

Regioselective and Solvent Dependent Photoisomerization Induced Internal Conversion in Red Fluorescent Protein Chromophore Analogues

Basanta Kumar Rajbongshi,^{*,†} Sheikh Abdullah,[†] Bittu Lama,^{#,‡} Himangshu
Pratim Bhattacharyya,^{#,‡} and Manabendra Sarma^{*,‡}

[†]Department of Chemistry, Cotton University, Guwahati, Assam-781001, India

[‡]Department of Chemistry, Indian Institute of Technology Guwahati, Assam-781039, India

E-mail: basanta.rajbongshi@cottonuniversity.ac.in; msarma@iitg.ac.in

Materials and methods

4-Methoxybenzaldehyde, aniline, benzaldehyde, *trans*-cinnamaldehyde, anhydrous zinc chloride, anhydrous sodium acetate, N-acetyl glycine, petroleum ether (b.p. 60 °C – 80 °C), ethyl acetate and acetic anhydride were procured from SD Fine Chemicals Ltd. Petroleum ether and ethyl acetate were distilled before use. Acetic anhydride was distilled under vacuum and dried over 4Å molecular sieves before use. Anhydrous zinc chloride and anhydrous sodium acetate were dried under vacuum before use. Terephthalaldehyde was purchased from Sigma-Aldrich Chemicals. HPLC grade cyclohexane, ethyl acetate, acetonitrile, chloroform, dichloromethane and acetone were procured from SD Fine Chemicals for spectroscopic studies. For thin layer chromatography (TLC) aluminium sheets precoated with silica gel 60 F₂₅₄ were purchased from E. Merck India. For column chromatography silica gel (60-120 and 100-200 mesh) obtained from LOBA CHEMIE were used. Melting points were recorded in a melting point apparatus (JSGW) and the observed melting points are uncorrected. ¹H NMR spectra were recorded in a JEOL JNM LA 400 and JEOL 500 MHz FT NMR spectrometers in CDCl₃, with CDCl₃ as the internal reference. ¹³C NMR spectra were recorded with 100 MHz NMR spectrometers with complete proton decoupling. Infra red spectrum was recorded in a Bruker Vector 22 FT-IR spectrometer. Mass spectrum was recorded in a Waters ESI-QTOF instrument.

General procedure for synthesis of the RFP chromophore analogs (3a-c)

[#] The authors contributed equally to this work

Synthesis of oxazolone, 1: A procedure mentioned elsewhere was followed.^{1,2} A mixture of 4-methoxybenzaldehyde (2 mL, 16.4 mmol, 1 equiv.), N-acetyl glycine (2.5 g, 21.3 mmol, 1.3 equiv.), anhydrous sodium acetate (1.74 g, 21.3 mmol, 1.3 equiv.) and freshly distilled acetic anhydride was refluxed at 140 °C for 1.5 h. The reaction mixture was cooled to around 50 °C and it was quenched with ice and vigorously stirred for 1 h to allow precipitation. The precipitate was filtered in a buchner funnel under suction and washed with ice-cold water (100 mL) and diethyl ether (20 mL). The crude product obtained this way was dried in a vacuum desiccator and purified by column chromatography. Color: Yellow; Isolated yield: 1.64 g (46%); m.p. 89-91 °C. ¹H NMR (400 MHz, CDCl₃, Figure S1): δ 2.39 (s, 3H, CH₃), 3.87 (s, 3H, OCH₃), 6.96 (d, *J* = 8 Hz, 2H, ArH), 7.11 (s, 1H, =CHAr), 8.06 (d, *J* = 8.8 Hz, 2H, ArH).

Synthesis of imidazolinone, 2: Imidazolinone, **2** was synthesized by solvent free Lewis acid catalyzed reaction.²⁻⁴ Synthesis of imidazolinone, **2** has been reported in reference 2. A mixture of oxazolone, **1** (2 g, 9.2 mmol, 1 equiv.), aniline (0.9 mL, 10.12 mmol, 1.1 equiv.) and anhydrous zinc chloride (0.16 g, 10 mmol%) was fused at 90 °C under nitrogen atmosphere. The reaction was monitored by thin layer chromatography. The brown colored reaction mixture was quenched with water and extracted with chloroform. The organic layer was collected and washed with saturated solution of sodium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using 100-200 mesh silica gel and ethyl acetate and petroleum ether (b.p. 60-80 °C). A pale yellow solid was obtained. Isolated yield: 1.40 g (52%); m.p. 130-132 °C;

¹H NMR (CDCl₃, 300 MHz, Figure S2): δ 8.17 (d, *J* = 9 Hz, 2H), 7.43-7.54 (m, 3H), 7.24-7.27 (m, 2H), 7.18 (s, 1H), 6.97 (d, *J* = 9 Hz, 2H), 3.88 (s, 3H), 2.27 (s, 3H).

Synthesis of 3a:

The three RFP chromophores were synthesized by solvent free Lewis acid catalyzed condensation of imidazolinone, **2** and an aromatic aldehyde by following reported procedure.^{2,4,5} Synthesis of **3a** has been reported in reference 5. A mixture of **2** (0.2 g, 0.68 mmol, 1 equiv.), freshly distilled benzaldehyde (0.07 mL, 0.68 mmol, 1 equiv.) and anhydrous zinc chloride (0.009 g, 10 mmol%) was fused under nitrogen atmosphere at 110 °C for 1.5 hour. The reaction was monitored by thin layer chromatography. The brown colored reaction mixture was quenched with water and extracted

with chloroform. The organic layer was collected and washed with saturated solution of sodium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using 100-200 mesh silica gel and ethyl acetate and petroleum ether (b.p. 60-80 °C). A yellow solid was obtained. Isolated yield: 0.20 g (76%); m.p. 143-144 °C. ¹H NMR (400 MHz, CDCl₃, Figure S3): δ 3.90 (s, 3H, OCH₃), 6.61 (d, *J* = 16 Hz, 1H, HC=CH), 7.02 (d, *J* = 8.0 Hz, 2H, ArH), 7.24 (s, 1H, =CHAr), 7.32-7.37 (m, 5H, ArH), 7.48-7.50 (m, 3H, ArH), 7.53-7.57 (m, 2H, ArH), 8.01 (d, *J* = 16 Hz, 1H, HC=CH), 8.28 (d, *J* = 8 Hz, 2H, ArH).

Synthesis of 3b:

A mixture of imidazolinone **2** (0.2 g, 0.68 mmol, 1 equiv.), terephthalaldehyde (0.091 g, 0.68 mmol, 1 equiv.) and anhydrous zinc chloride (0.009 g, 10 mmol%) was fused at 160 °C under nitrogen atmosphere for 1.5 h. The reaction was monitored by thin layer chromatography. The brown colored reaction mixture was quenched with water and extracted with chloroform. The organic layer was collected and washed with saturated solution of sodium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using 100-200 mesh silica gel and ethyl acetate and petroleum ether (b.p. 60-80 °C). An orange solid was obtained. Isolated yield: 0.11 g (35%); m.p. 165-167 °C. *R_f* 0.5 (30% Ethyl acetate-Petroleum ether). IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$: 1715, 1694, 1627, 1594, 1504, 1385, 1306, 1255, 1162. ¹H NMR (500 MHz, CDCl₃, Figure S4): δ 3.90 (s, 3H, OCH₃), 6.74 (d, *J* = 15.8 Hz, 1H, HC=CH), 7.02 (d, *J* = 8.5 Hz, 2H, ArH), 7.29 (s, 1H, =CHAr), 7.33 (d, *J* = 7.9 Hz, 2H, ArH), 7.48-7.51 (m, 1H, ArH), 7.55-7.58 (m, 2H, ArH), 7.63 (d, *J* = 7.9 Hz, 2H, ArH), 7.87 (d, *J* = 8.2 Hz, 2H, ArH), 8.02 (d, *J* = 15.8 Hz, 1H, HC=CH), 8.28 (d, *J* = 8.6 Hz, 2H, ArH), 10.01 (s, 1H, CHO). ¹³C NMR (CDCl₃, 100 MHz, Figure S5): δ 55.6, 114.6, 117.2, 127.6, 127.7, 128.4, 128.9, 129.4, 129.9, 130.3, 133.5, 134.8, 137.0, 137.1, 138.6, 141.0, 157.1, 161.9, 169.9, 191.4. ESI-MS+ *m/z* Calcd. for C₂₆H₂₁N₂O₃: 409.1552 [M+H], found 409.1559.

Synthesis of 3c:

Synthesis of **3c** is reported in reference 2. A mixture of imidazolinone, **2** (0.2 g, 0.68 mmol, 1 equiv.), *trans*-cinnamaldehyde (0.086 mL, 0.68 mmol, 1 equiv.) and anhydrous zinc chloride (0.009 g, 10 mmol%) was fused at 100 °C for 4 h under nitrogen atmosphere. The reaction was

monitored by thin layer chromatography. The brown colored reaction mixture was quenched with water and extracted with chloroform. The organic layer was collected and washed with saturated solution of sodium bicarbonate and dried over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography using 100-200 mesh silica gel and ethyl acetate and petroleum ether (b.p. 60-80 °C). An orange solid was obtained. Isolated yield: 0.17 g (60%); m.p. 148-150 °C. ¹H NMR (500 MHz, CDCl₃, Figure S6) δ3.90 (s, 3H, OCH₃), 6.16 (d, *J* = 15 Hz, 1H, HC=CH), 6.88 (dd, *J*₁ = 10 Hz, *J*₂ = 10 Hz, 1H, HC=CH), 6.97 (d, *J* = 15 Hz, 1H, HC=CH), 7.01 (d, *J* = 10 Hz, 2H, ArH), 7.21 (s, 1H, =CHAr), 7.29-7.36 (m, 5H, ArH), 7.44 -7.48 (m, 3H, ArH), 7.52 -7.56 (m, 2H, ArH), 7.82 (dd, *J*₁ = 15 Hz, *J*₂ = 15 Hz, 1H, HC=CH), 8.27 (d, *J* = 10 Hz, 2H, ArH).

