

Supporting Information

Viscosity-Responsive Styryl Benzoxazole Probes for Lipid Droplet Visualization

Vijay Sai Krishna Cheerala[‡], Simran[‡], Prateek Sarkar, Deeksha Rajput and Sriram Kanvah*

Department of Chemistry, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar
382055, India. E-mail: sriram@iitgn.ac.in

[‡]Equal Authorship

<i>Table of Contents</i>		
No.	Content	Page No.
1	Characterization Details	03
2	Absorption and emission spectra	04
3	DLS Spectra	07
3	MTT assay	08
4	Live Cell Imaging	07
5	Other Experiment	11
6	Viscosity dependent Fluorescence Enhancement	12
8	NMR Spectra	14
9	Mass Spectra	22

1. Characterization Details

(E)-4-(2-(benzo[d]oxazol-2-yl)vinyl)-N,N-dimethylaniline (R1)

¹H NMR (500 MHz, CDCl₃) δ 7.73 (d, *J* = 16.2 Hz, 1H), 7.67 (m, 1H), 7.49 (m, 3H), 7.29 (m, 2H), 6.85 (d, *J* = 16.2 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 2H), 3.03 (s, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 164.15, 151.55, 150.49, 142.60, 140.12, 129.26 (2C), 124.58, 124.35, 123.24, 119.46, 112.14 (2C), 110.16, 108.66, 40.31 (2C). HRMS (ESI-TOF) *m/z* [M+H]⁺ calculated for C₁₇H₁₇N₂O⁺ 265.1335 ; found 265.1337.

(E)-2-(4-(pyrrolidin-1-yl)styryl)benzo[d]oxazole (R2)

¹H NMR (500 MHz, CDCl₃) δ 7.72 (d, *J* = 16.2 Hz, 1H), 7.65 (m, 1H), 7.48 (m, 3H), 7.28 (m, 2H), 6.81 (d, *J* = 16.2 Hz, 1H), 6.56 (d, *J* = 8.7 Hz, 2H), 3.35 (m, 4H), 2.03 (m, 4H). **¹³C NMR (126 MHz, CDCl₃)** δ 164.22, 150.37, 148.99, 142.54, 140.32, 129.33 (2C), 124.36, 124.20, 122.42, 119.27, 111.78 (2C), 110.01, 107.77, 47.58 (2C), 25.48 (2C). **HRMS (ESI-TOF)** *m/z* [M+H]⁺ calculated for C₁₉H₁₉N₂O⁺ 291.1492 ; found 291.1493.

***(E)*-2-(4-(piperidin-1-yl)styryl)benzo[d]oxazole (R3)**

¹H NMR (500 MHz, CDCl₃) δ 7.72 (d, *J* = 16.2 Hz, 1H), 7.68 (m, 1H), 7.49 (m, 1H), 7.48 (d, *J* = 8.8 Hz, 2H), 7.30 (m, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 16.2 Hz, 1H), 3.29 (t, *J* = 5.5 Hz, 4H), 1.67 (m, 6H). **¹³C NMR (126 MHz, CDCl₃)** δ 163.94, 152.82, 150.50, 142.55, 139.81, 129.13 (2C), 125.09, 124.73, 124.41, 119.57, 115.30 (2C), 110.22, 109.68, 49.44 (2C), 25.62 (2C), 24.47. **HRMS (ESI-TOF)** *m/z* [M+H]⁺ calculated for C₂₀H₂₁N₂O⁺ 305.1648 ; found 305.1651.

***(E)*-2-(4-morpholinostyryl)benzo[d]oxazole (R4)**

¹H NMR (500 MHz, CDCl₃) δ 7.72 (d, *J* = 16.3 Hz, 1H), 7.68 (m, 1H), 7.51 (m, 3H) 7.30 (m, 2H), 6.90 (m, 3H), 3.87 (t, *J* = 4.9 Hz, 4H), 3.25 (dd, *J* = 4.9 Hz, 4H). **¹³C NMR (126 MHz, CDCl₃)** δ 163.67, 152.29, 150.50, 142.46, 139.47, 129.10 (2C), 126.48, 124.92, 124.50, 119.67, 115.08 (2C), 110.64, 110.28, 66.82 (2C), 48.39 (2C). **HRMS (ESI-TOF)** *m/z* [M+H]⁺ calculated for C₁₉H₁₉N₂O₂⁺ 307.1441 ; found 307.1461.

***(E)*-4-(2-(benzo[d]oxazol-2-yl)vinyl)-*N,N*-diphenylaniline (R5)**

¹H NMR (500 MHz, CDCl₃) δ 7.73 (d, *J* = 16.3 Hz, 1H), 7.68 (m, 1H), 7.5 (m, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.3 (m, 6H), 7.14 (dd, *J* = 8.6, 1.2 Hz, 4H), 7.09 (t, 2H), 7.05 (d, *J* = 8.6 Hz, 2H), 6.93 (d, *J* = 16.3 Hz, 1H). **¹³C NMR (126 MHz, CDCl₃)** δ 163.40, 150.42, 149.45, 147.03, 142.36, 139.10, 129.50, 128.67, 128.40, 125.31, 124.91, 124.43, 123.92, 122.09, 119.65, 111.32, 110.22. **HRMS (ESI-TOF)** *m/z* [M+H]⁺ calculated for C₂₇H₂₁N₂O⁺ 389.1648 ; found 389.1665.

***(E)*-2-(4-(9H-carbazol-9-yl)styryl)benzo[d]oxazole (R6)**

¹H NMR (500 MHz, CDCl₃) δ 8.16 (d, *J* = 7.7 Hz, 2H), 7.90 (d, *J* = 16.4 Hz, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.76 (m, 1H), 7.65 (d, *J* = 8.4 Hz, 2H), 7.57 (m, 1H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.44 (m, 2H), 7.37 (m, 2H), 7.32 (m, 2H), 7.17 (d, *J* = 16.4 Hz, 1H). **¹³C NMR (126 MHz, CDCl₃)** δ 162.76, 150.61, 142.34, 140.62, 139.09, 138.41, 134.17, 129.11 (2C), 127.36 (2C),

126.23 (2C), 125.53, 124.77, 123.77, 120.53 (2C), 120.44 (2C), 120.11, 114.70, 110.53, 109.93 (2C). **HRMS** (ESI-TOF) m/z $[M+H]^+$ calculated for $C_{27}H_{19}N_2O^+$ 387.1492 ; found 387.1513.

***(E)-2-(2-(9-ethyl-9H-carbazol-2-yl)vinyl)benzo[d]oxazole* (R7)**

1H NMR (500 MHz, $CDCl_3$) δ 8.31 (d, $J = 1.6$ Hz, 1H), 8.14 (d, $J = 7.7$ Hz, 1H), 8.00 (d, $J = 16.2$ Hz, 1H), 7.75 (dd, $J = 8.5, 1.8$ Hz, 1H), 7.72 (m, 1H), 7.53 (m, 1H), 7.51 (ddd, $J = 8.3, 7.0, 1.2$ Hz, 1H), 7.43 (dd, $J = 8.5, 1.6$ Hz, 2H), 7.32 (m, 2H), 7.29 (ddd, $J = 7.9, 7.0, 1.2$ Hz, 1H), 7.10 (d, $J = 16.2$ Hz, 1H), 4.37 (q, $J = 7.3$ Hz, 2H), 1.45 (t, $J = 7.3$ Hz, 3H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 163.66, 150.44, 142.43, 140.94, 140.82, 140.47, 126.30, 126.28, 125.22, 124.77, 124.37, 123.46, 122.87, 120.64, 120.60, 119.58, 110.71, 110.20, 108.97, 108.87, 37.78, 13.87. **HRMS** (ESI-TOF) m/z $[M+H]^+$ calculated for $C_{23}H_{19}N_2O^+$ 339.1492 ; found 339.1498.

***4-((1E,3E)-4-(benzo[d]oxazol-2-yl)buta-1,3-dien-1-yl)-N,N-dimethylaniline* (R8)**

1H NMR (500 MHz, $CDCl_3$) δ 7.67 (m, 1H), 7.57 (ddd, $J = 15.5, 7.5, 2.8$ Hz, 1H), 7.48 (m, 1H), 7.40 (d, $J = 8.9$ Hz, 2H), 7.29 (m, 2H), (6.83 (m, 2H), 6.69 (d, $J = 8.9$ Hz, 2H), 6.51 (d, $J = 15.5$ Hz, 1H), 3.00 (s, 6H). **^{13}C NMR (126 MHz, $CDCl_3$)** δ 163.65, 150.94, 150.50, 142.57, 141.10, 139.65, 128.66 (2C), 124.79, 124.54, 124.42, 122.93, 119.58, 114.05, 112.23 (2C), 110.21, 40.36 (2C). **HRMS** (ESI-TOF) m/z $[M+H]^+$ calculated for $C_{19}H_{19}N_2O^+$ 291.1492 ; found 291.1493.

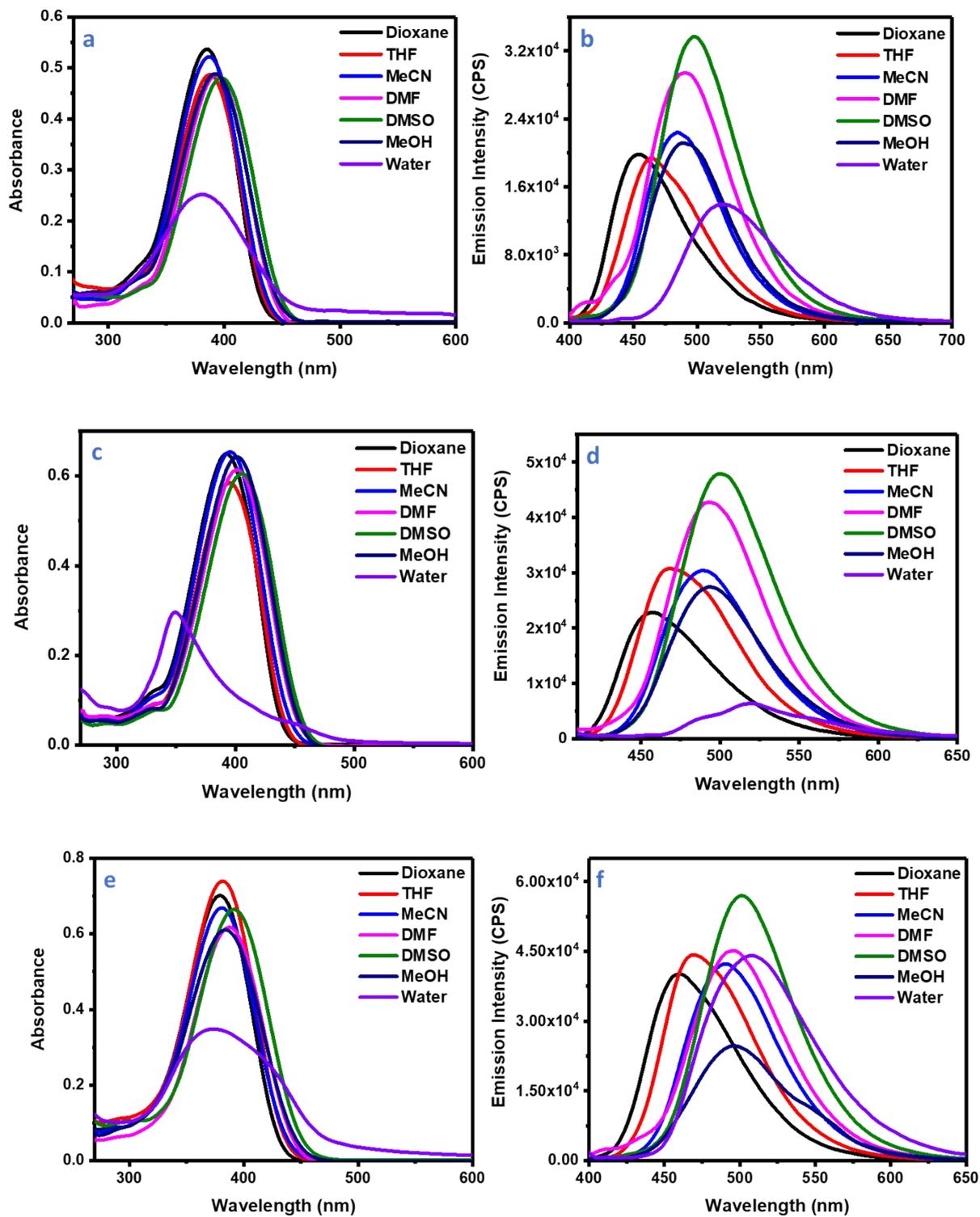


Figure S1a: Absorption and emission of the synthesized compounds **R1** (a,b) -**R2** (c,d) **R3** (e,f), respectively in various solvents. [Concentration= $10 \mu\text{M}$]

