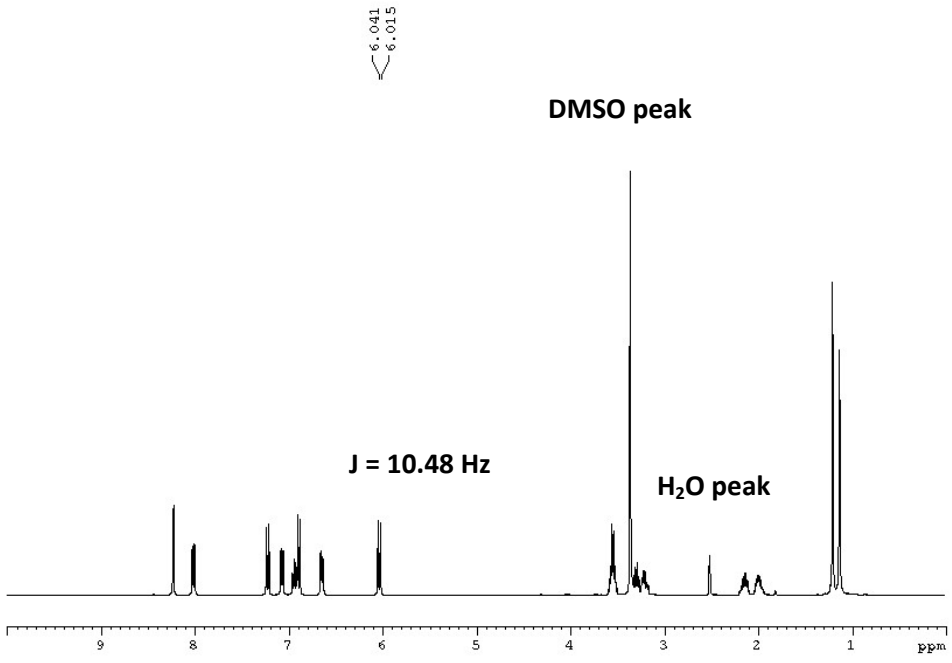


Fig S5. ^{1}H NMR spectra of compound **2a** (DMSO-d_6 as a solvent) before and after UV irradiation.

6. Antibacterial studies

The antibacterial activity of the compounds was evaluated against *Staphylococcus aureus* (ATCC 25923) bacteria. Initially, the bacteria were revived from freeze-dried stock by plating on agar and incubating at 37°C. The resulting colonies were cultured overnight in Luria broth (LB, HiMedia – 20 g/L) to establish a primary culture. A secondary culture was prepared by inoculating 50 µL of the primary culture into 5 mL of fresh LB and incubating at 37°C until the culture reached the mid-log phase (OD_{620nm} ~ 0.3). The bacterial suspension was then adjusted to an optical density of OD_{620nm} = 0.01 (approximately 10^6 to 10^7 CFU/mL) for subsequent experiments. The compound was first dissolved in 100% DMSO. It was then irradiated with UV light to generate the MC form of spiropyran. Immediately after obtaining the MC form, it was added to the PBS buffer containing the bacterial suspension for further testing. In a 96-well plate, 100 µL of the bacterial suspension was mixed with 100 µL of the compound solutions. Bacterial growth was monitored continuously for 12 hours using a microplate reader equipped with a shaker and incubator, maintained at 37°C. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the compound at which no increase in bacterial growth was observed.