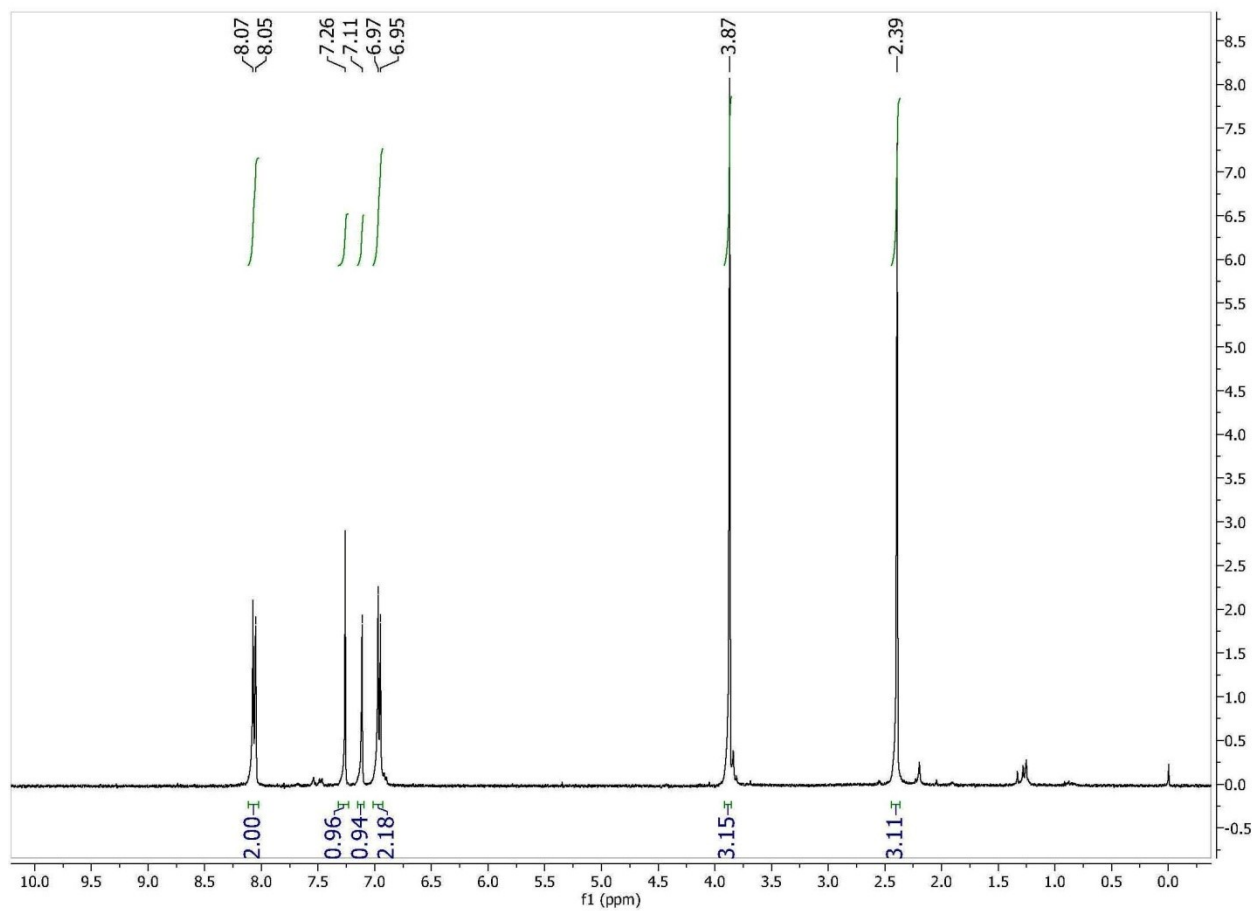


Figure S1. ^1H NMR (CDCl_3 , 400 MHz) of oxazolone, **1**

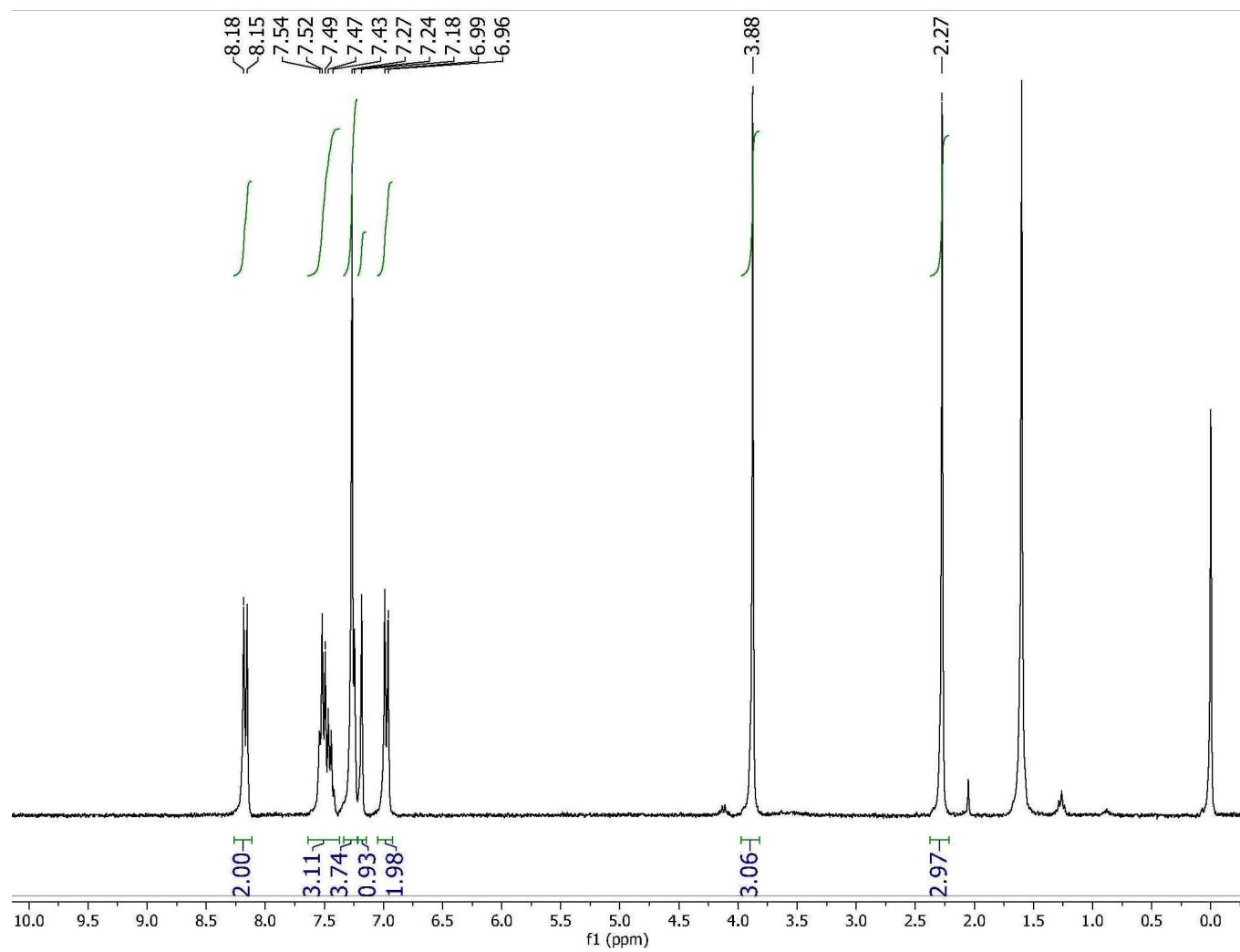


Figure S2. ^1H NMR (CDCl_3 , 300 MHz) of imidazolinone, **2**

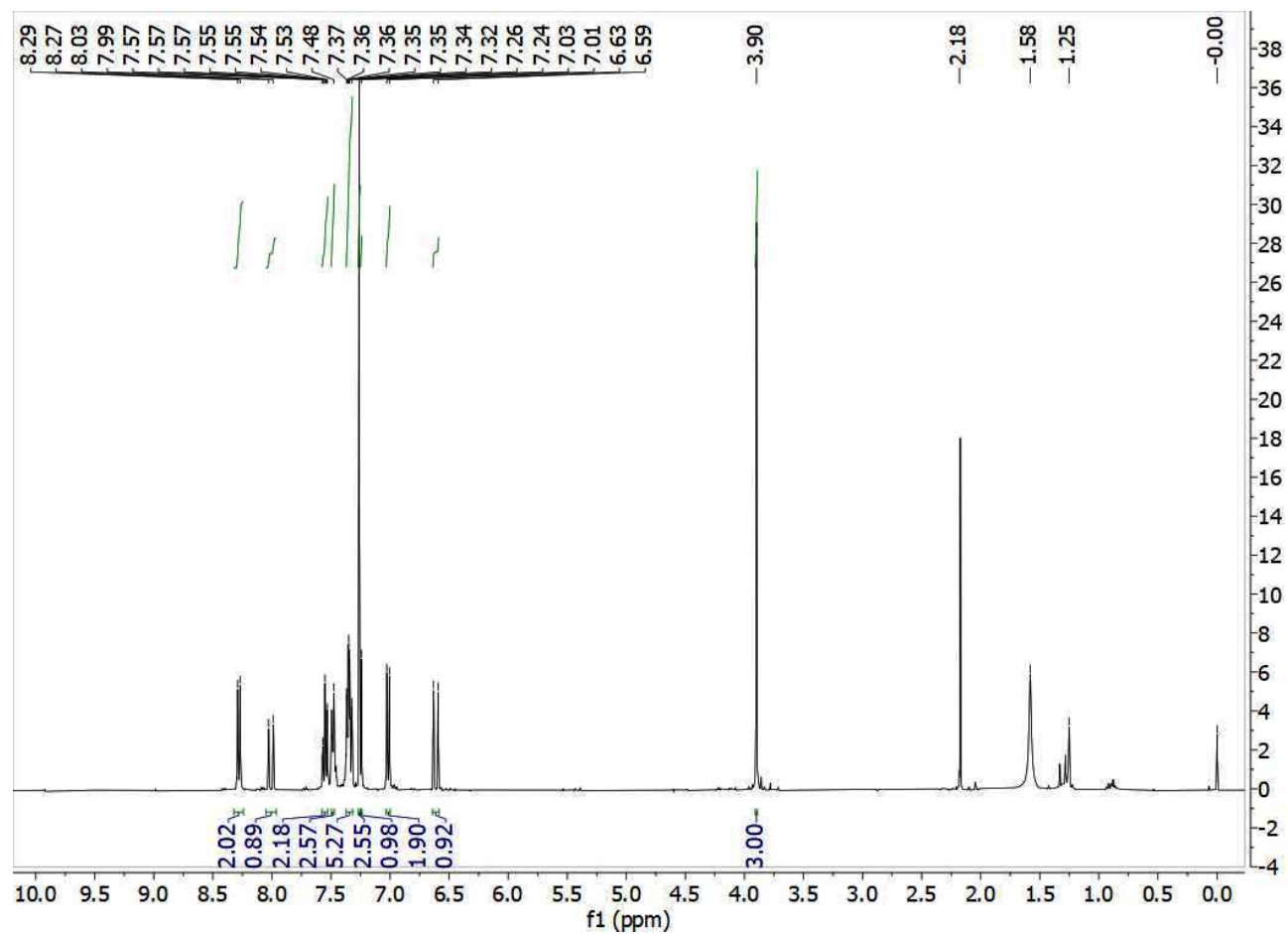