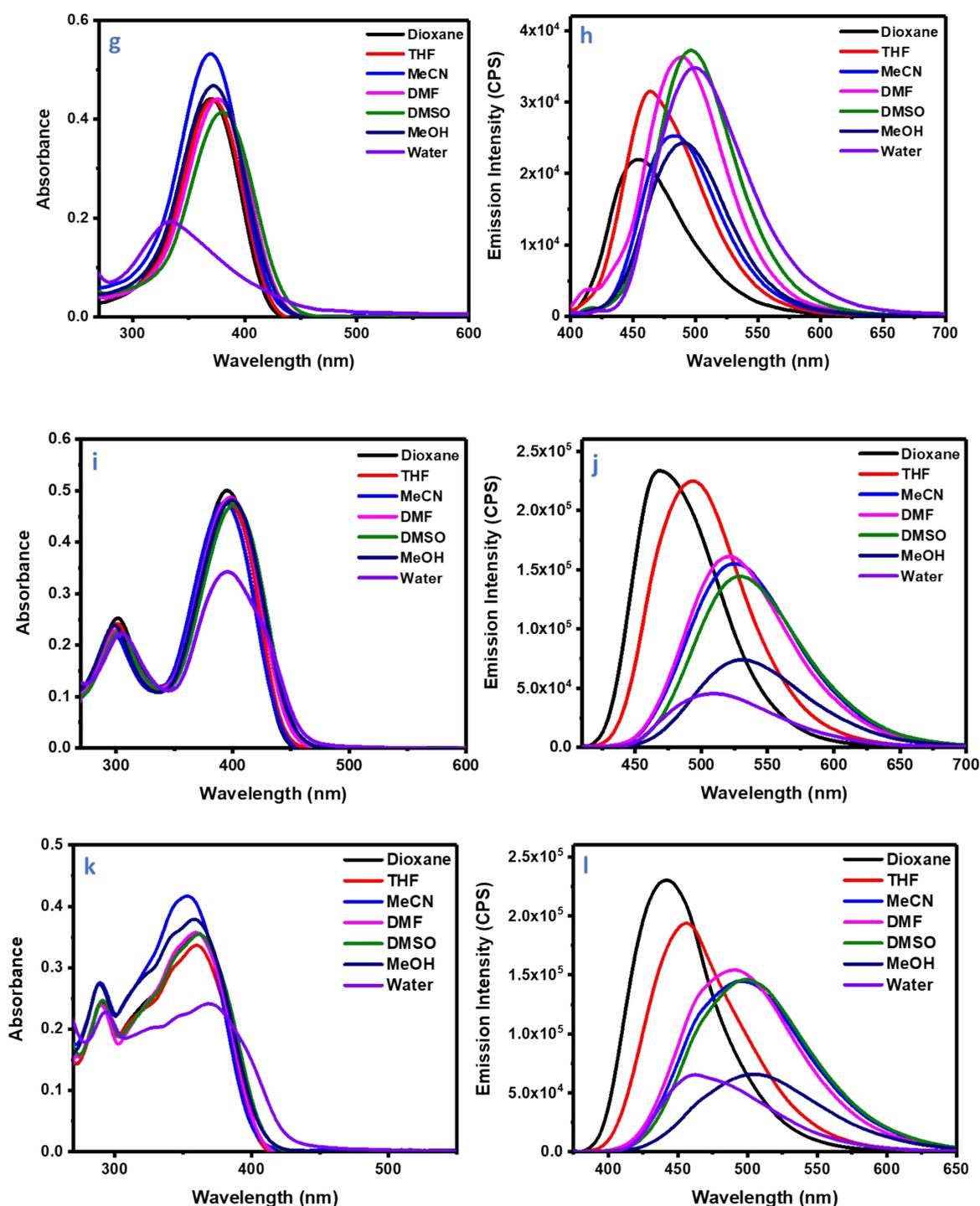


Figure S1b: Absorption and emission of the synthesized compounds **R4** (g,h), **R5** (i,j), **R6** (k, l) in different solvents of varying polarity, [Concentration= 10 μ M]

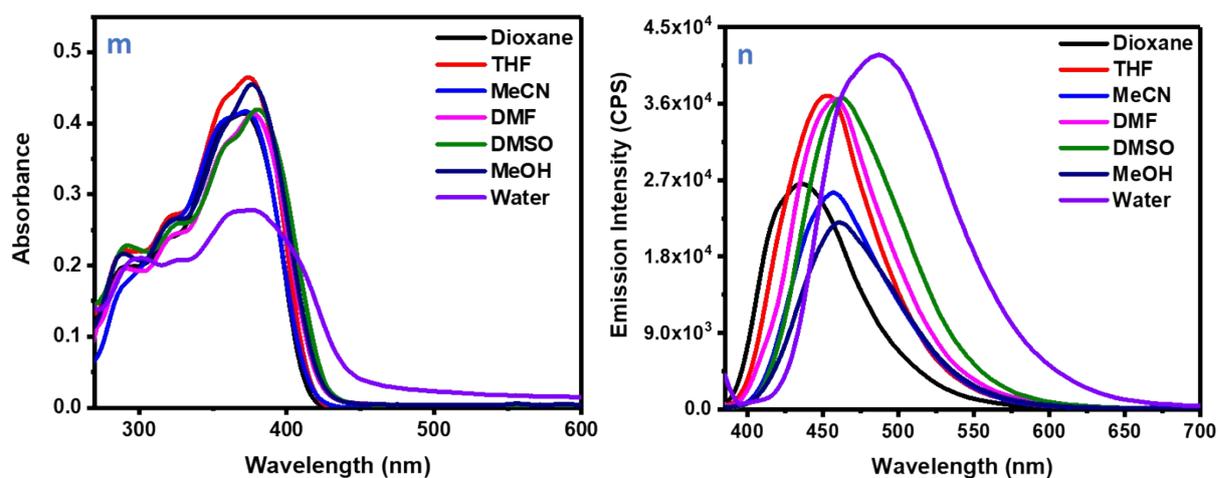


Fig S1c Absorption and emission of the synthesized compounds **R7** (m, n) in solvents of varying polarity, [Concentration= 10 μ M]

DLS Spectra

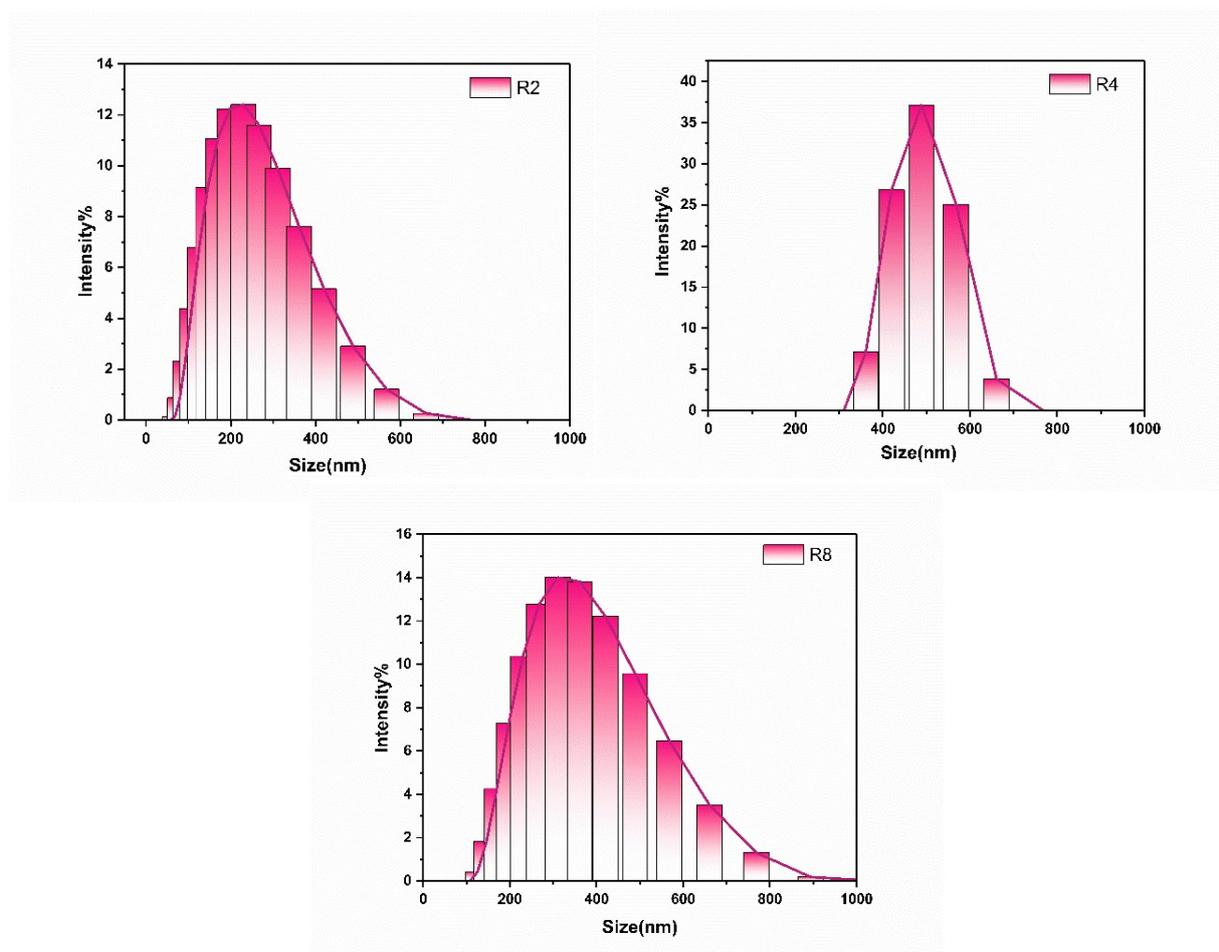


Fig S3: Dynamic Light Scattering Spectra of **R2**, **R4** & **R8**

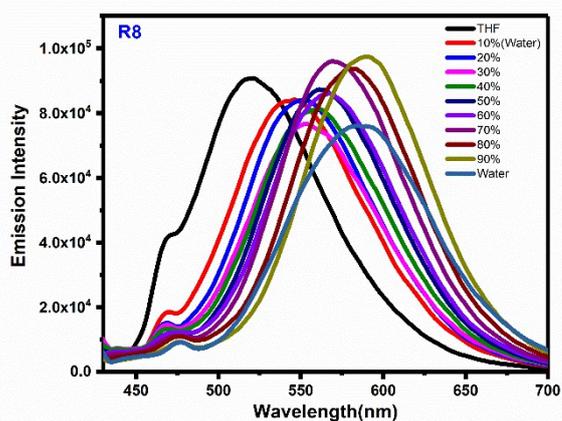
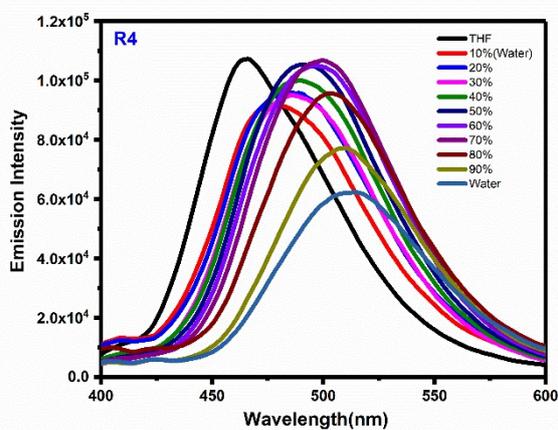
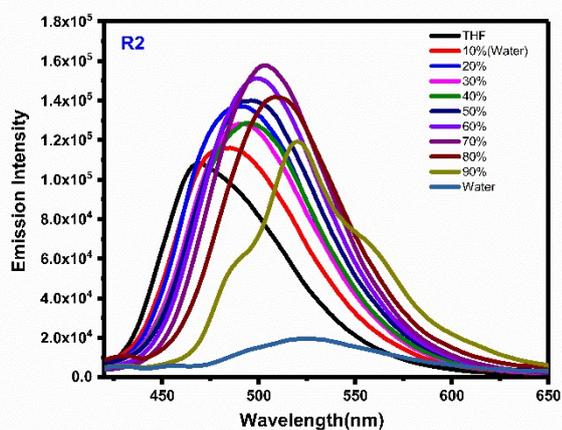


Fig S3: Emission intensity of compounds **R2**, **R4** and **R8** in THF: water binary mixtures with increasing water fraction.