7. UV-Vis spectra of all the spiropyran in acetonitrile

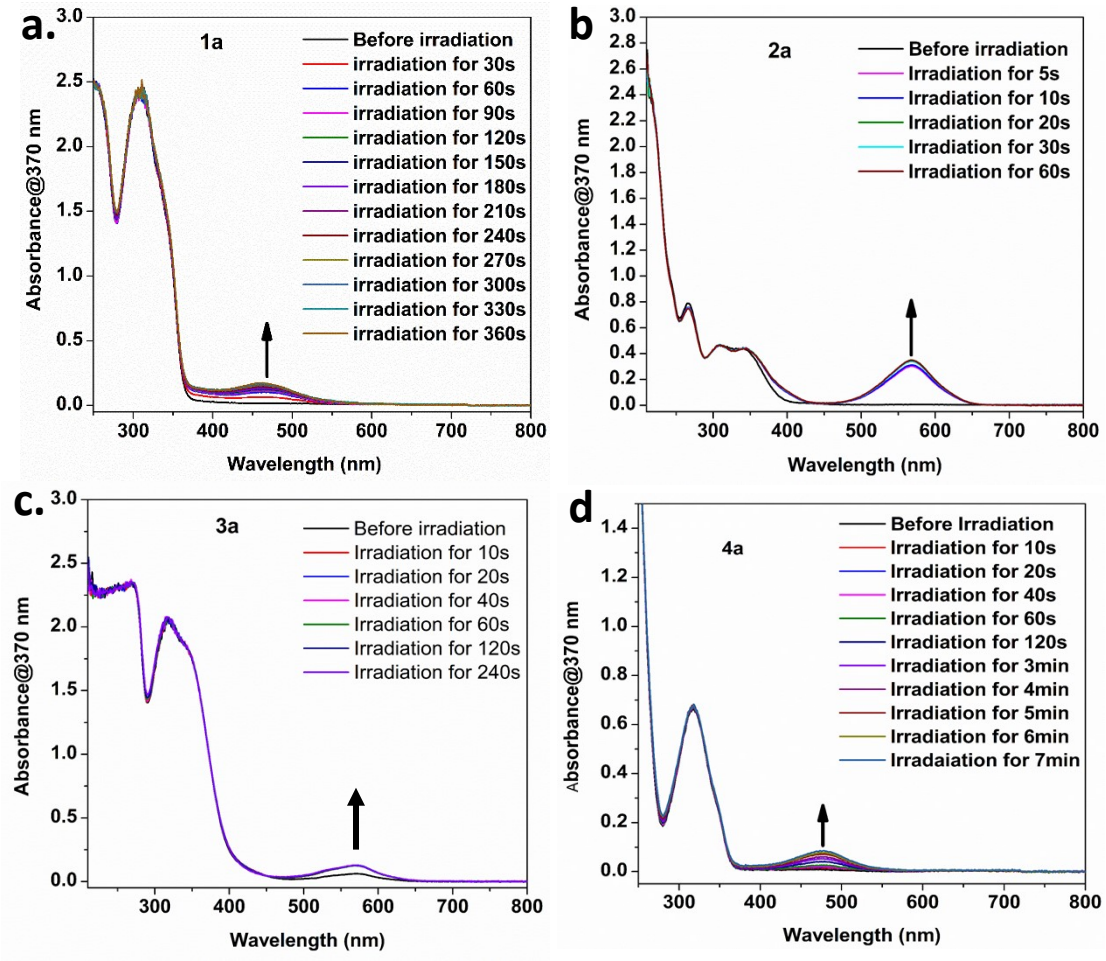


Fig S6. UV-Vis absorption spectra (1×10^{-6} M) in acetonitrile solvent: (a) **1a**; (b) **2a**; (c) **3a**; (d) **4a.**

8. Kinetics of dark relaxation of all the spiropyran

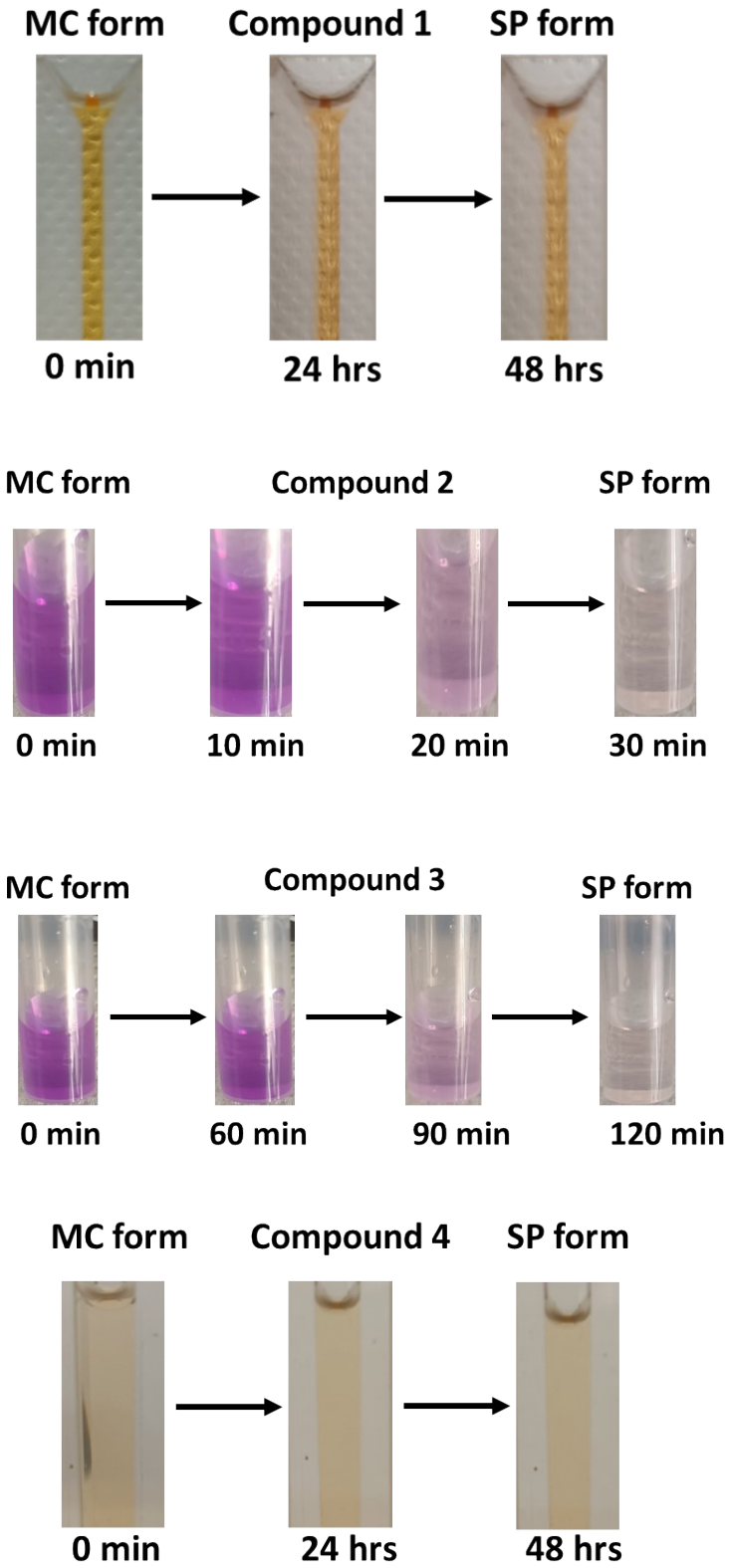


Fig S7. Kinetics of dark relaxation for all the spiropyran.