Figure S3. ^1H NMR (CDCl_3 , 400 MHz) of **3a**. The signal at δ 2.18 ppm is from acetone and the signal at δ 1.25 ppm is from the grease (Gottlieb *et al.*, Journal of Organic Chemistry 1997, 62, 7512-7515).

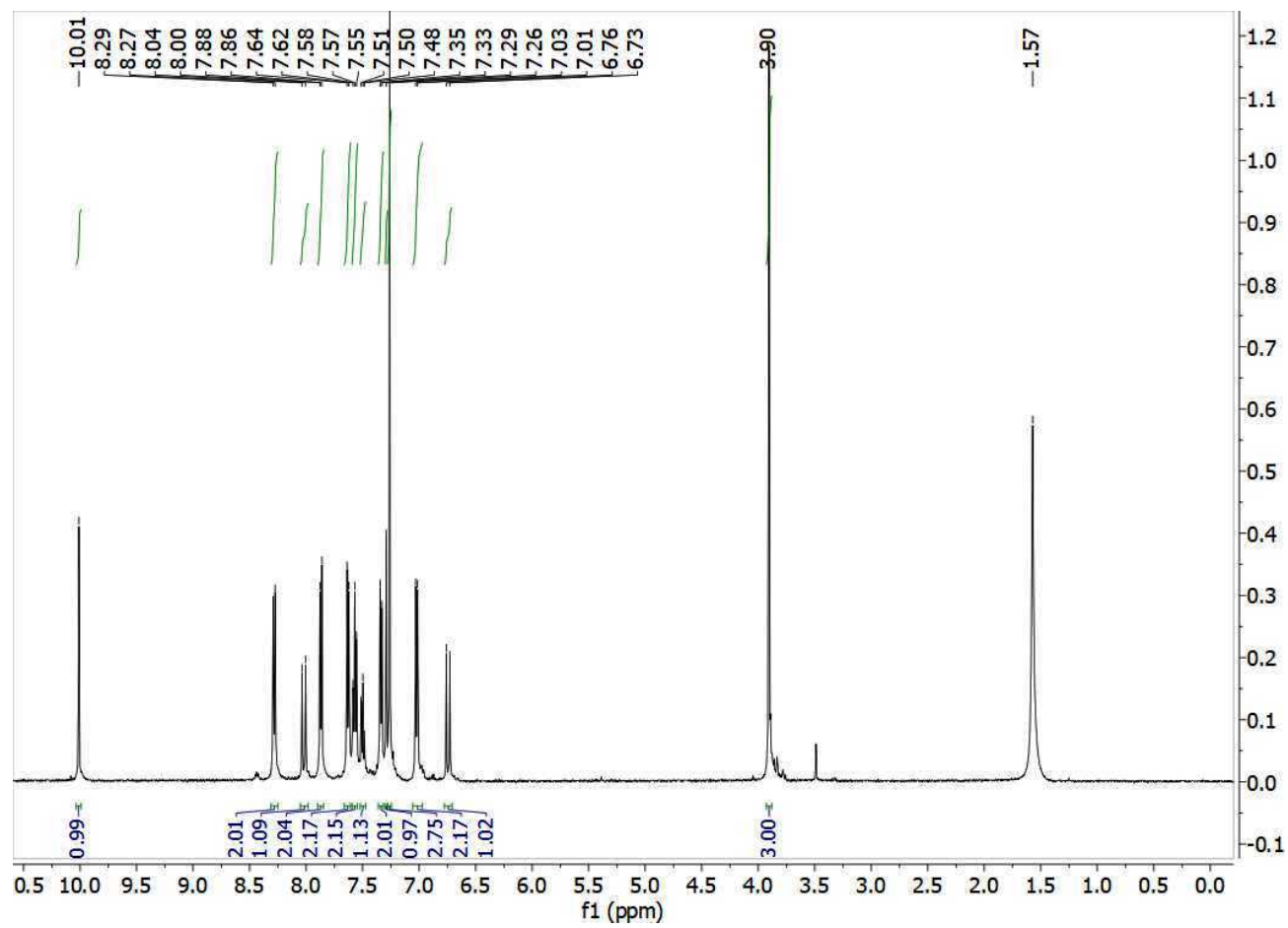


Figure S4. ^1H NMR (CDCl_3 , 500 MHz) of **3b**.