Cytotoxicity assay

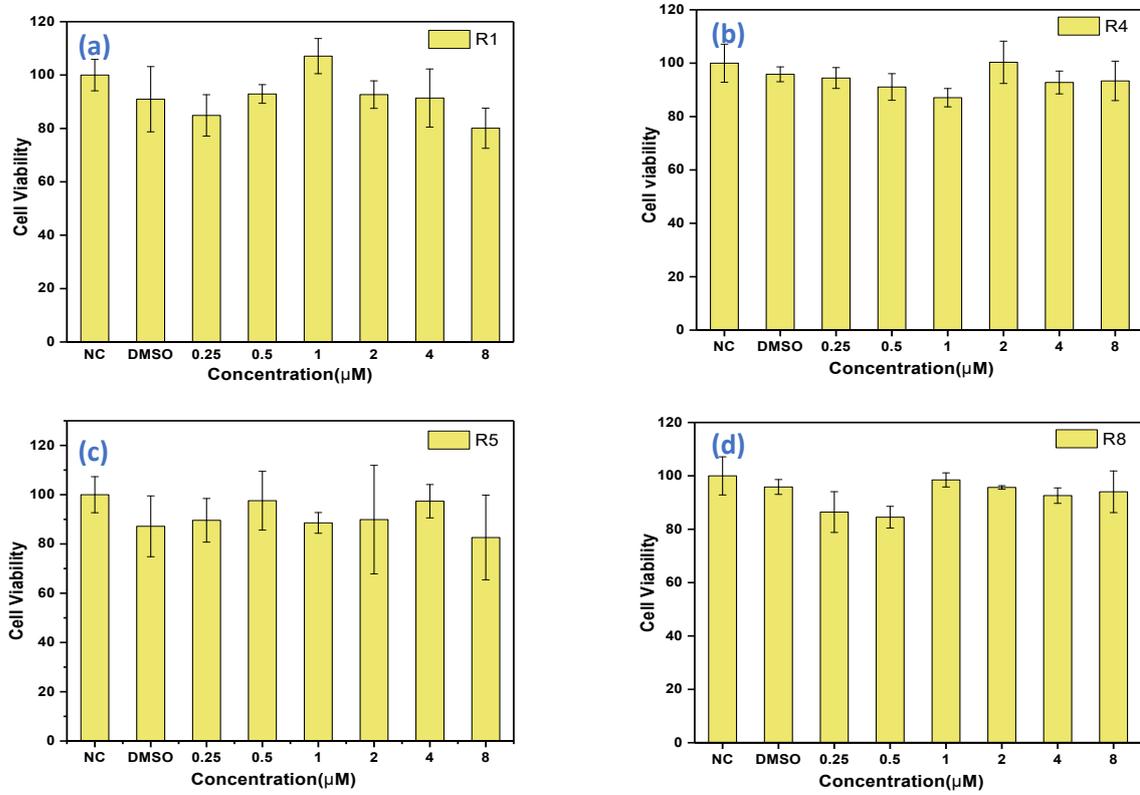


Fig S4: Cytotoxicity assay for R1, R4, R5 and R8 at various concentrations.

Live Cell Imaging

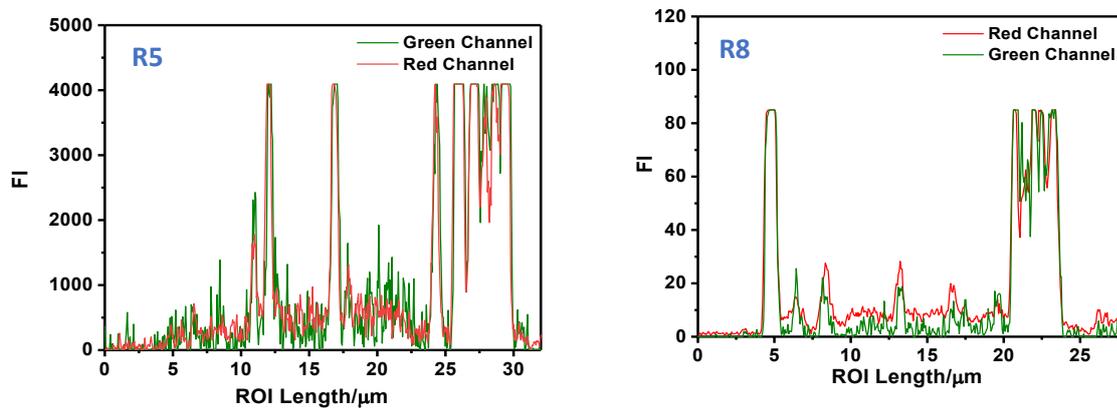


Fig S5: Line profile for **R5** and **R8** showing the extent of localization

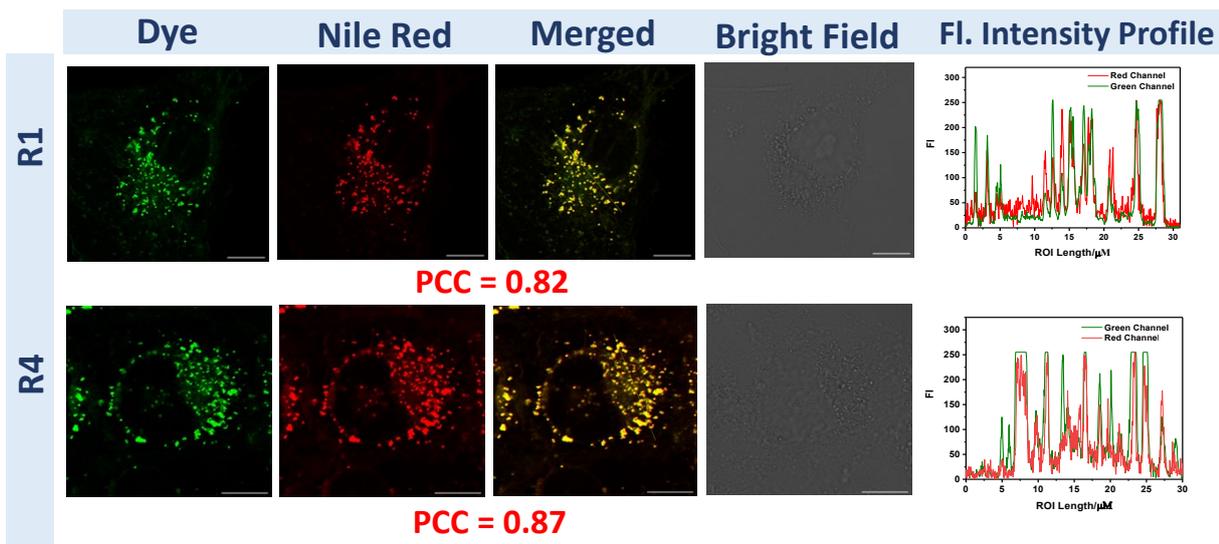


Fig S6: Colocalization of **R1** and **R4** in COS-7 cells. CLSM image of **R1** (Row-1) and **R4** (Row-2) with Nile Red. [Concentration= 2 μM]

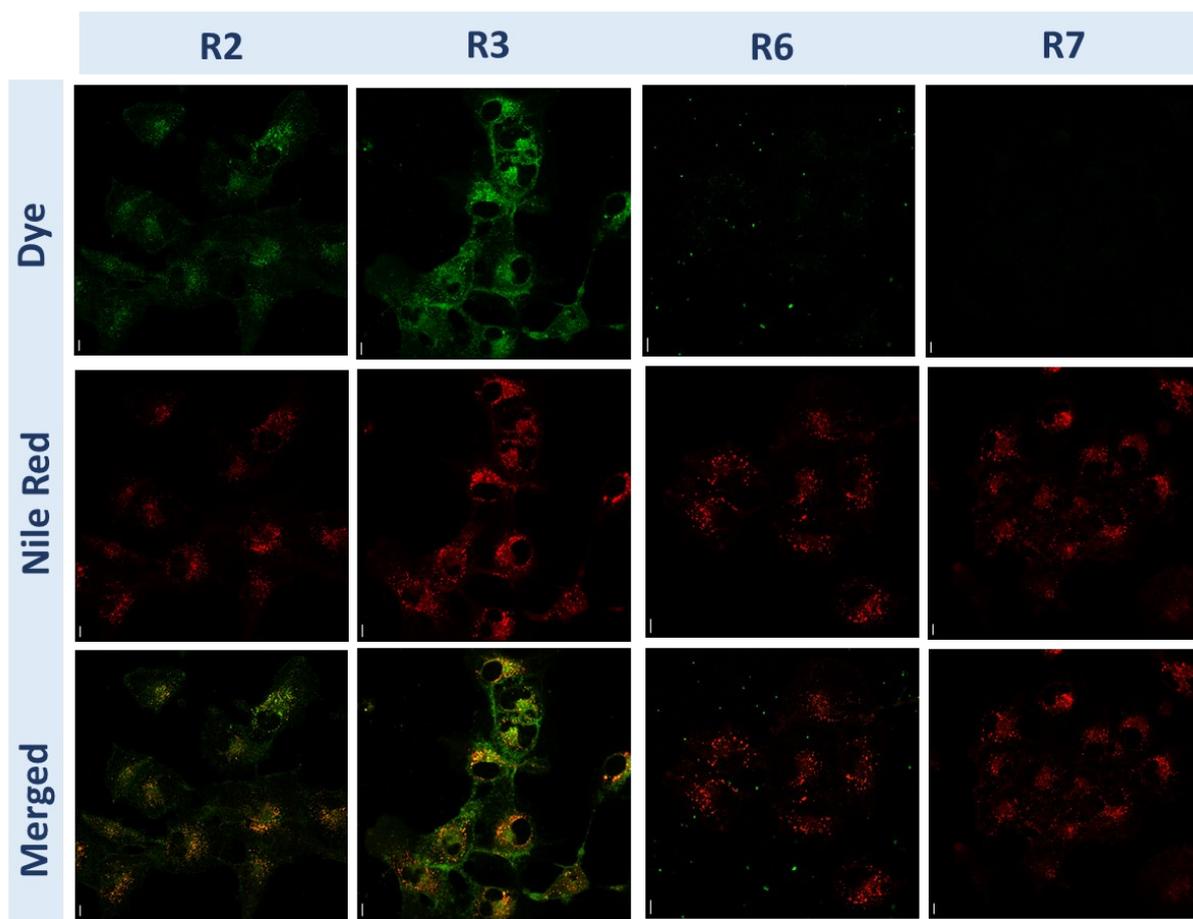


Figure S7: Colocalization of **R2**, **R3**, **R6** and **R7** in COS-7 cells. CLSM image of R2 (Column-1), R3 (Column-2), R6 (Column-3) and R7 (Column-4) with Nile Red [Concentration= 2 μ M]