9. Checking of full reversibility of compounds 1 and 4 by UV-Vis experiment

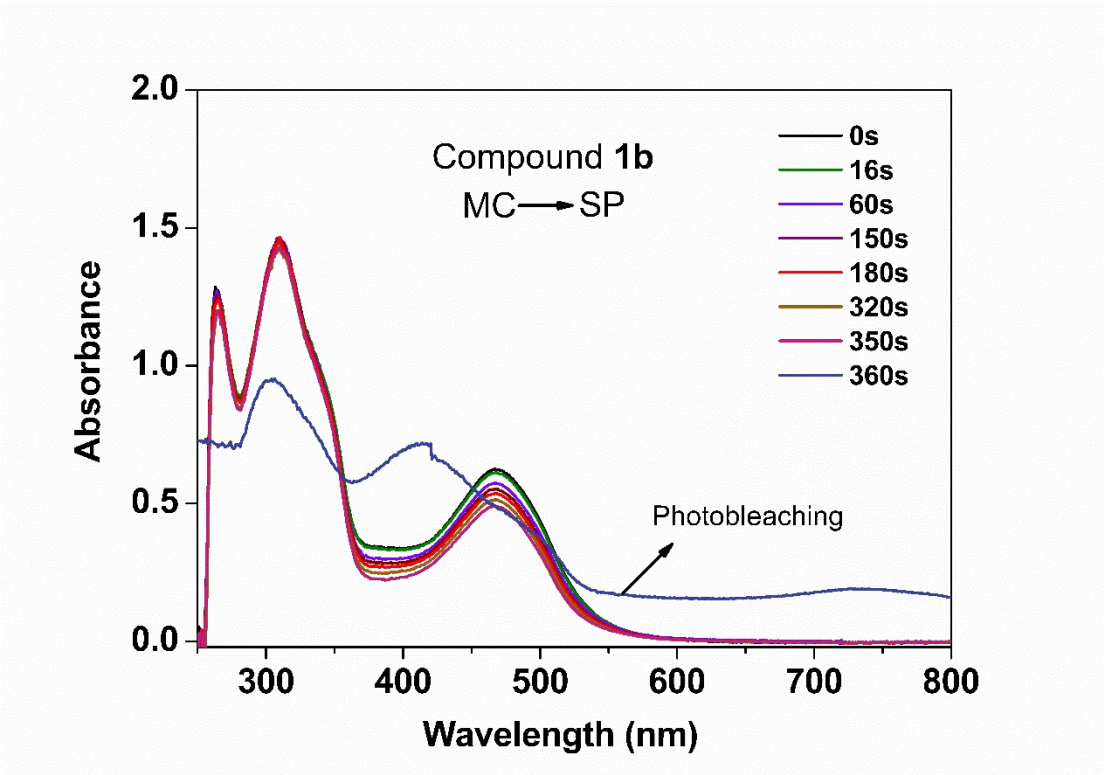


Fig S8. UV-Vis absorption spectra (1×10^{-6} M) in DMSO solvent for compound **1b**.

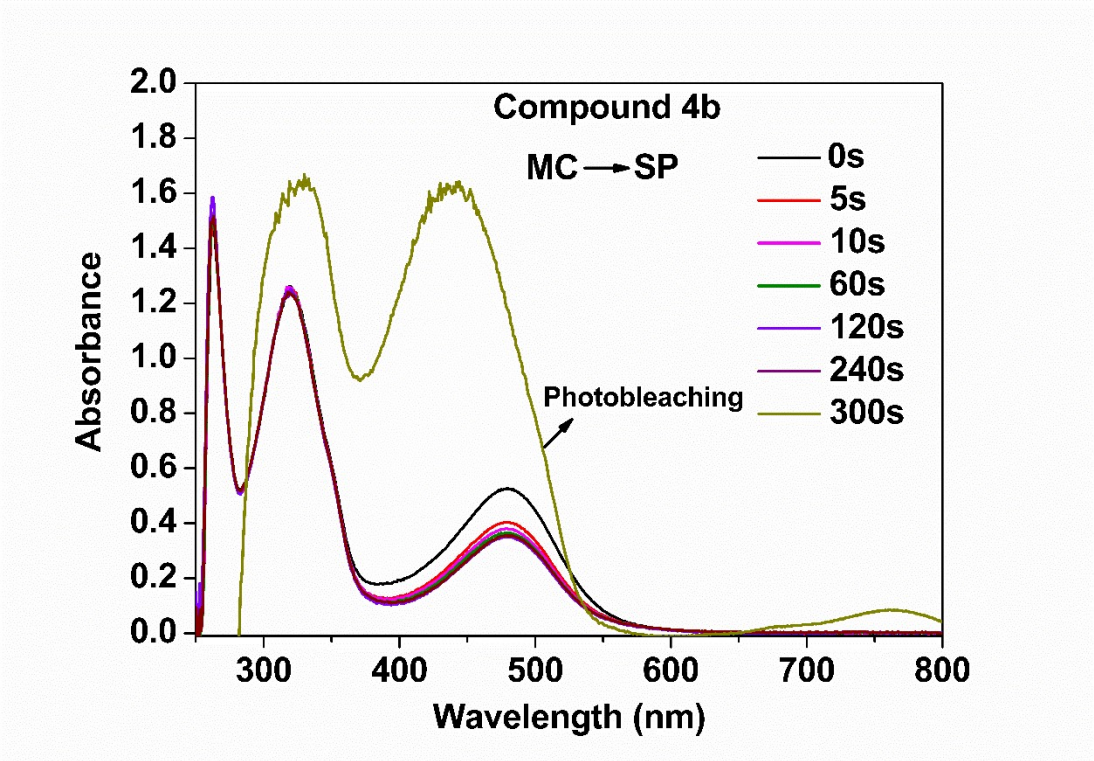
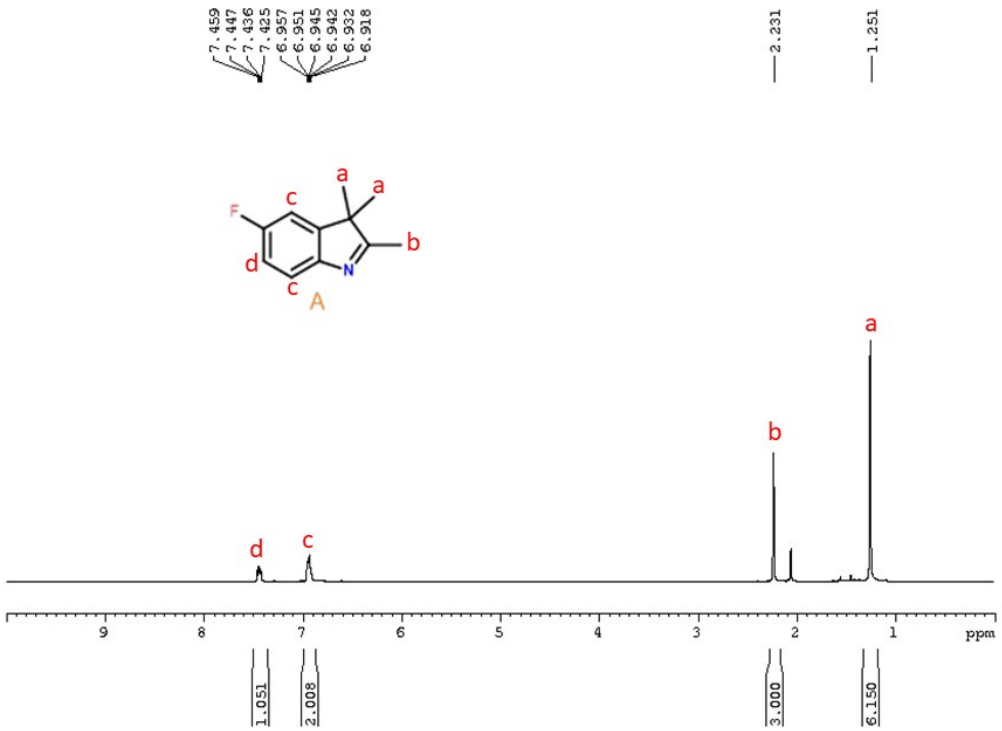