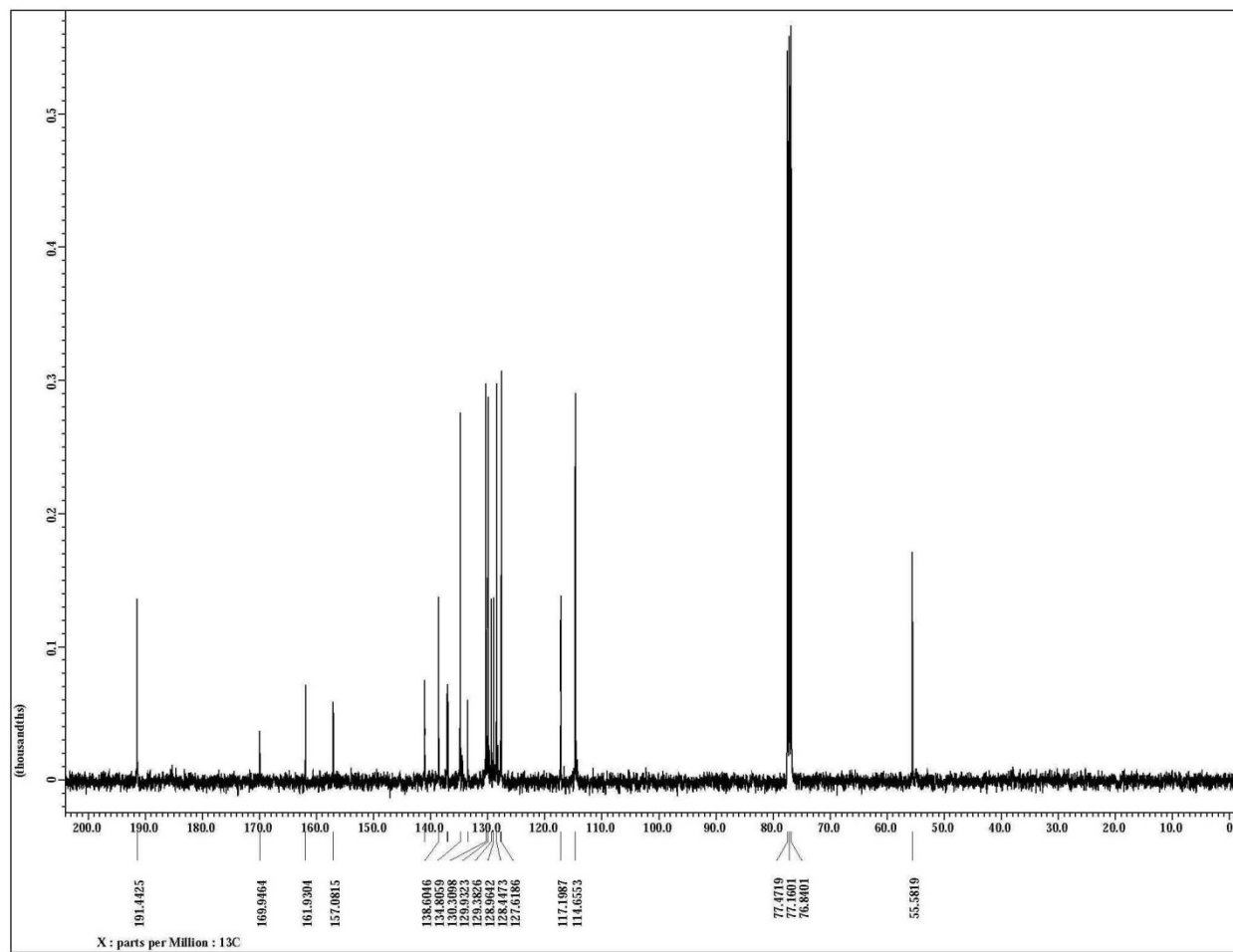


Figure S5. ^{13}C NMR (CDCl_3 , 100 MHz) of **3b**.

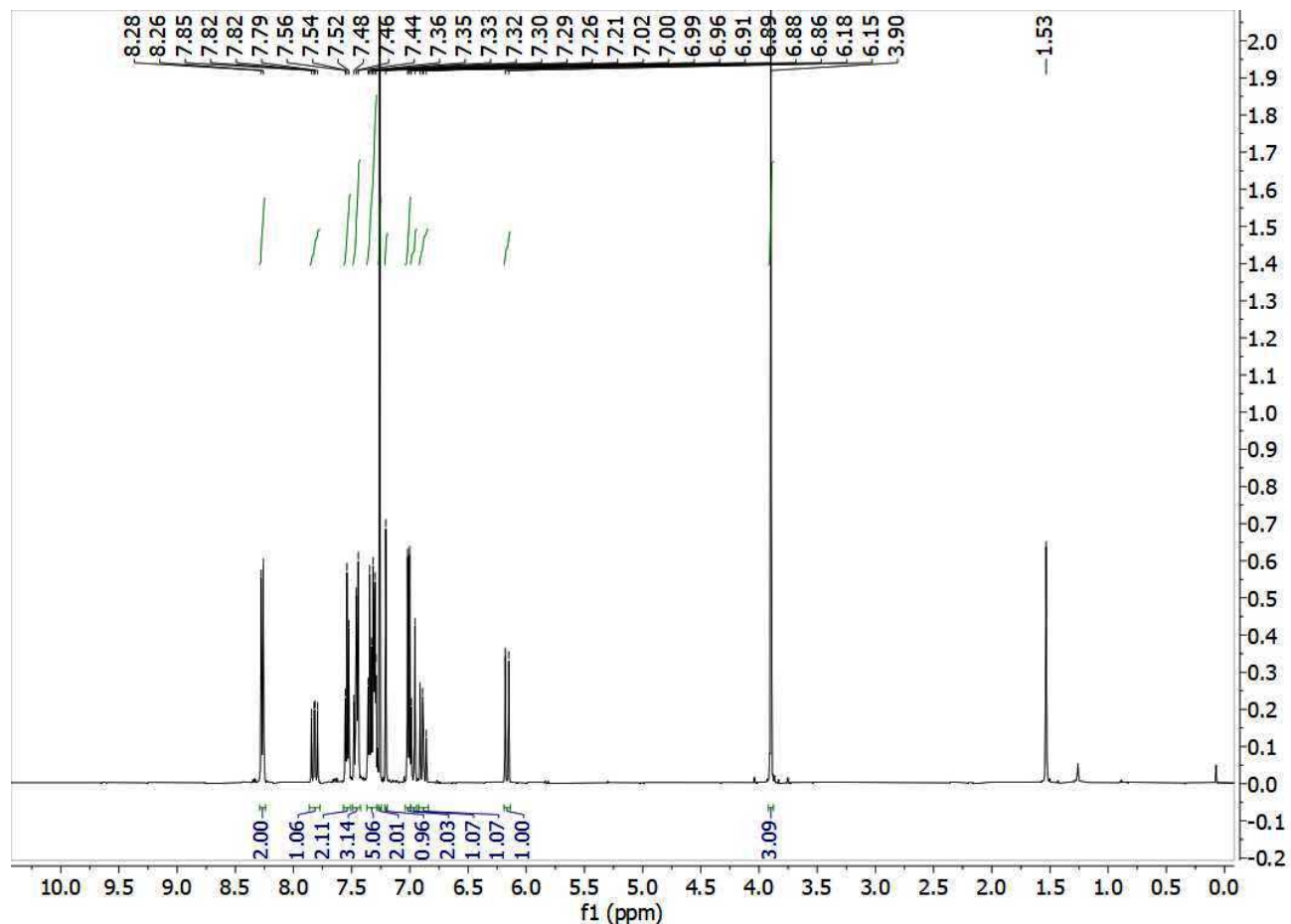


Figure S6. ^1H NMR (CDCl_3 , 500 MHz) of **3c**

Photoirradiation experiment:

An acetonitrile solution of RFP chromophore analog **3a** was taken in a pyrex tube and then purged with nitrogen gas for 15 minutes. This solution was irradiated in a Luzchem photoreactor fitted with 350 nm (OD at 350 nm \sim 1.02) lamps for 13 h. Steady state absorption spectra were recorded at times 0h, 4h, 9h, 11h, and 13h. For recording of ^1H NMR spectrum after photoirradiation, **3a** was dissolved in CDCl_3 in an NMR tube and purged with N_2 gas for 15 min and irradiated at 350 nm for 17h in a Luzchem photoreactor. After 17h of irradiation, ^1H NMR was recorded in a JEOL 500 MHz NMR spectrometer.

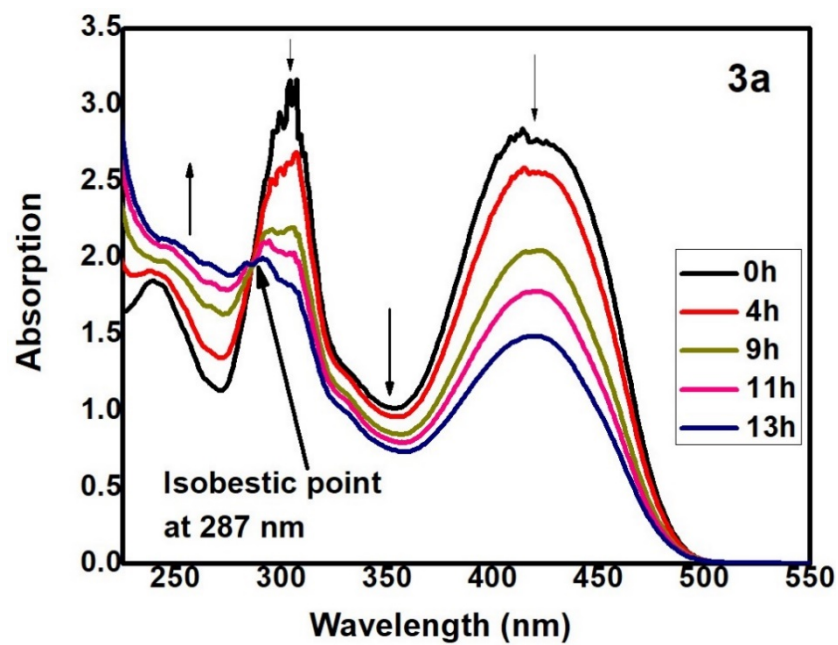


Figure S7. Absorption spectra of molecule **3a** in acetonitrile before irradiation and during irradiation at times 0h, 4h, 9h, 11h and 13h. **3a** was irradiated in a Luzchem photoreactor fitted with 350 nm (OD at 350 nm ~1.02) lamps for 13 h