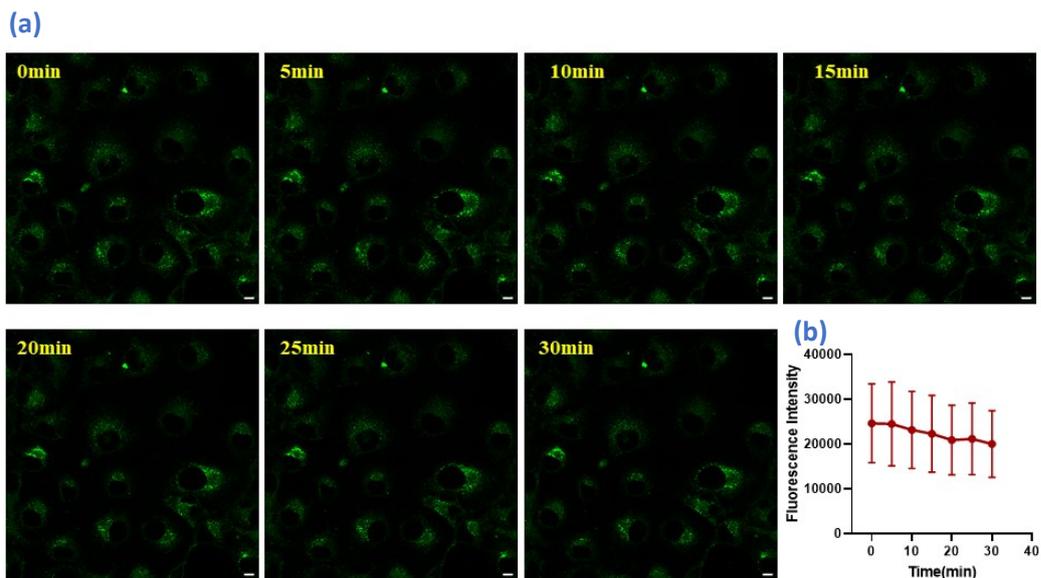


Fig S8: Photo stability Experiment: (a) CLSM images of **R8** upon continuous exposure to 405 nm laser for 30-minute duration. (b) Fluorescence intensity vs time plot at different time intervals. (Scale Bar: 10 μm)

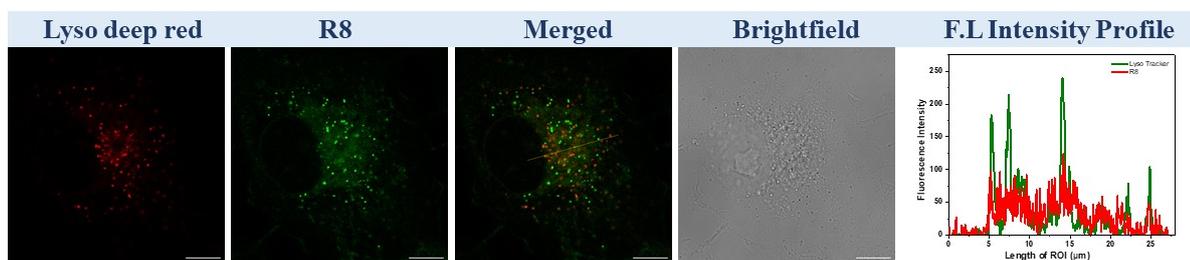


Fig S9: CLSM image of Colocalization of **R8** with lyso deep Red in COS-7 cells. [Concentration= 1 μM] (Scale Bar 10 μm)

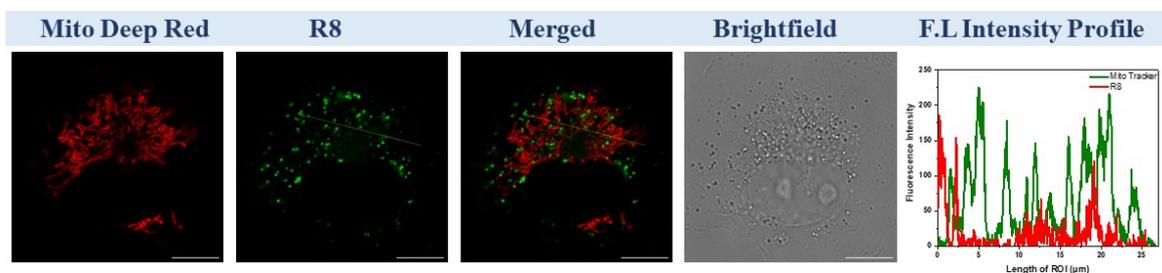


Fig S10: CLSM image of Colocalization of **R8** with Mito deep Red in COS-7 cells. [Concentration= 1 μM] (Scale Bar 10 μm)

pH Study

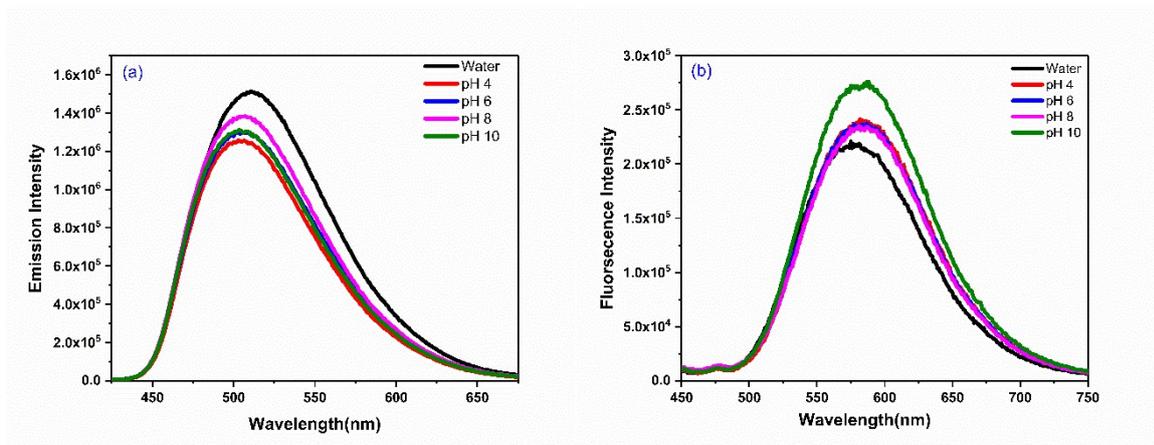


Fig S11: Emission intensity spectra of (a) **R5** & (b) **R8** (10 μM) in water across different pH values.

Analyte Titration

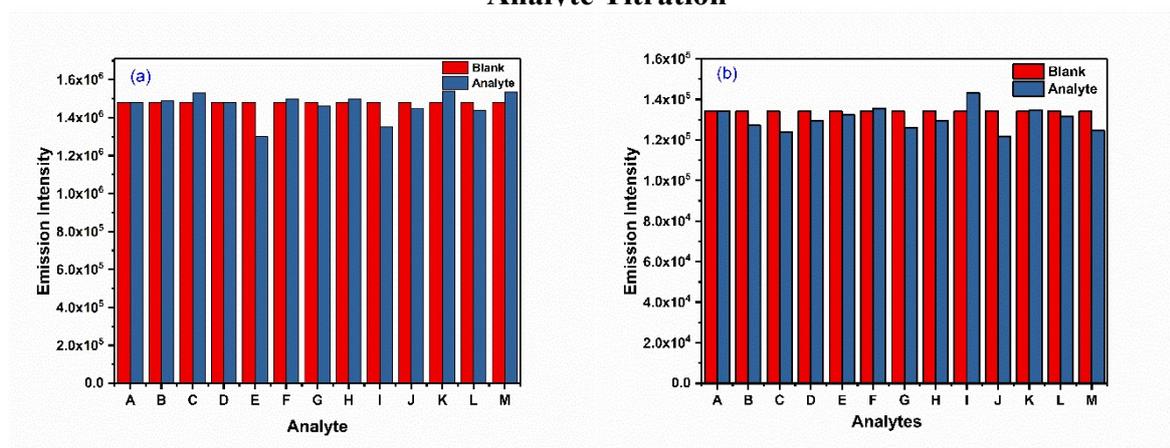


Fig S12: Emission intensity changes for (a) **R5** & (b) **R8** (10 μM) with Fe²⁺ different analytes [A to M = None, Mg²⁺, Na⁺, Glutathione, Fe³⁺, K⁺, PO₄³⁻, Ca²⁺, Al³⁺, Bisuphite, Cystine, S²⁻, H₂O₂]

Viscosity dependence in Glycerol-water binary mixture

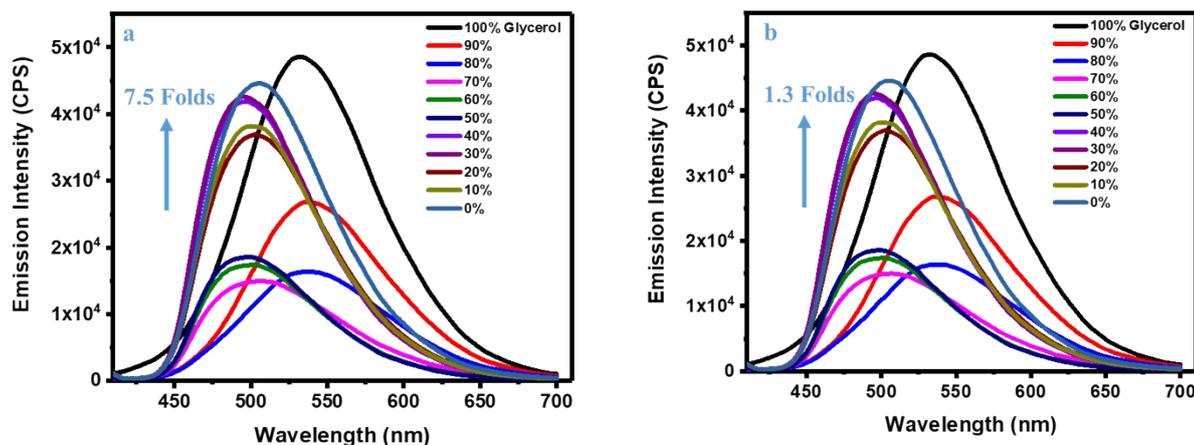


Fig S13: Effect of viscosity on the emission of **R4** and **R5** with gradual addition of glycerol. [Concentration= 10 μ M]