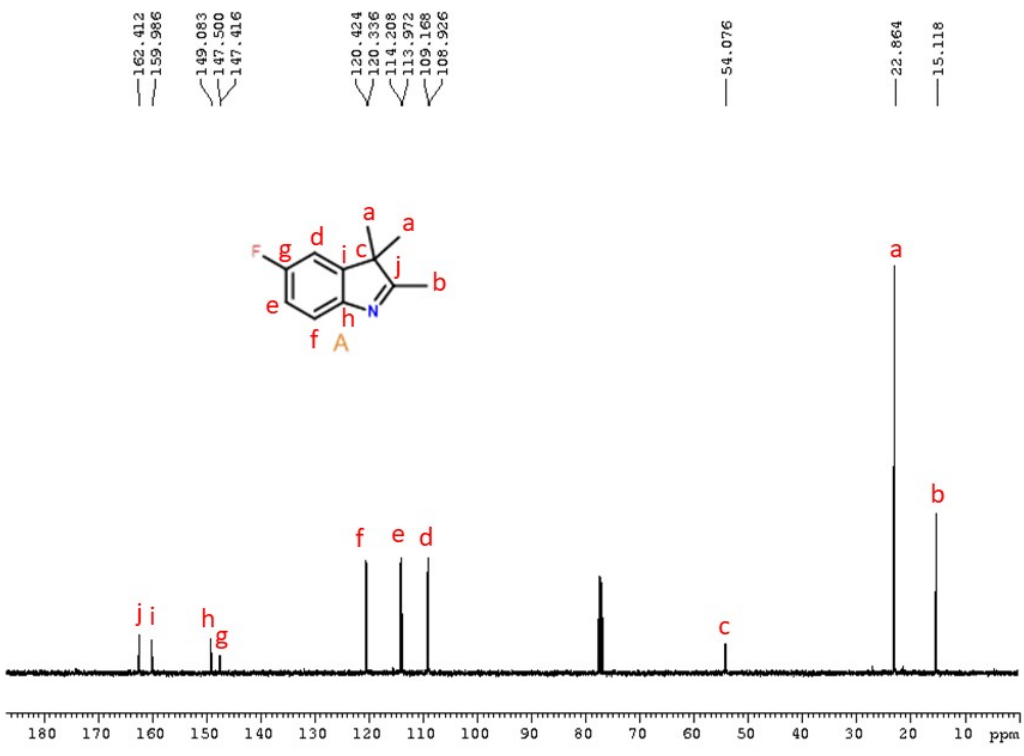
Fig S9. UV-Vis absorption spectra (1×10^{-6} M) in DMSO solvent for compound **4b**.