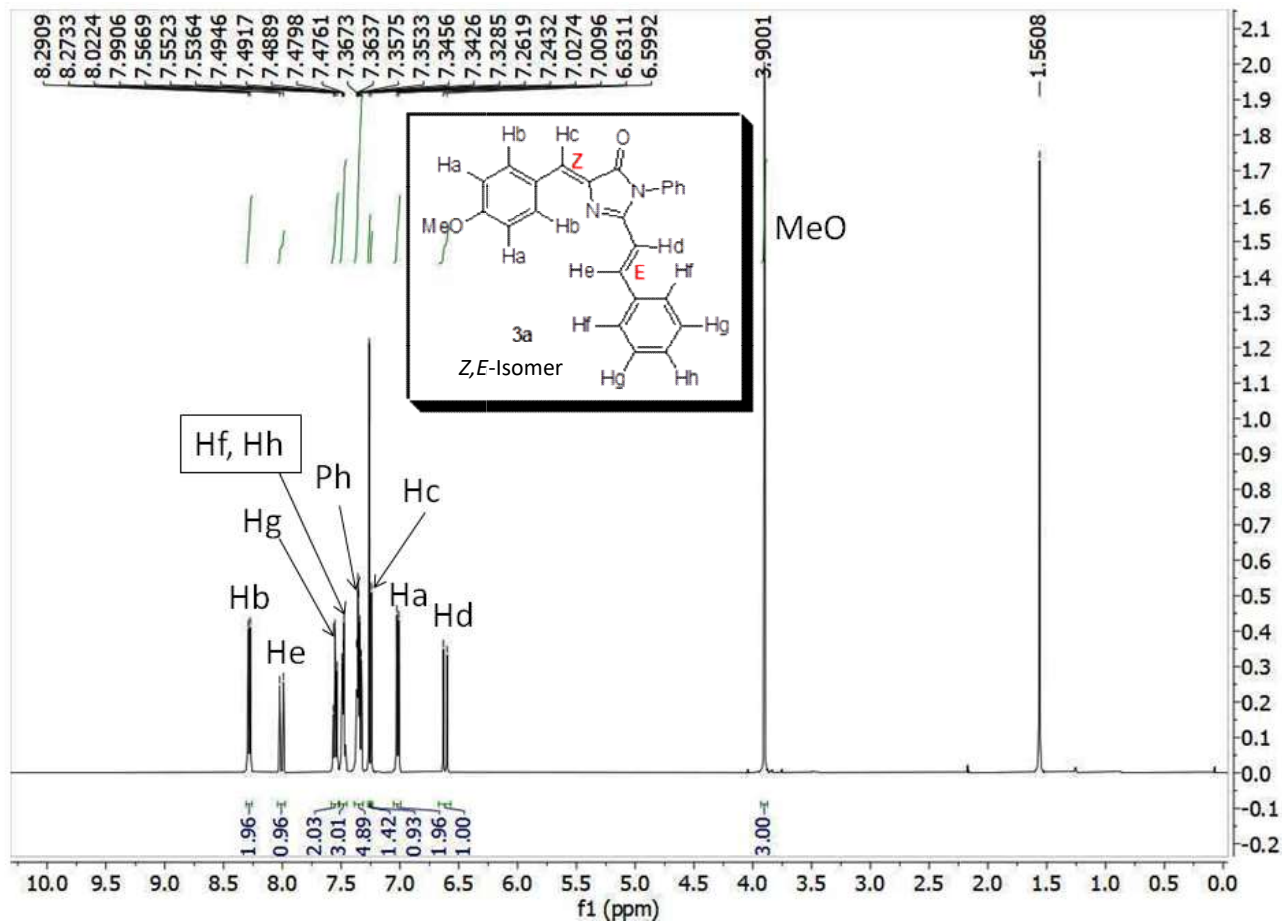


Figure S8. ^1H NMR (CDCl_3 , 500 MHz) of **3a** (full spectrum) before irradiation.

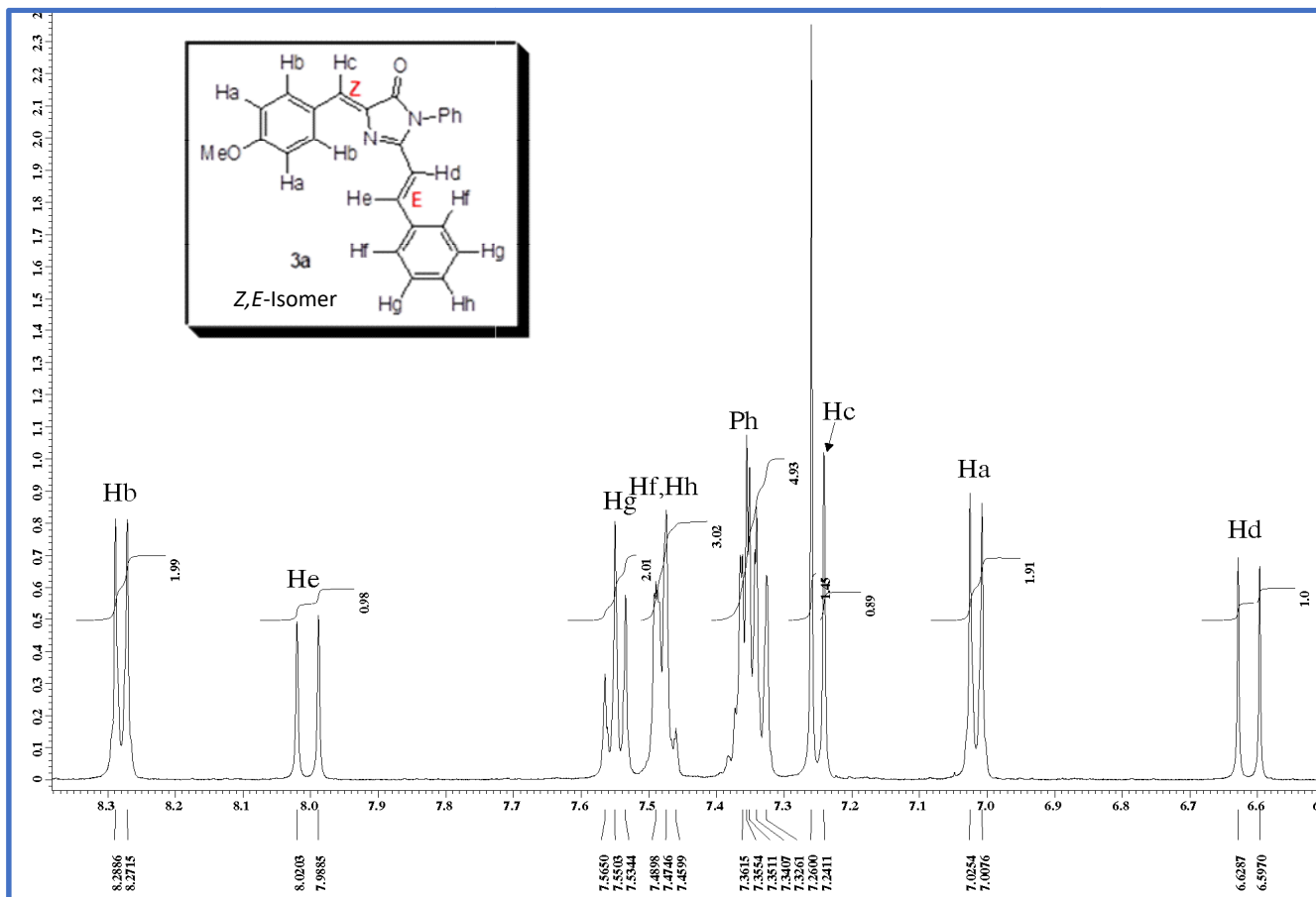


Figure S9. ¹H NMR (CDCl₃, 500 MHz) of 3a (Zoomed) before irradiation.

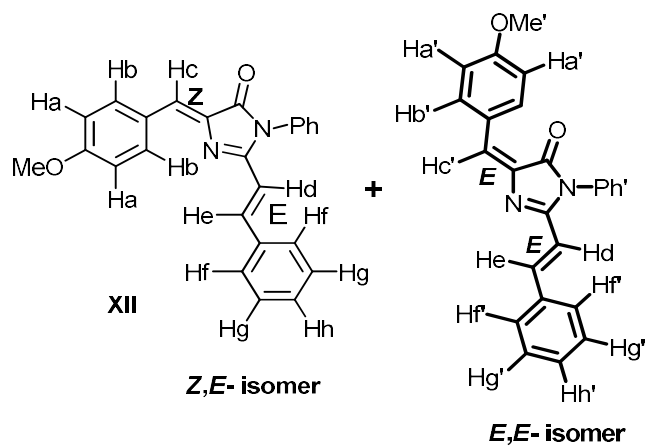
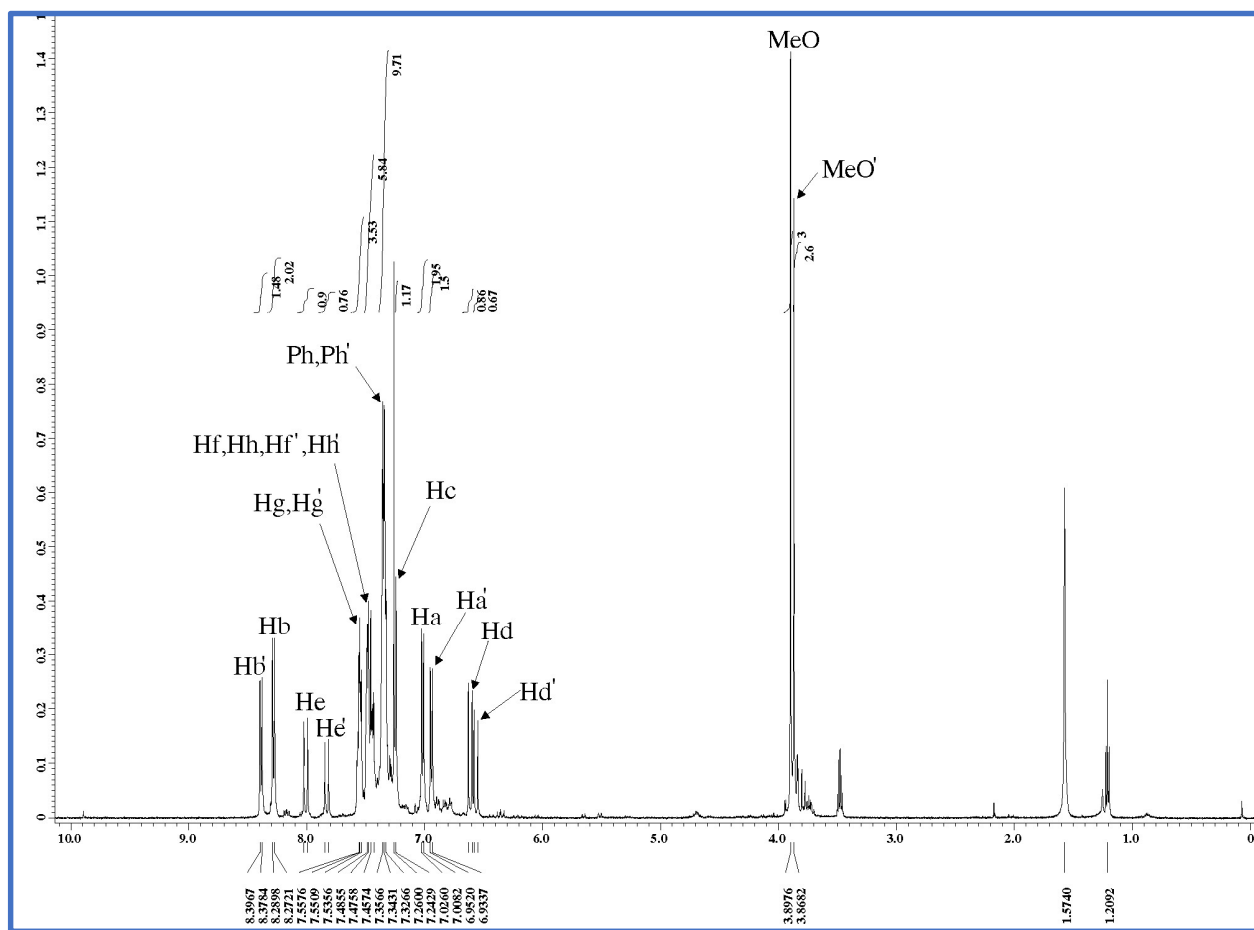