Förster Hoffman Plots

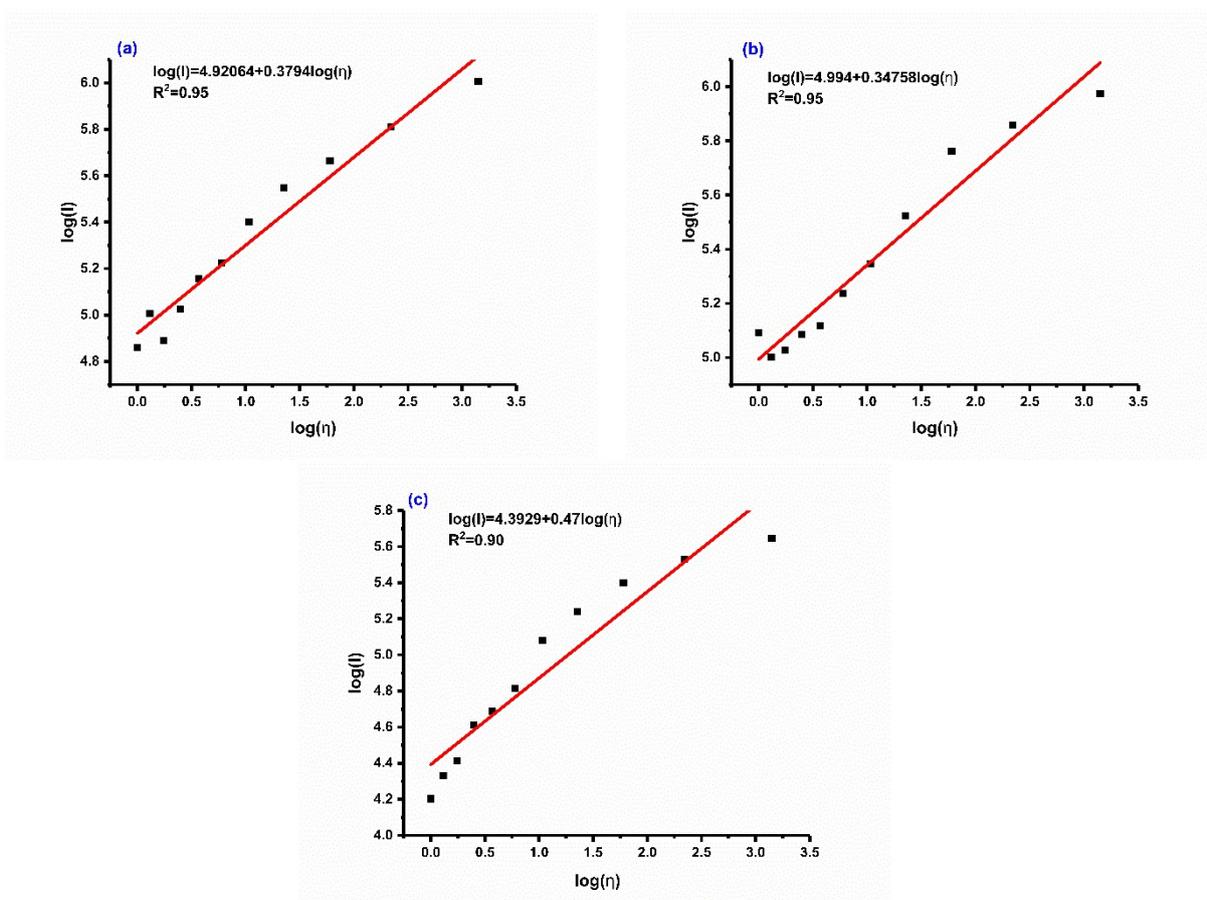
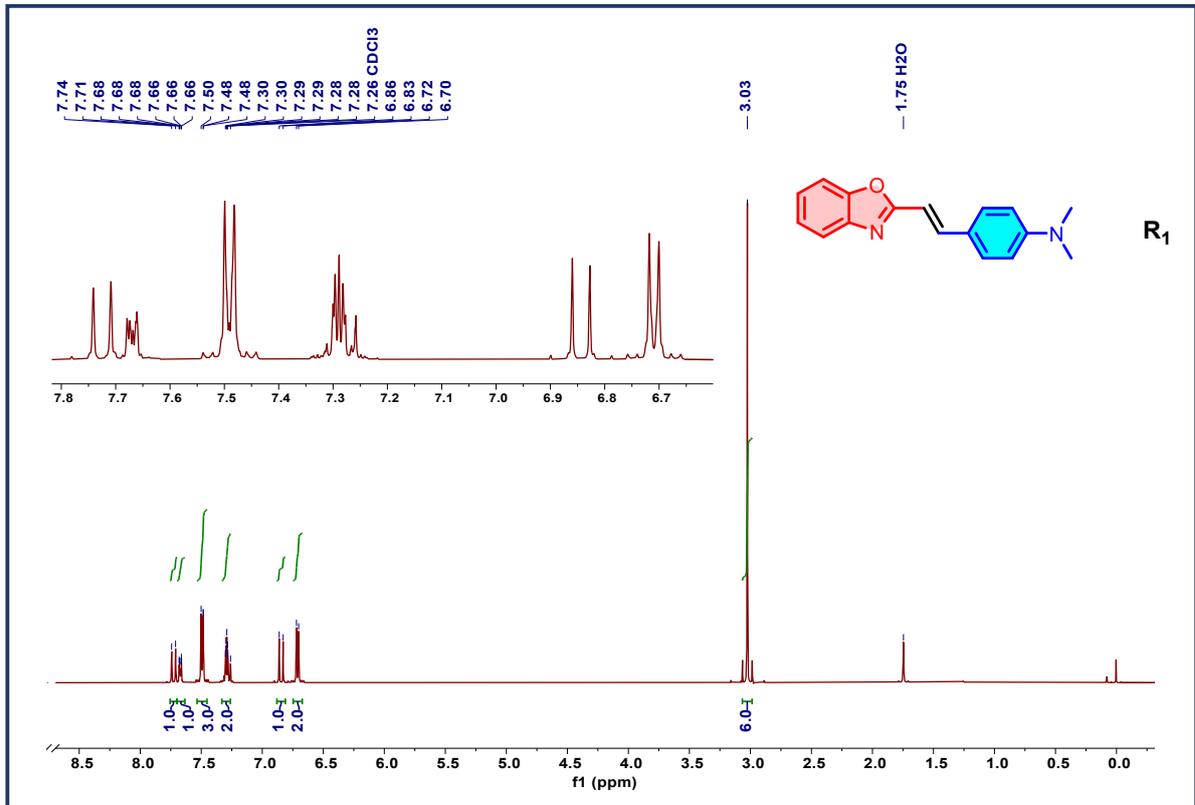


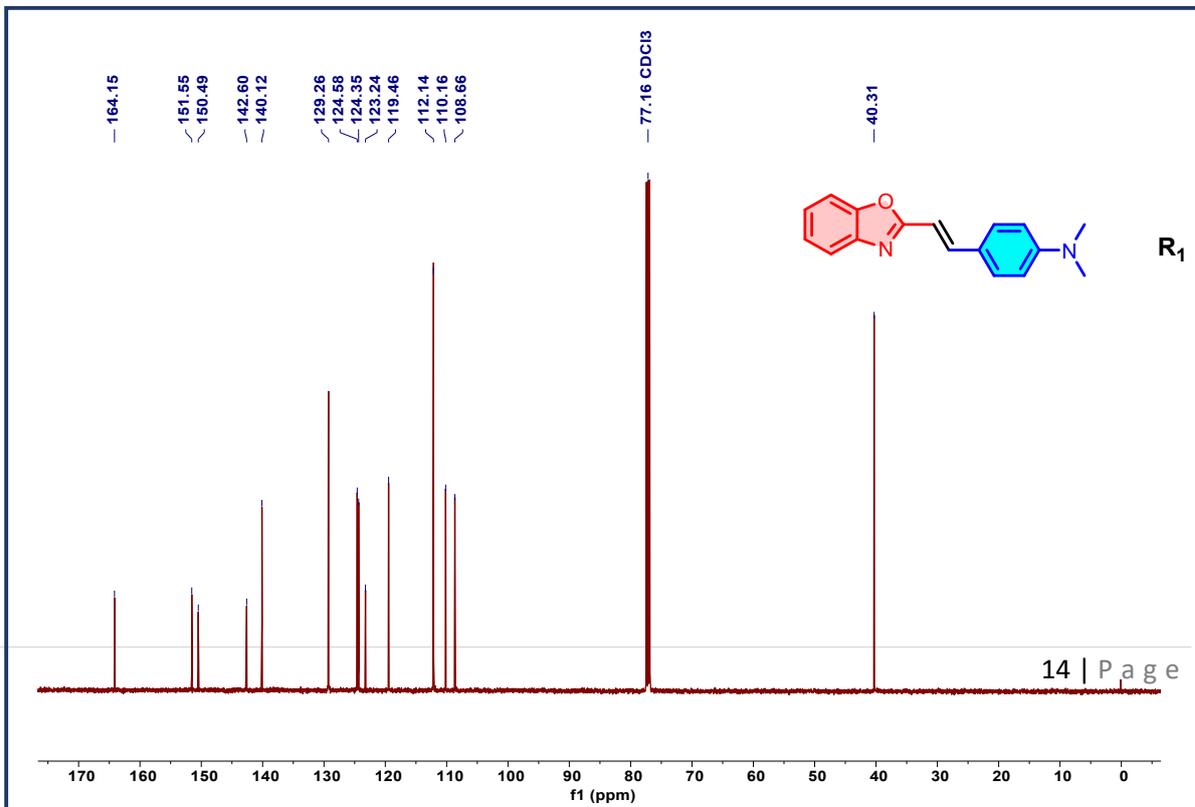
Fig S14: Plot of a logarithm of Emission intensity and logarithm of viscosity values obtained from the water-glycerol mixture for compound **R1** (a), **R4** (b), & **R8** (c)

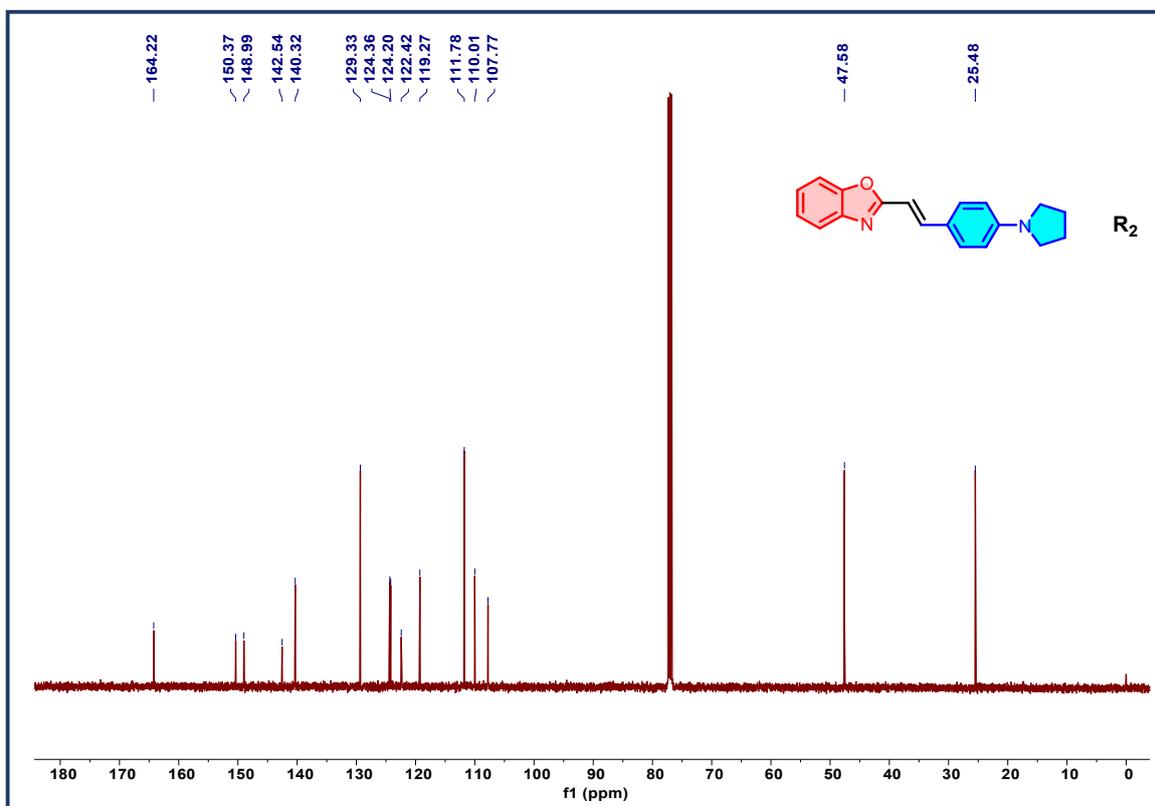
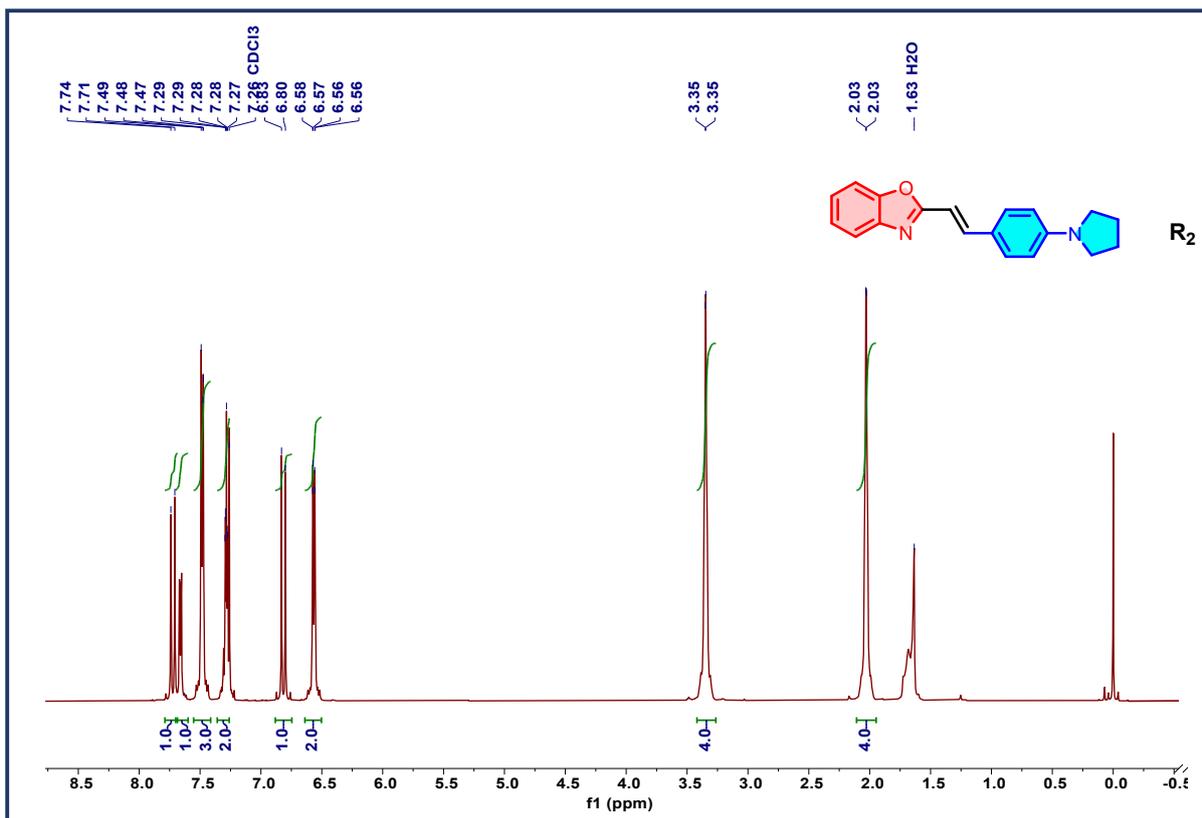
Characterization Spectral Details