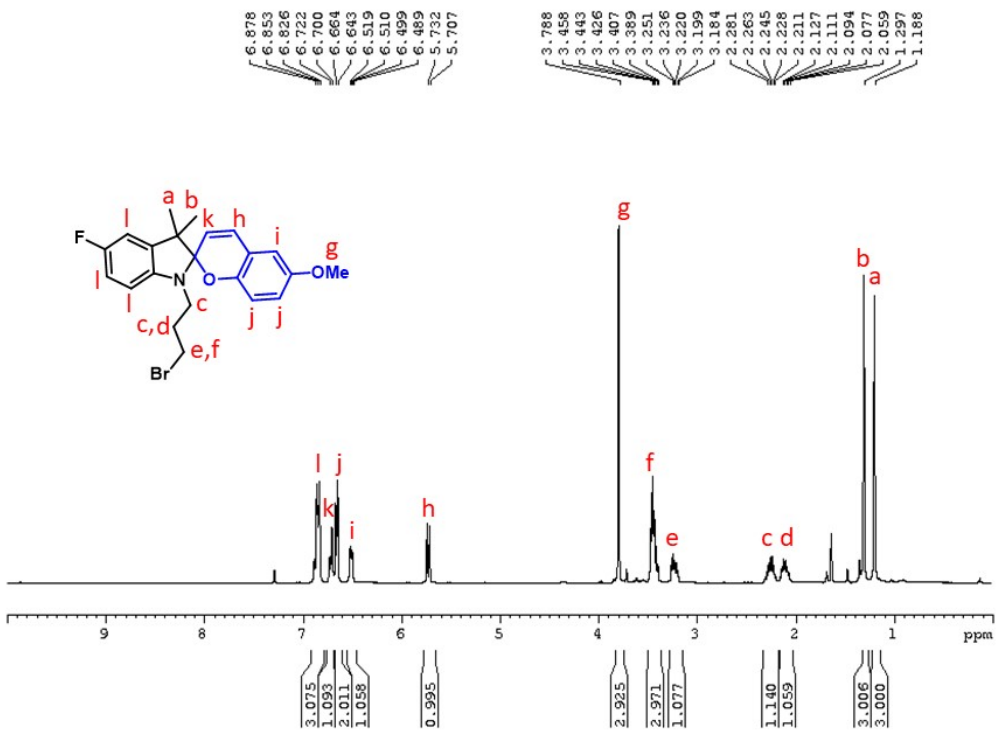
10. ^{1}H, ^{13}C and ^{19}F NMR copies



^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **A**

^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **A**

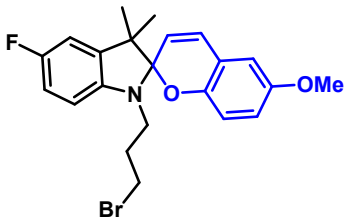


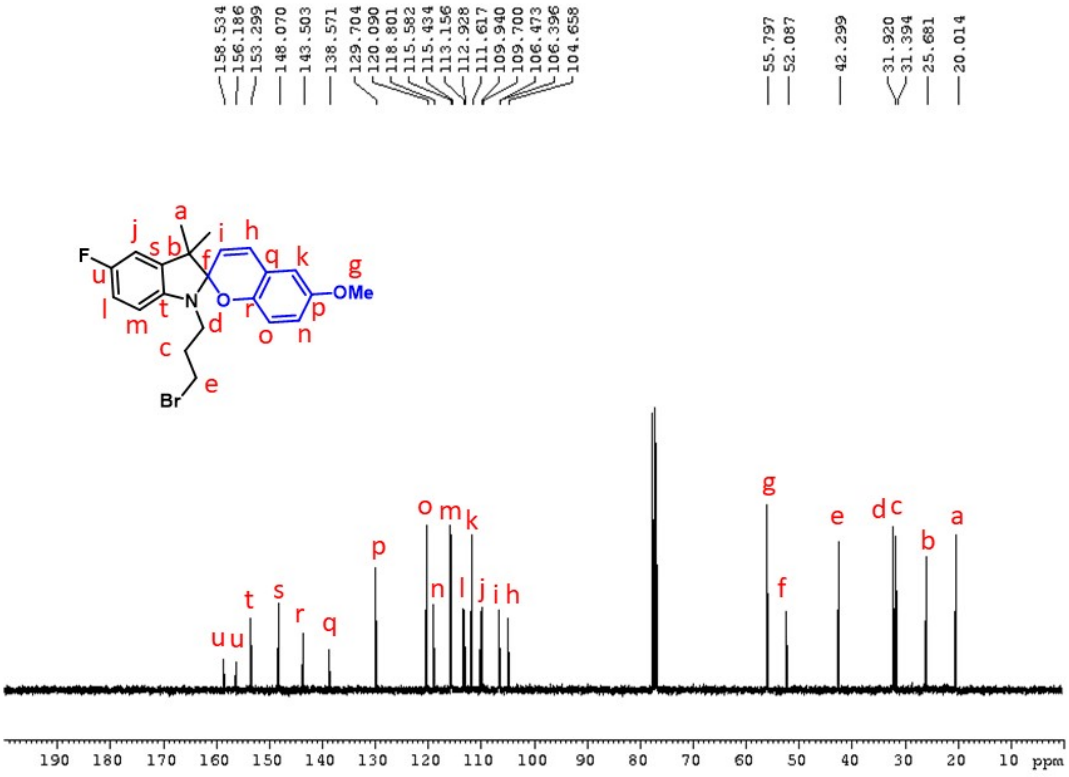


^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **1a**

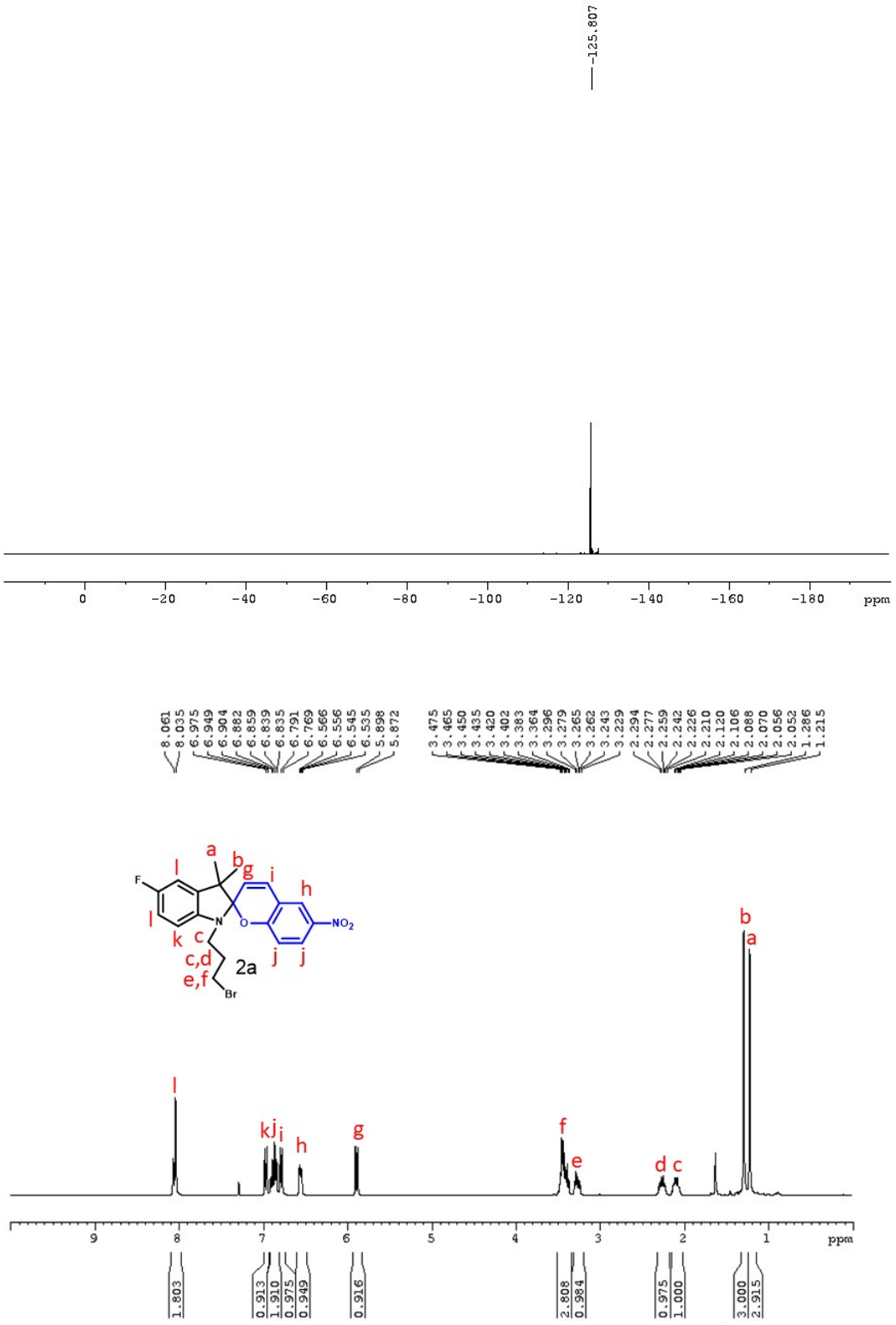
^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **1a**