Figure S10. ^1H NMR (CDCl_3 , 500 MHz) of **3a** (full spectrum) after irradiation for 17h in a Luzchem photoreactor fitted with 350 nm lamps. The structures of the *Z,E* and *E,E* isomers are shown below the ^1H NMR spectrum.

All the signals appear doubles.

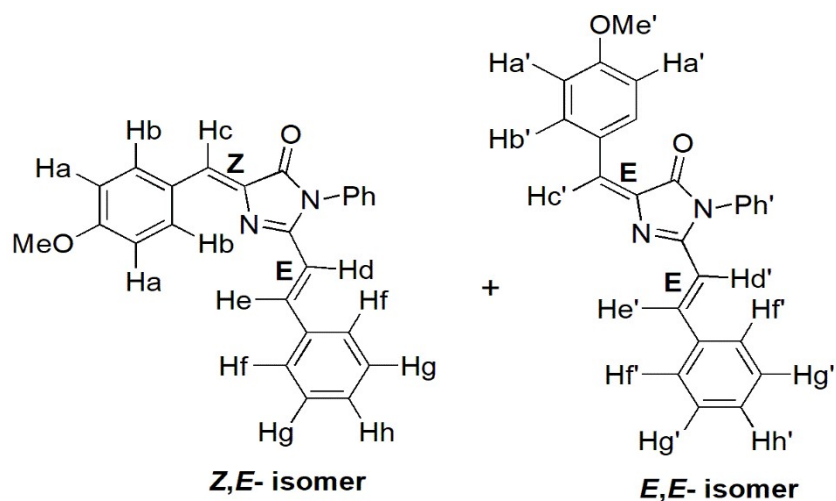
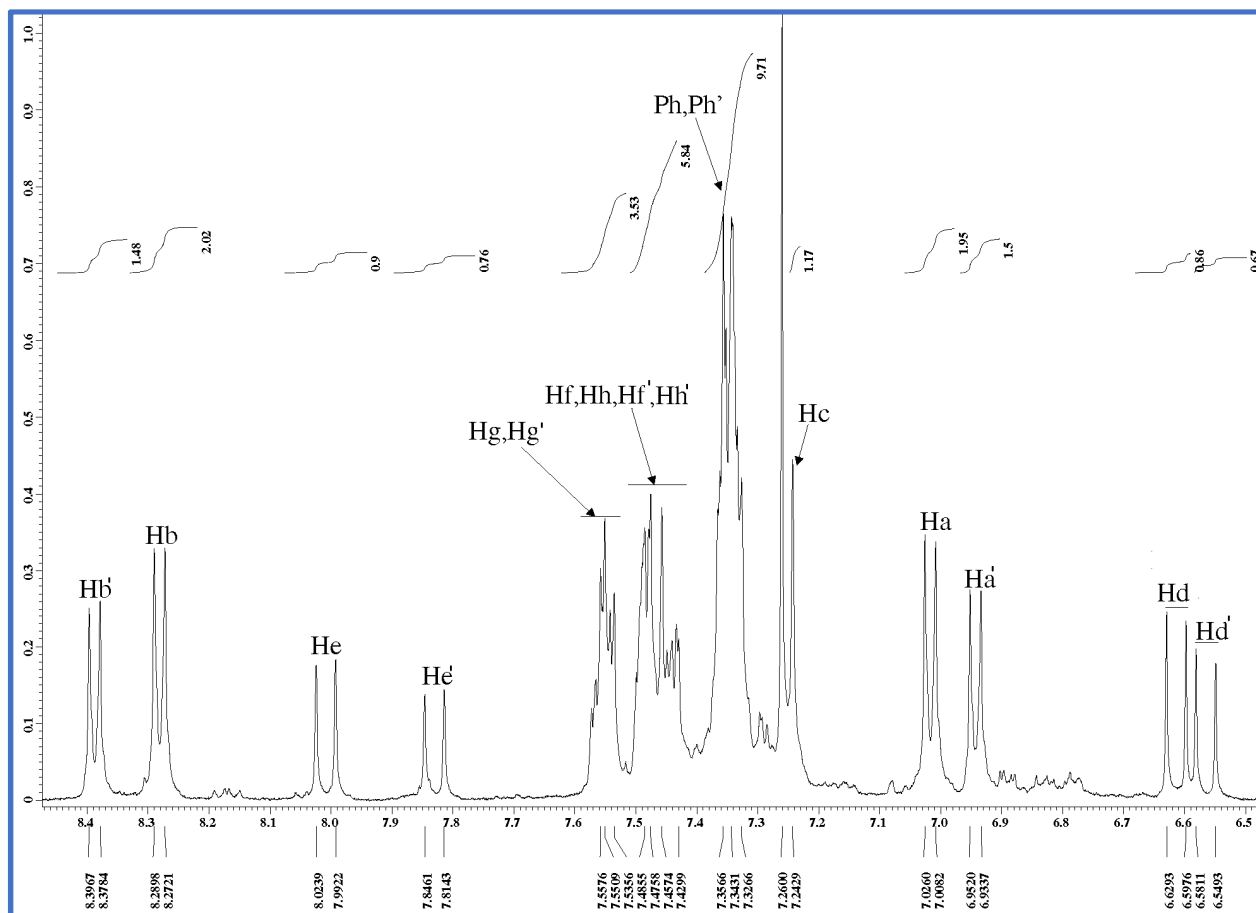


Figure S11. ^1H NMR (CDCl_3 , 500 MHz) of **3a** (Zoomed) after irradiation for 17h in a Luzchem photoreactor fitted with 350 nm lamps. The structures of the *Z,E* and *E,E* isomers are shown below the ^1H NMR spectrum.

All the signals appear double signifying the presence of two isomers.