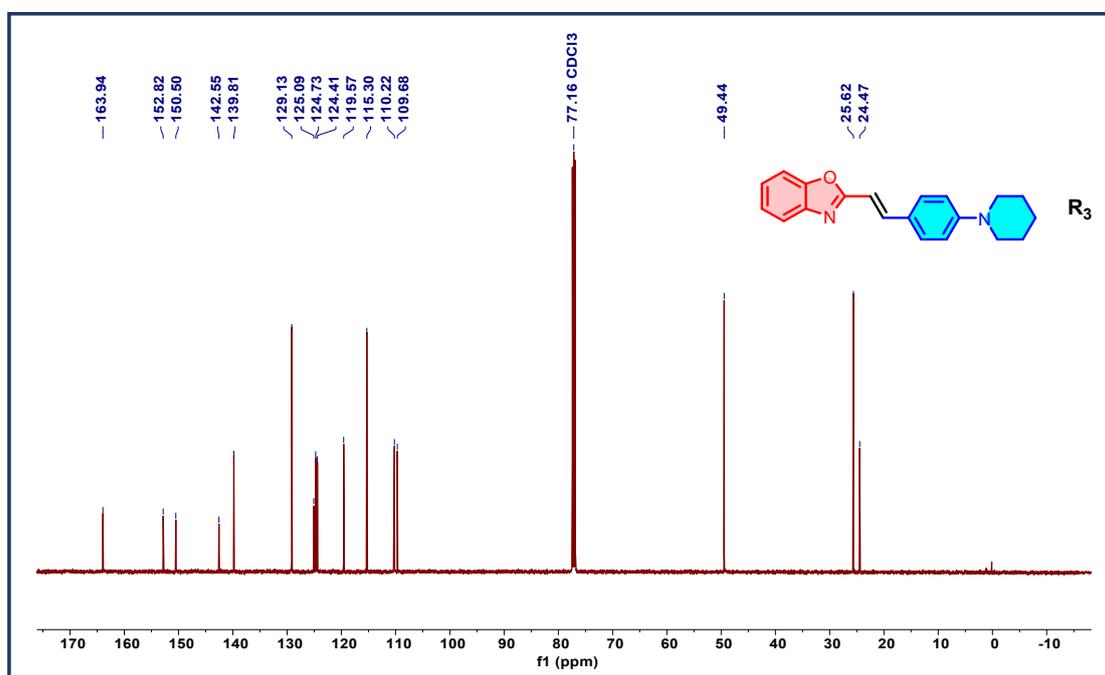
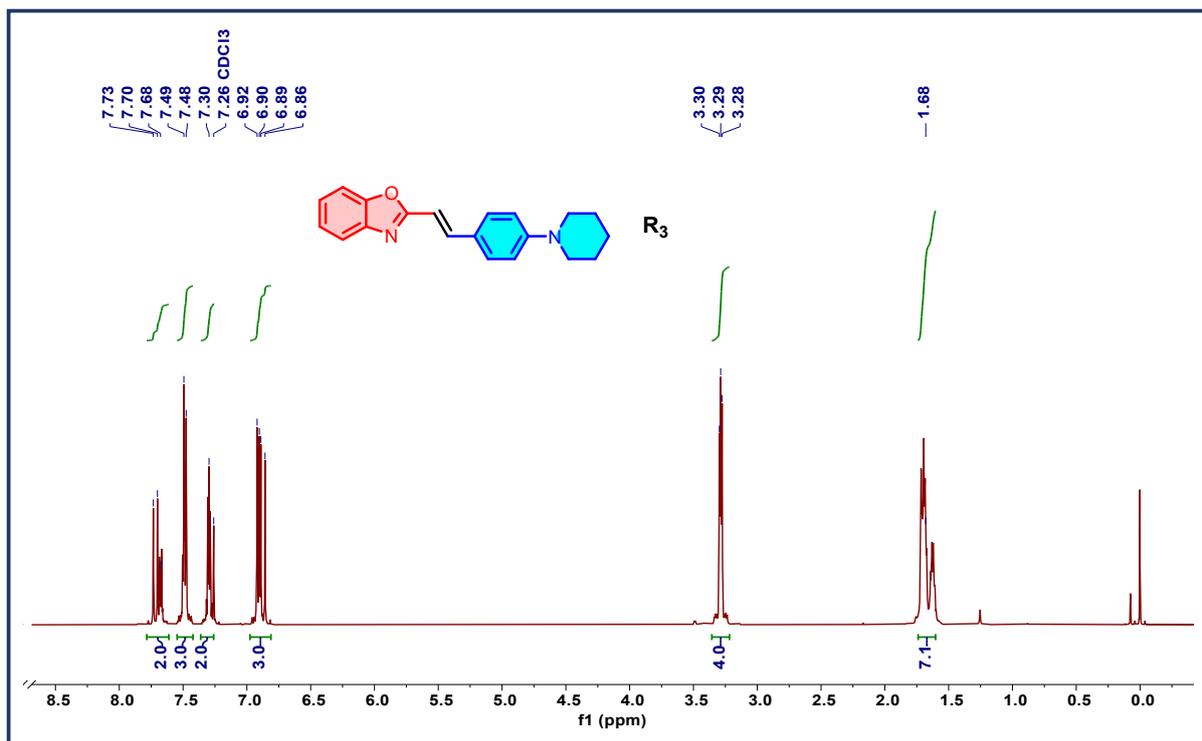
¹H/¹³C NMR Spectra of R₁

¹H/¹³C NMR Spectra of R₂

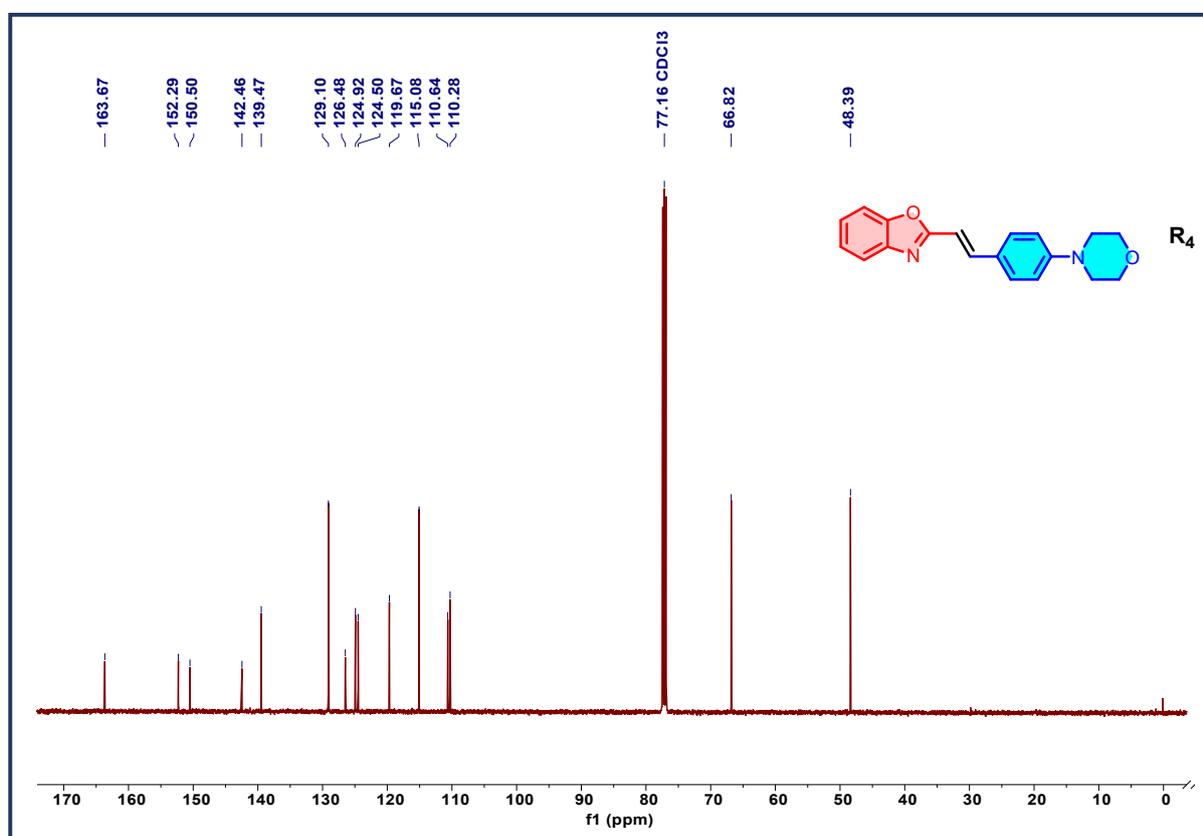
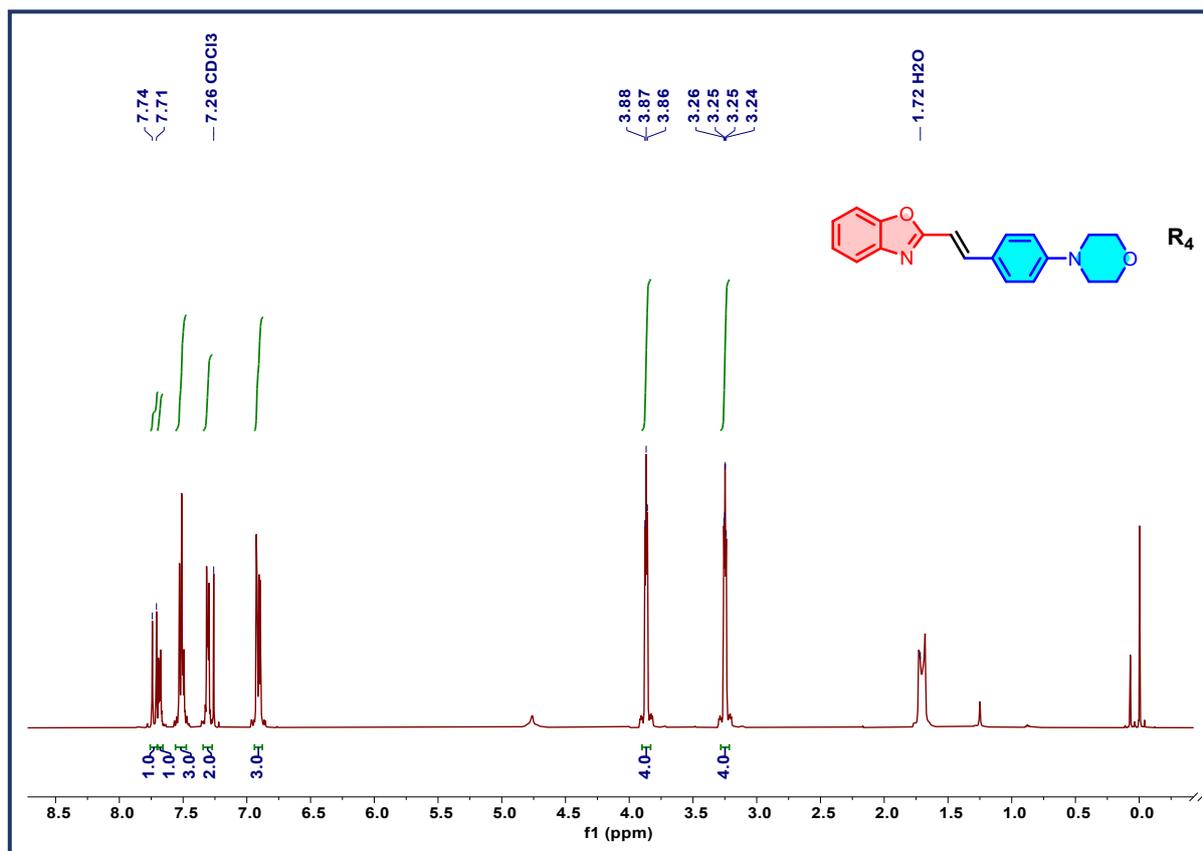