^{19}F NMR (101 MHz, $CDCl_3$) spectra of compound **1a**

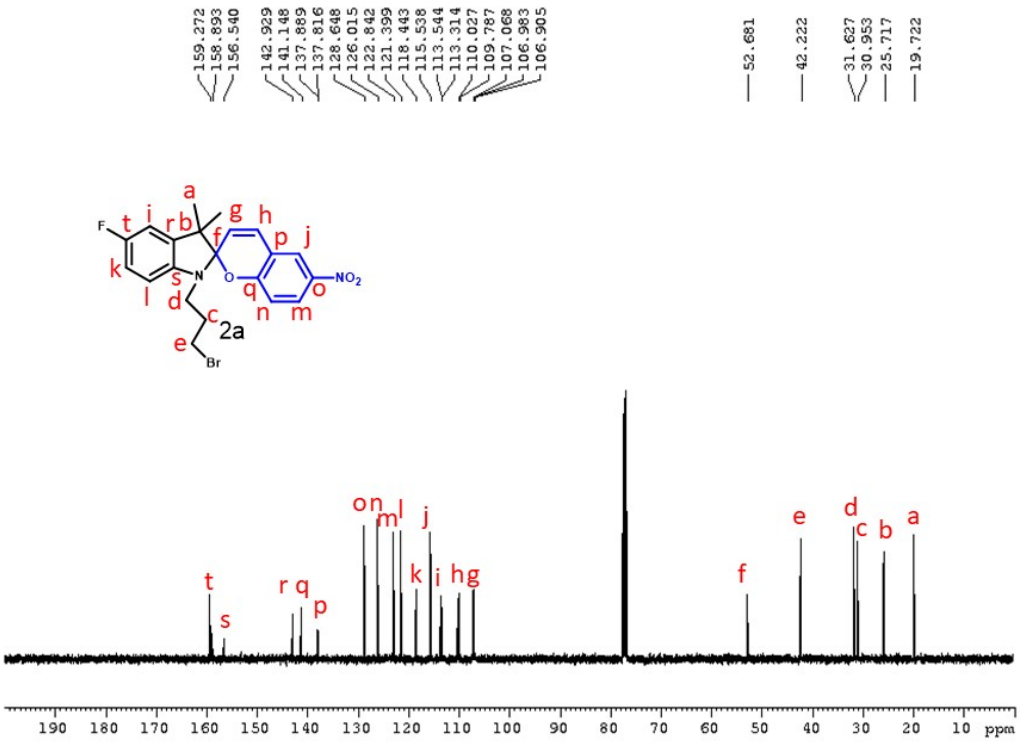




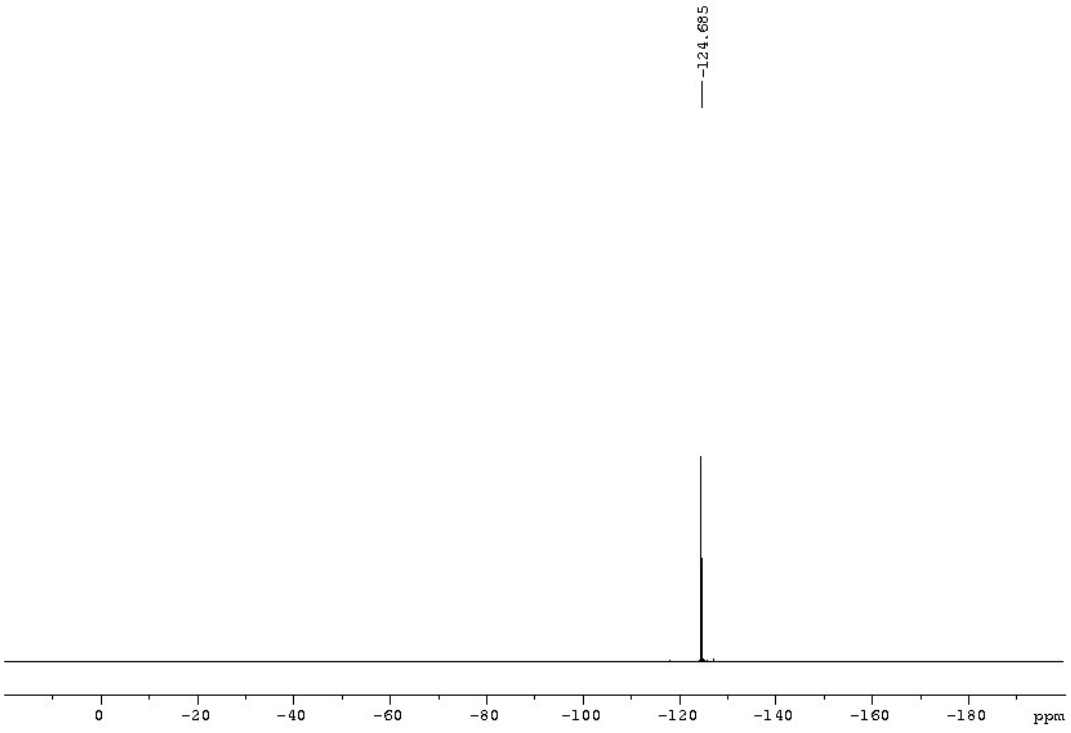
^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **2a**