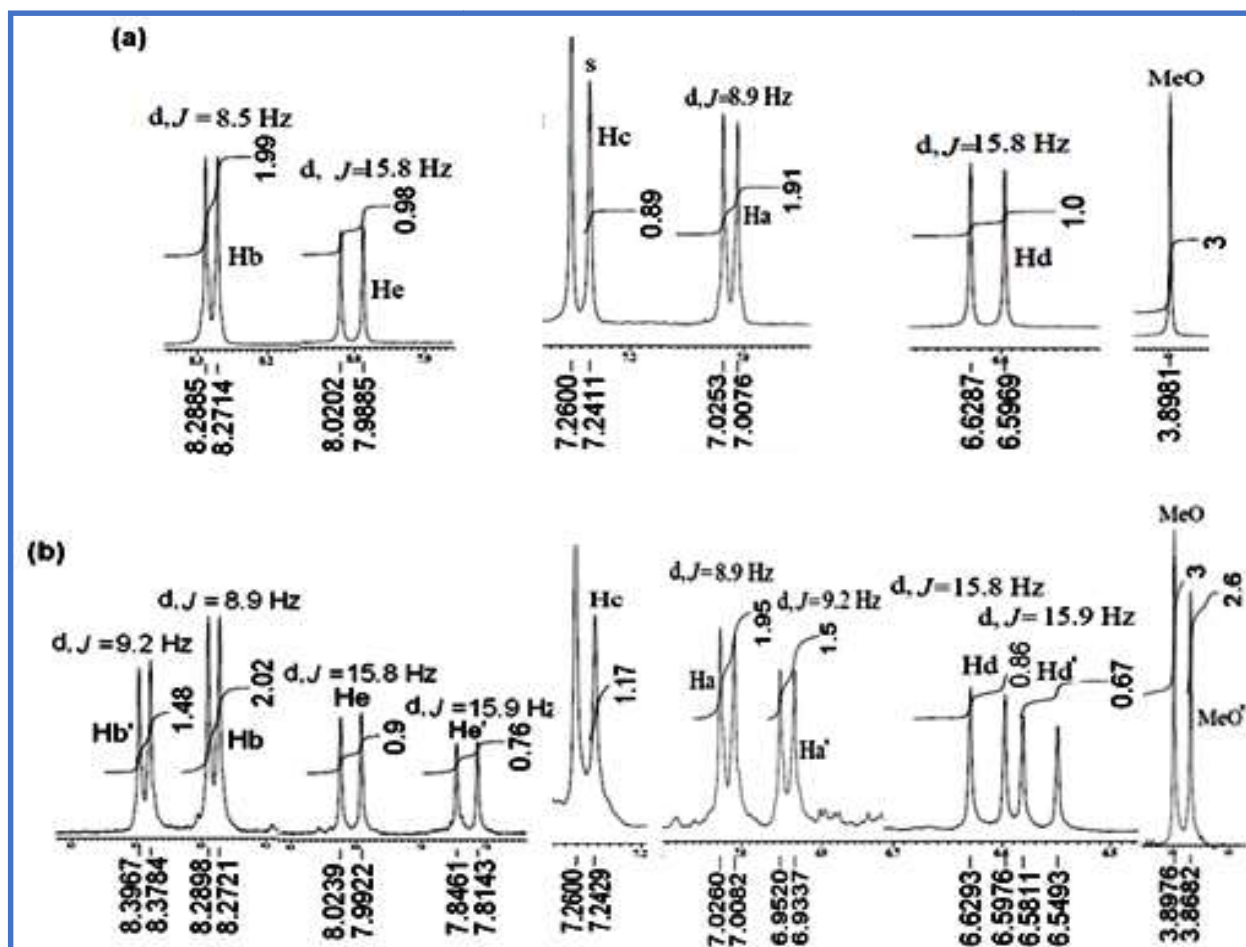


Figure S12. (a) ¹H NMR (CDCl₃, 500 MHz) of molecule **3a** before irradiation. (b) ¹H NMR (CDCl₃, 500 MHz) after irradiation for 17h in a Luzchem photoreactor fitted with 350 nm lamps. The multiplets arising from the N-Ph and styrenyl benzene ring are removed for clarity. Notation: d – doublet, s- singlet. ¹H NMR signals of figure **a** correspond to *Z,E*- isomer. ¹H NMR signals of figure **b** correspond to a mixture of *Z,E*- and *E,E*-isomers. The protons of the *E,E*- isomer are labeled with prime symbol (') to distinguish from the protons of the *Z,E*-isomer.

All the signals appear double signifying the presence of two isomers. As seen in Figure S6, a new aromatic doublet at δ 8.38 ppm ($J = 9.2$ Hz, 2H, Hb') is downfield from the doublet of Hb protons (δ 8.28 ppm, d, $J = 8.9$ Hz, 2H). Supposing that the molecule **3a** (*Z,E*- isomer) undergoes isomerization about the exo-methylene double bond (*Z*-stereochemistry) to form the *E,E*-isomer, the new aromatic doublet at δ 8.38 ppm can be assigned to the Hb' protons because of the possibility of formation of an intramolecular C-H \cdots O hydrogen bond.¹ A new olefinic doublet at δ 7.83 ppm ($J = 15.9$ Hz, 1H) which is upfield from the doublet of He protons (δ 8.01 ppm, d, $J = 15.8$ Hz, 1H) can

be assigned to the He' protons of the *E,E*-isomer. A new aromatic doublet at $\delta 6.94$ ppm ($J = 9.2$ Hz, 2H) which is upfield from the Ha protons ($\delta 7.02$ ppm, d, $J = 8.9$ Hz, 2H) can be assigned to the Ha' protons of the *E,E*-isomer. A new olefinic doublet at $\delta 6.56$ ppm ($J = 15.9$ Hz, 1H) which is upfield from the Hd protons ($\delta 6.61$ ppm, d, $J = 15.8$ Hz, 1H) can be assigned to the Hd' protons of the *E,E*-isomer. A new singlet signal at $\delta 3.87$ ppm (3H) ppm which is upfield from the protons of the methoxy group ($\delta 3.89$ ppm, s, 3H) can be assigned to the MeO' group of the *E,E*- isomer. Since the Hb' protons are downfield and the olefinic Hd' and He' protons are appearing as trans-coupled protons, it is evident that isomerization of molecule **3a** has occurred about the exo-methyle double bond (*Z*-stereochemistry). The ratio of the integrations of the corresponding protons (MeO, MeO'; Hb, Hb' etc.) shows isomerization of almost 46% of the *Z,E*- isomer to the *E,E*- isomer after 17 h irradiation.

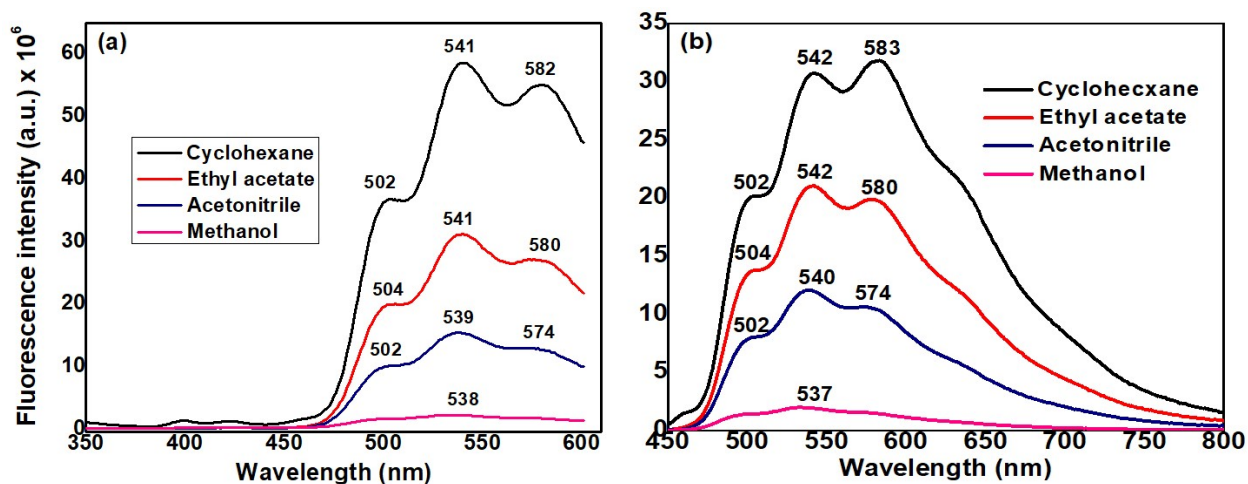


Figure S13. Steady state fluorescence spectra of **3a** in cyclohexane, ethyl acetate, acetonitrile and methanol at two different excitation wavelengths: (a) excitation wavelength 305 nm, (b) excitation wavelength 423 nm. Concentration of the solution is 1×10^{-5} M, band pass = 3,3 nm

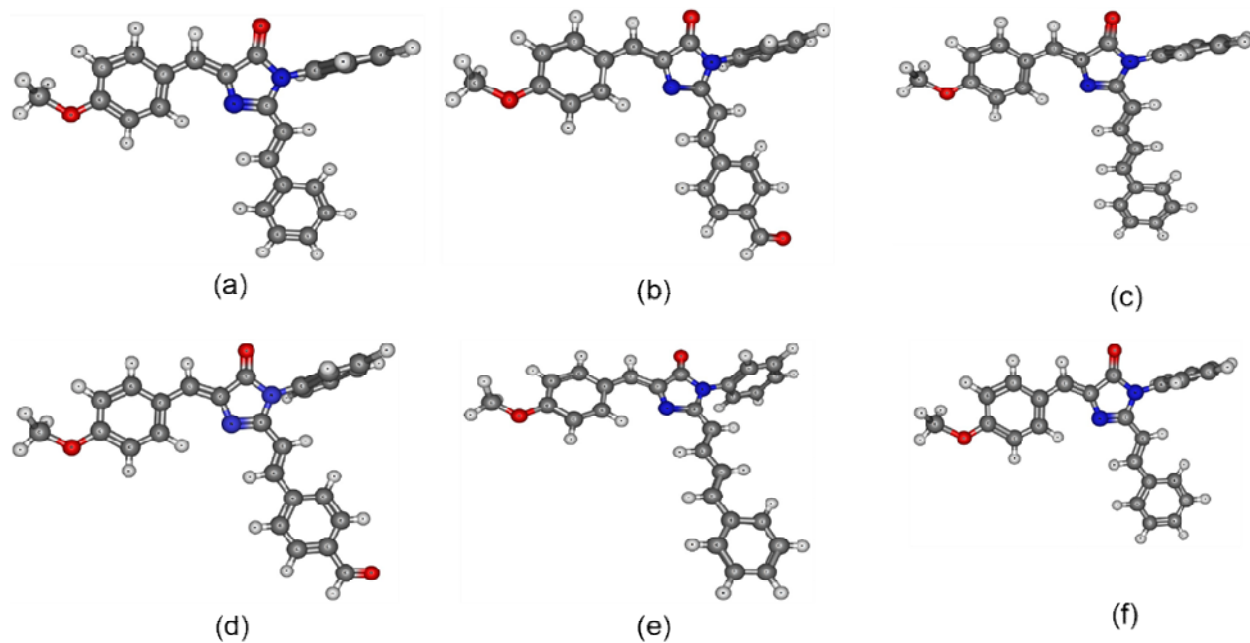


Figure S14: Optimized structure of (a) **3a**, (b) **3b**, and (c) **3c** in the ground-state (S_0) and (d) **3a**, (e) **3b**, and (f) **3c** in the excited-state (S_1) of the compounds **3a-c** calculated at B3LYP/TZVP and TD-DFT/CAM-B3LYP/TZVP level in the acetonitrile solvent.