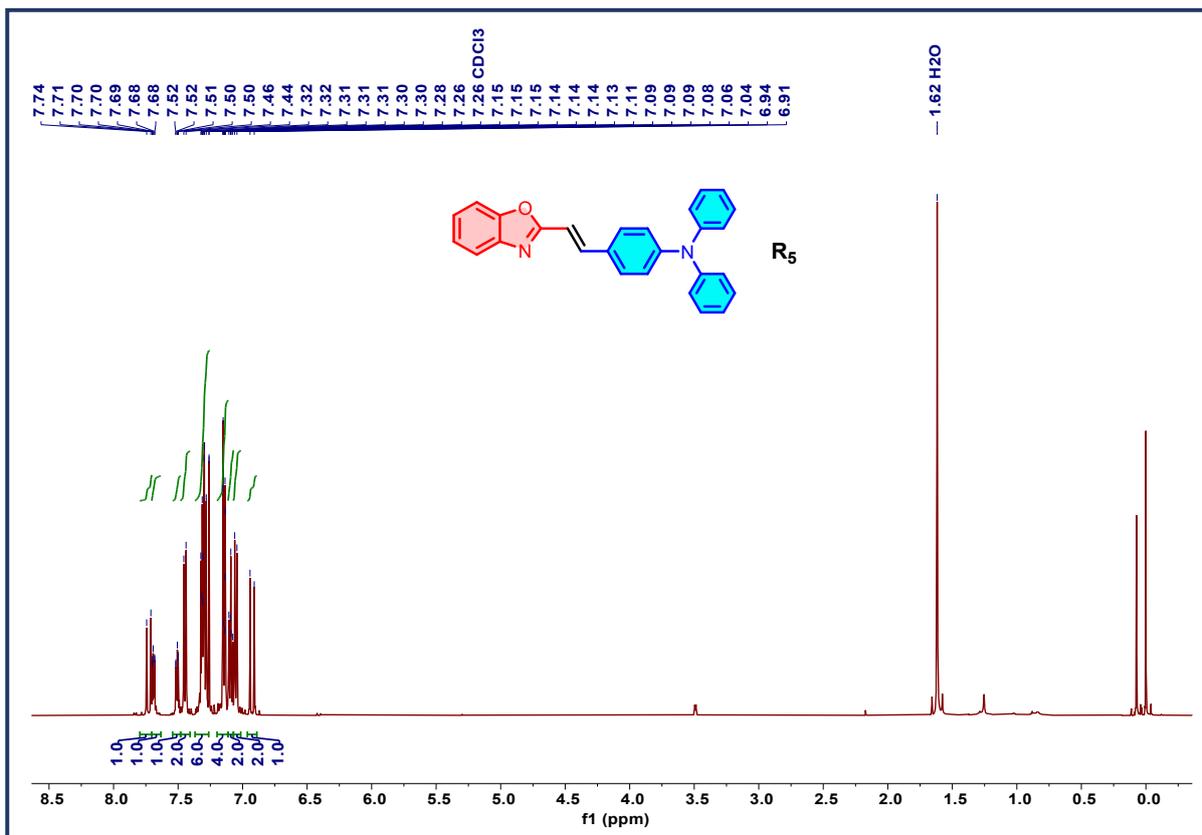


$^1\text{H}/^{13}\text{C}$ NMR Spectra of R3

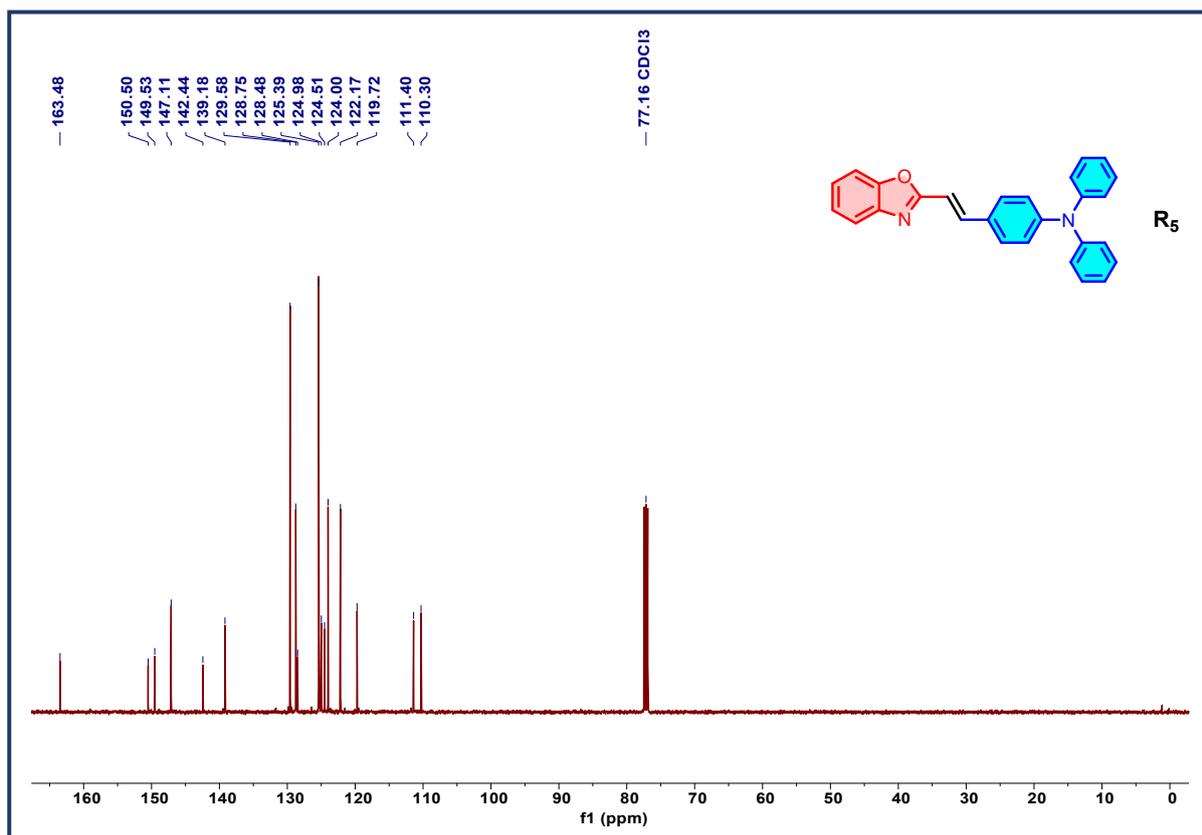


$^1\text{H}/^{13}\text{C}$ NMR Spectra of R4

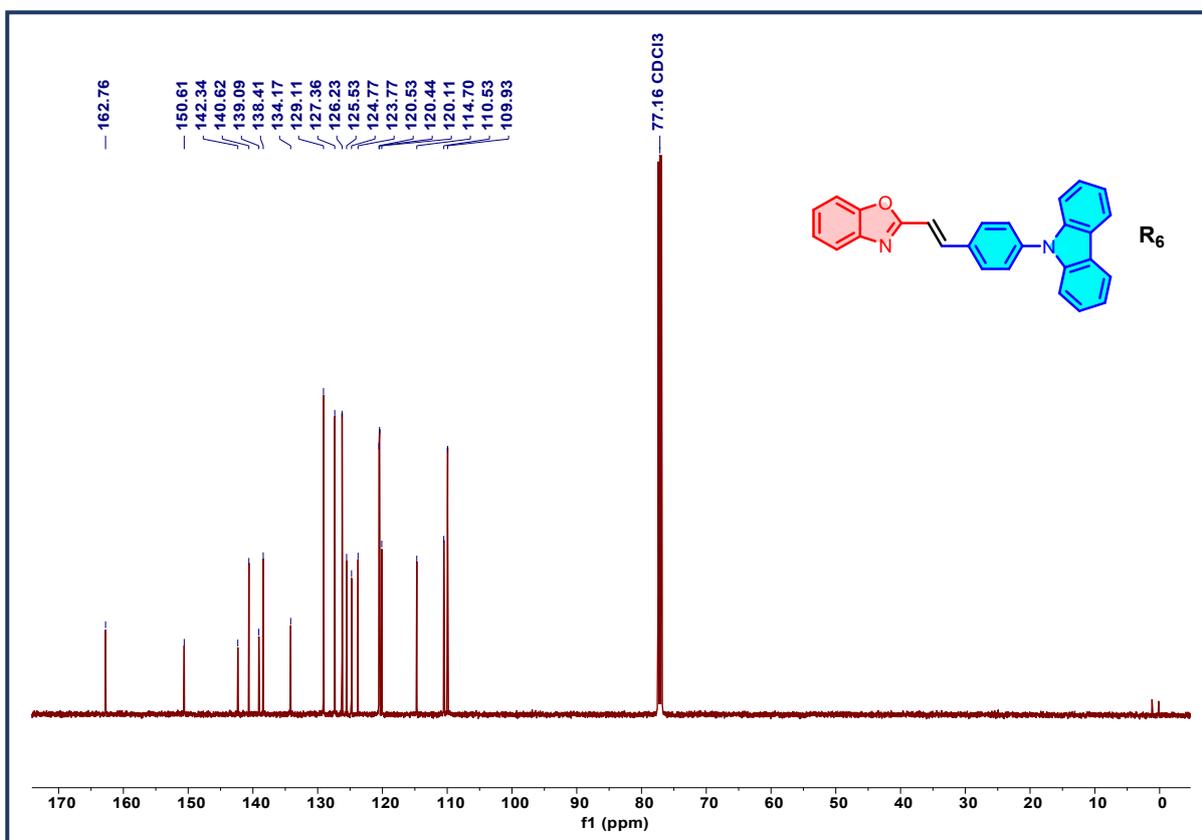
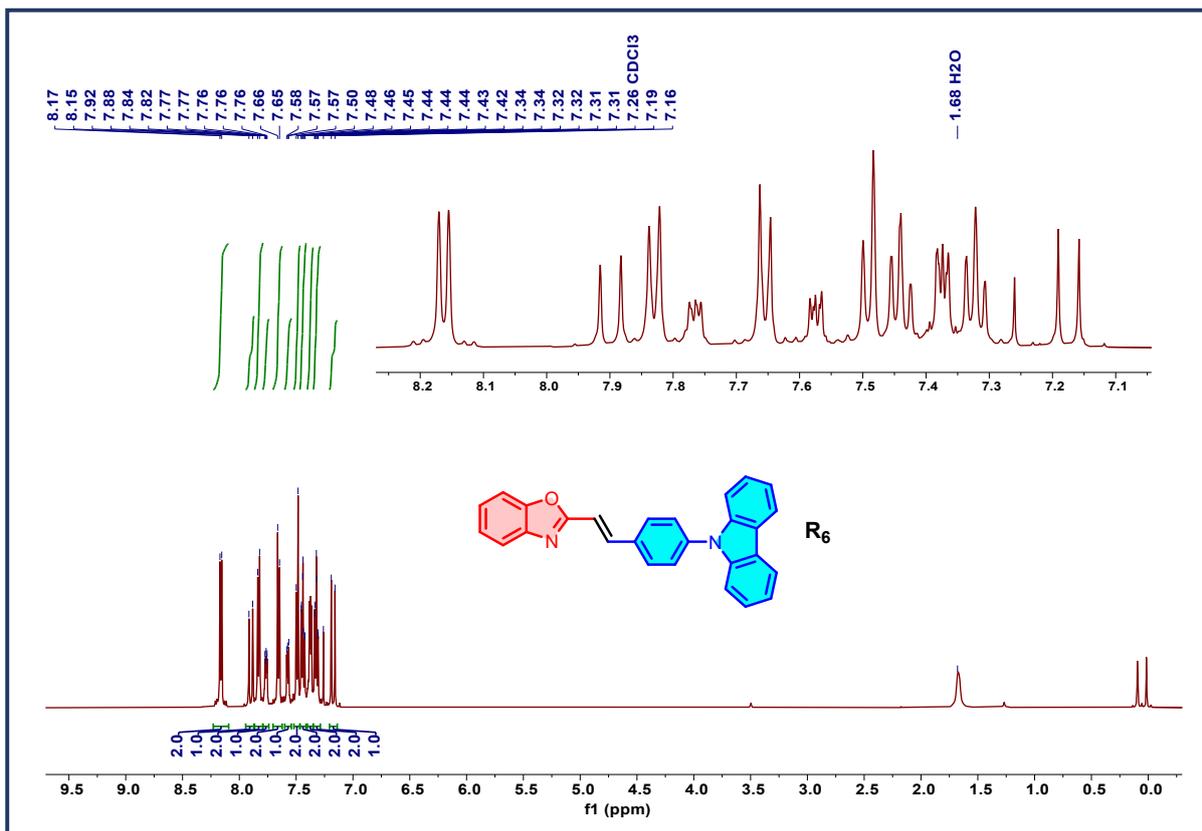