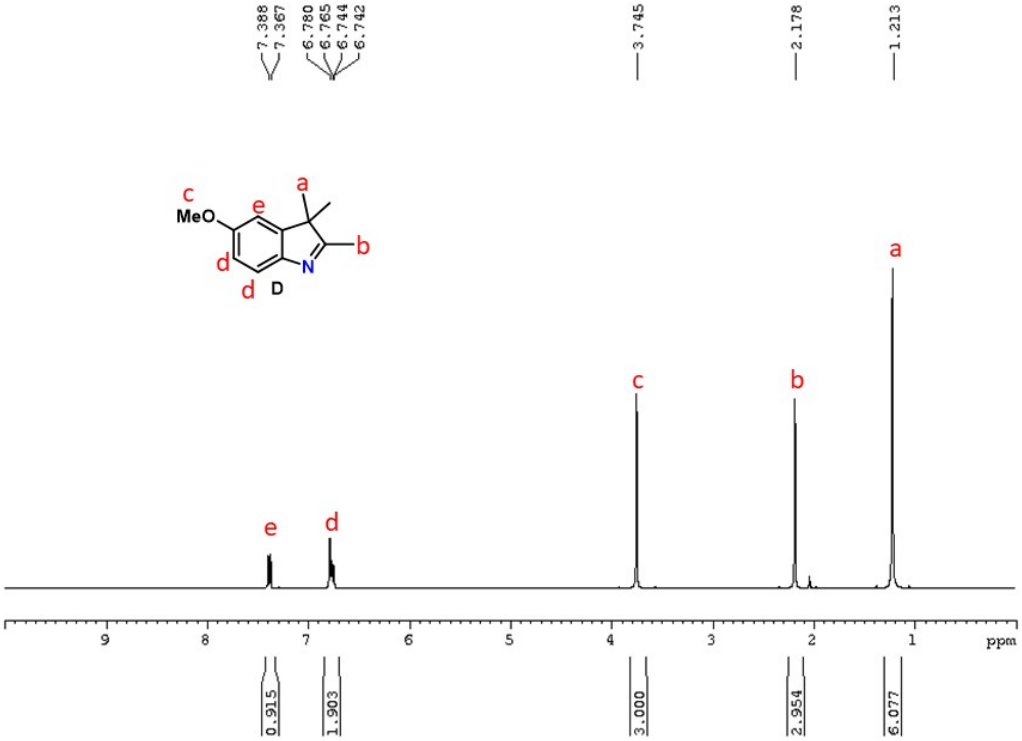
^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **2a**



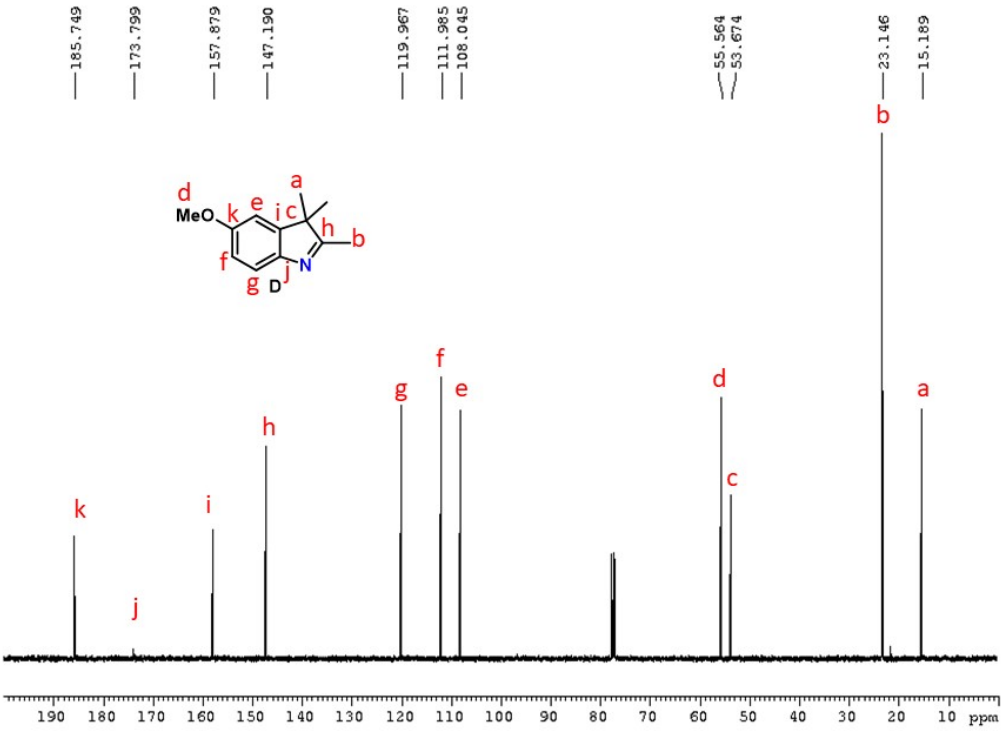
^{19}F NMR (101 MHz, $CDCl_3$) spectra of compound **2a**



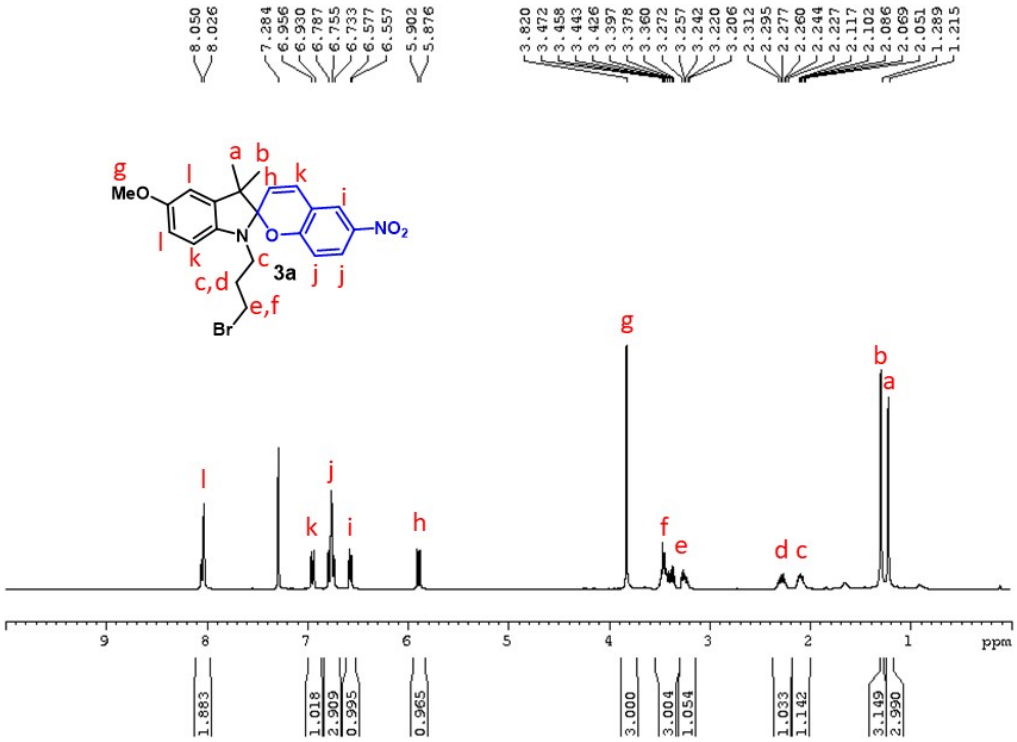
^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **D**