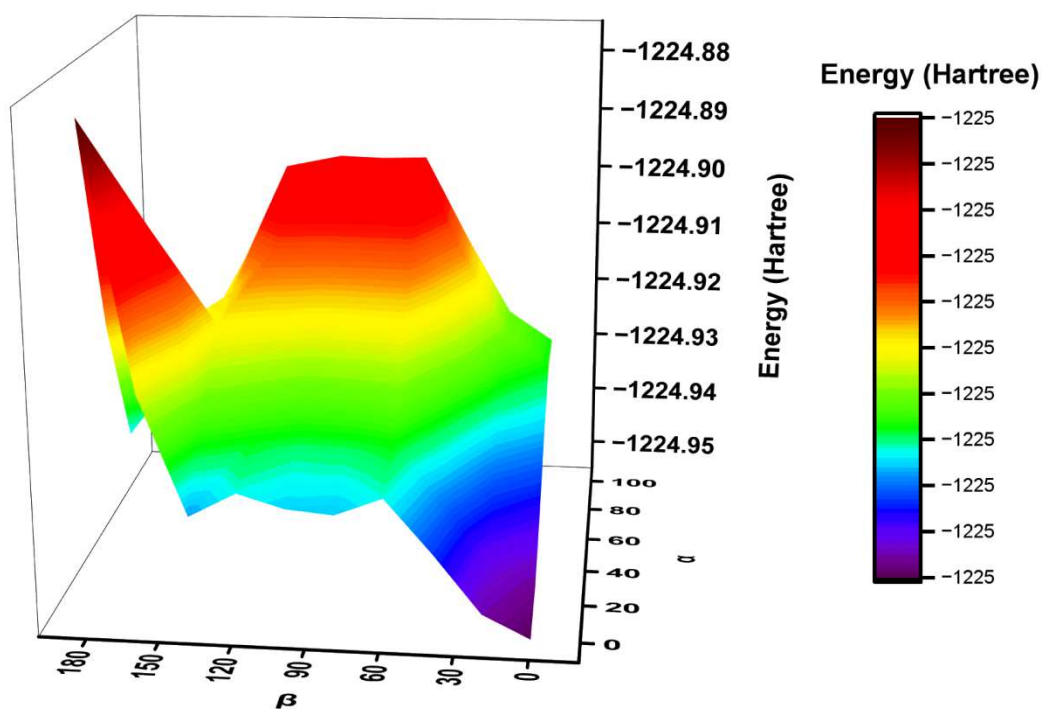


Figure S15. Three-dimensional potential energy surface of the S_1 state of **3a** as a function of dihedral angles α and β computed at TD-B3LYP/TZVP level of theory.

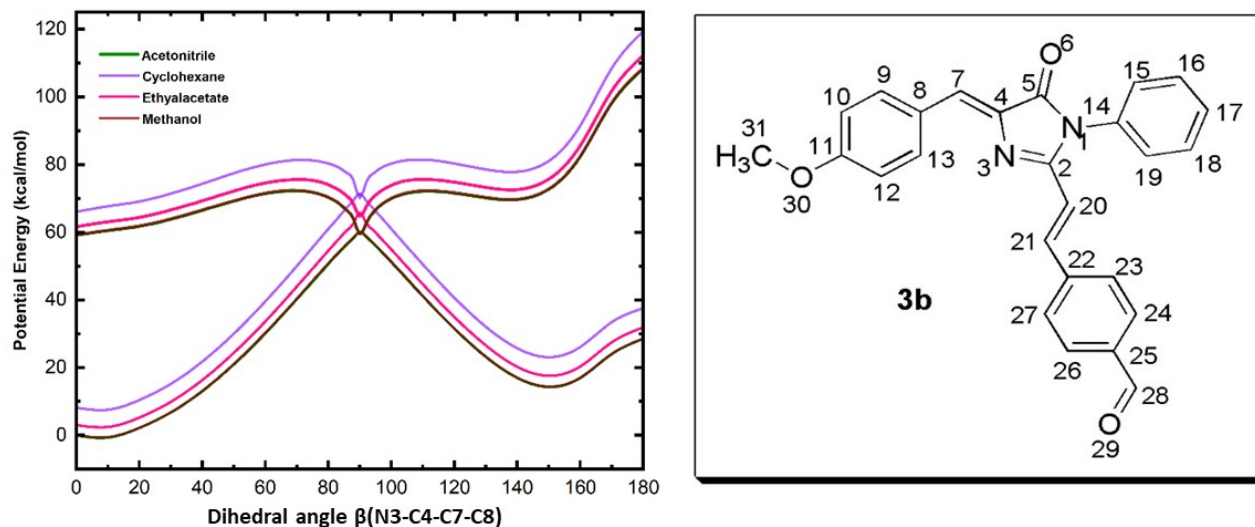


Figure S16. Potential energy curves of the S_0 and S_1 states of molecule **3b** for rotation along the dihedral angle β (N3-C4-C7-C8) (rotation along the Z-configuration C=C bond) in four solvents – cyclohexane, ethyl acetate, acetonitrile and methanol (left). On the right atom numbering of **3b** is shown.

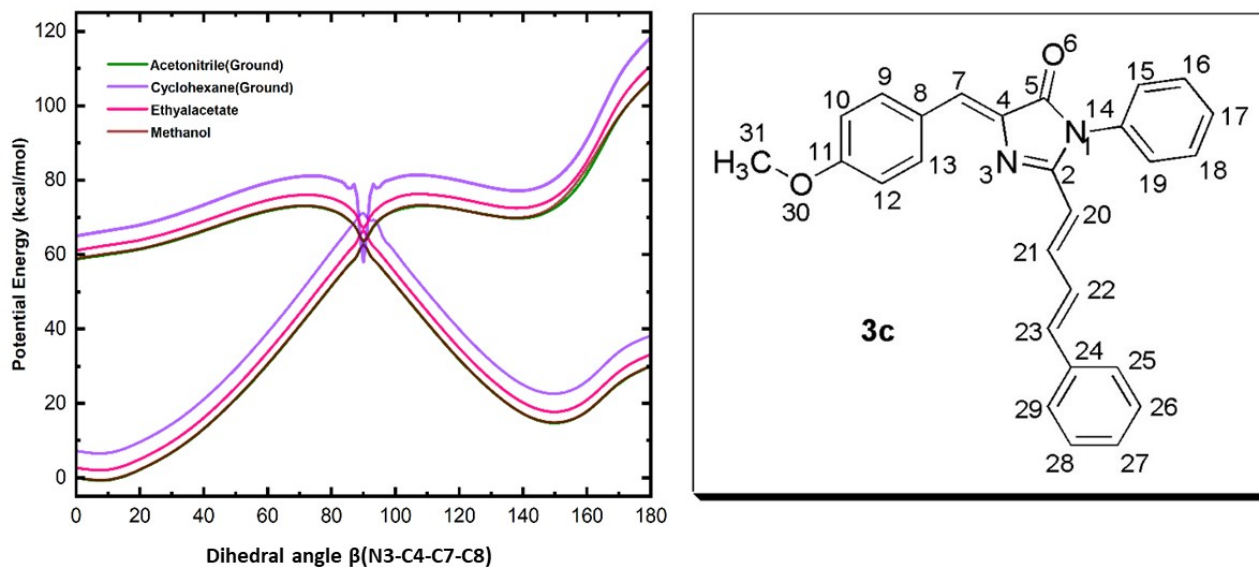


Figure S17. Potential energy curves of the S_0 and S_1 states of molecule **3c** for rotation along the dihedral angle β (N3-C4-C7-C8) (rotation along the Z-configuration C=C bond) (left) in four solvents – cyclohexane, ethyl acetate, acetonitrile and methanol. On the right atom numbering of **3c** is shown.

References:

- 1 S. Kojima, H. Ohkawa, T. Hirano, S. Maki, H. Niwa, M. Ohashi, S. Inouye and F. I. Tsuji, Fluorescent properties of model chromophores of tyrosine-66 substituted mutants of Aequorea green fluorescent protein (GFP), *Tetrahedron Lett.*, 1998, **39**, 5239–5242.
- 2 B. K. Rajbongshi, N. N. Nair, M. Nethaji and G. Ramanathan, Segregation into chiral enantiomeric conformations of an achiral molecule by concomitant polymorphism, *Cryst. Growth Des.*, 2012, **12**, 1823–1829.
- 3 M. Z. Badr, H. A. H. El-Sherief and M. E. Tadros, Studies on 2-Methyl- and 2-Phenyl-4-arylmethylene-2-imidazolin-5-ones and Related Compounds, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2267–2270.
- 4 G. Bhattacharjya, Sarit. S. Agasti and G. Ramanathan, Solvent free Lewis acid catalyzed vinylogous condensation, *ARKIVOC*, 2006, **2006**, 152–161.
- 5 G. Bhattacharjya, From Molecules To Materials: Design, synthesis, characterization and possible applications of imidazolin-5-ones, Ph.D Thesis (2006), Indian Institute of Technology Kanpur.