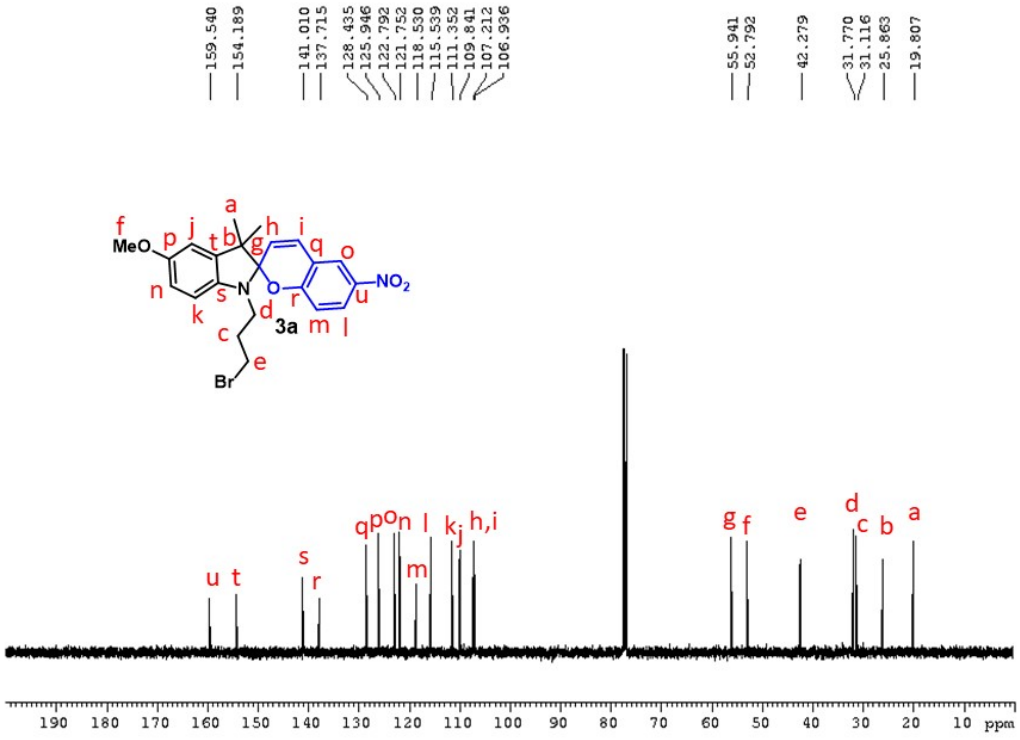


^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **D**



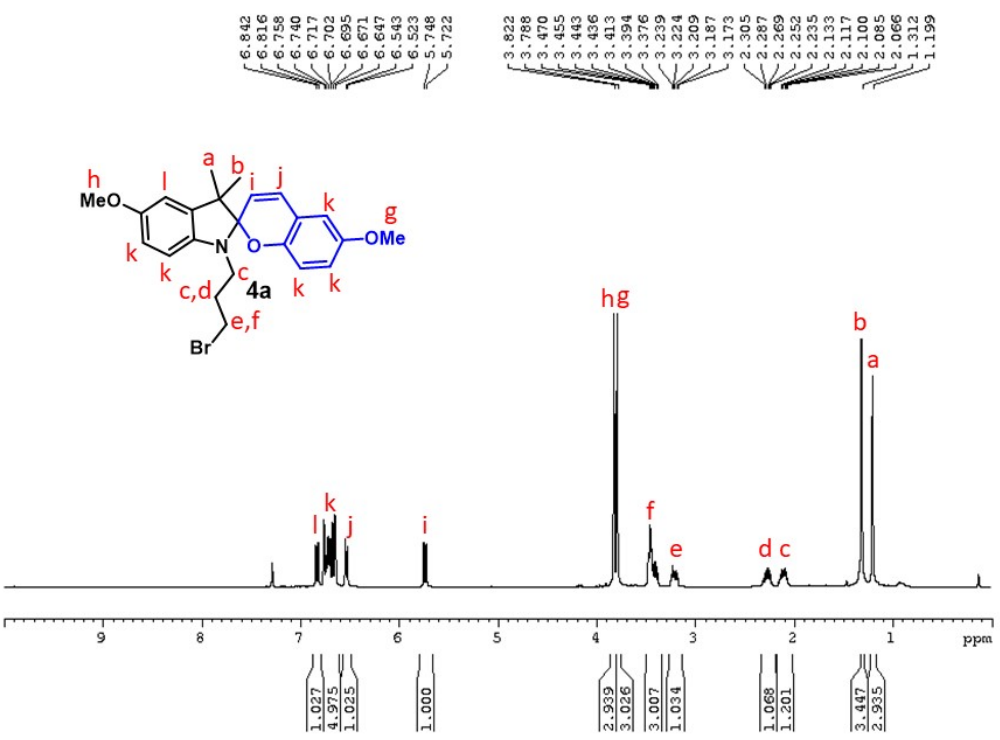
^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **3a**





^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **3a**

^{1}H NMR (400 MHz, $CDCl_3$) spectra of compound **4a**



^{13}C NMR (101 MHz, $CDCl_3$) spectra of compound **4a**