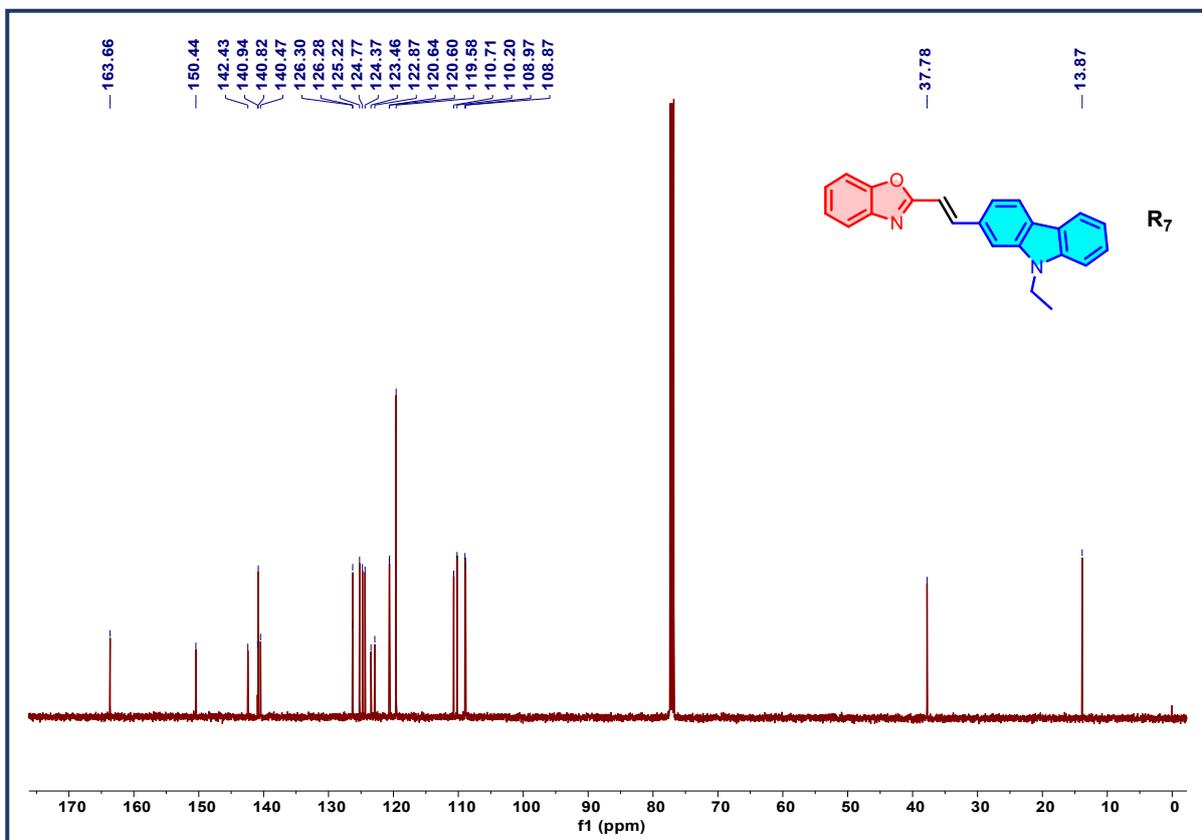
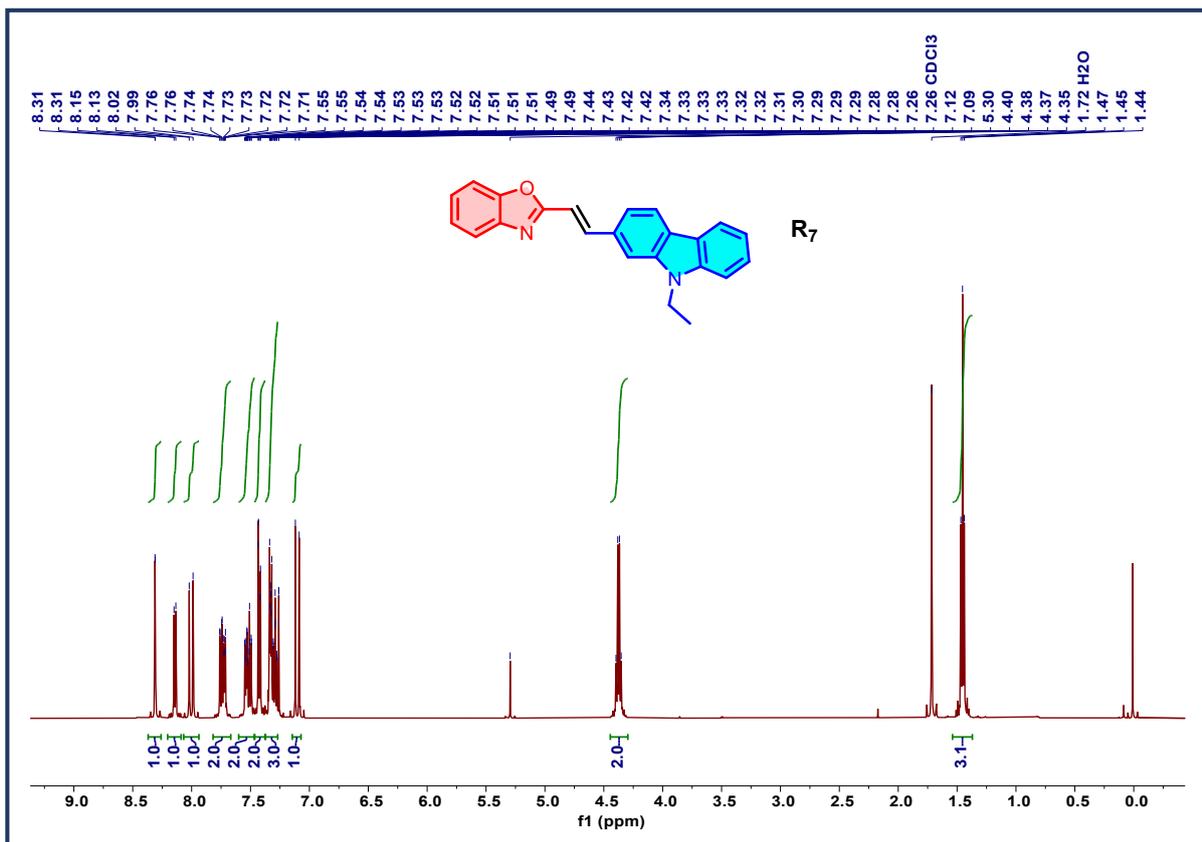
¹H/¹³C NMR Spectra of R5



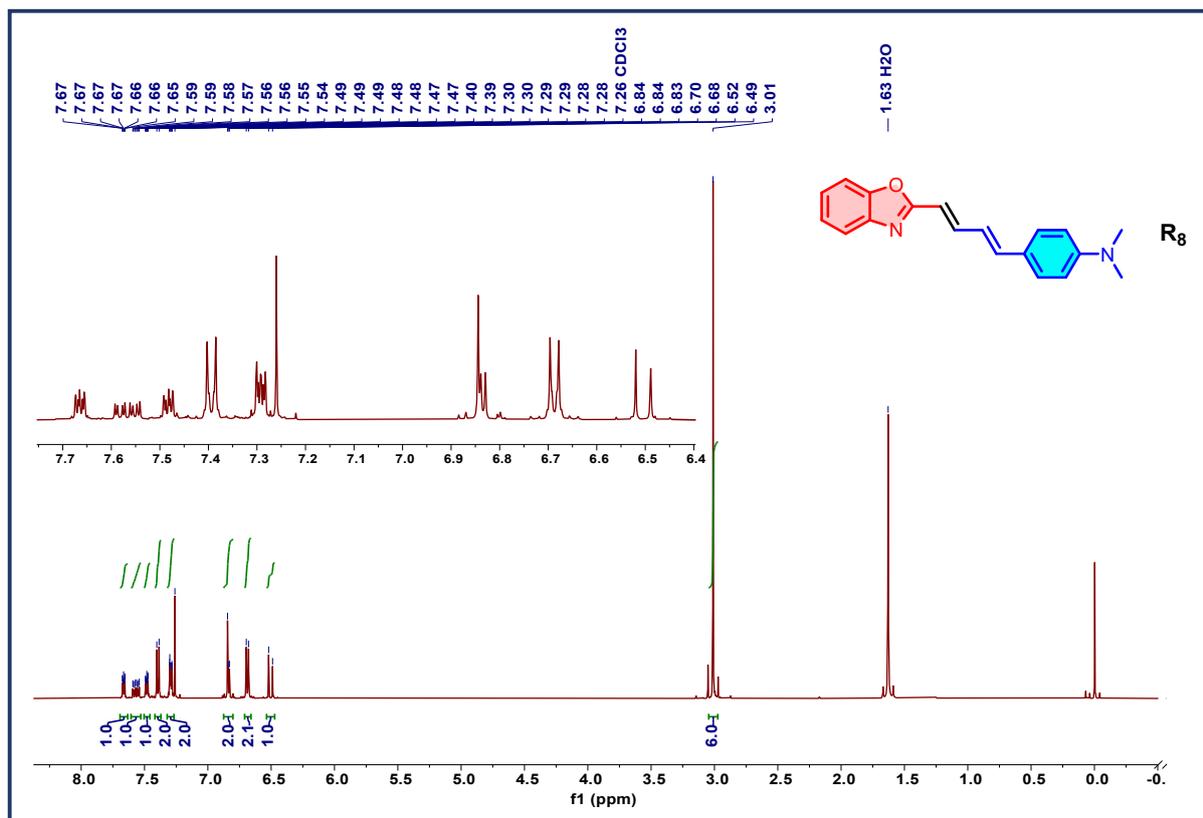
¹H/¹³C NMR Spectra of R6

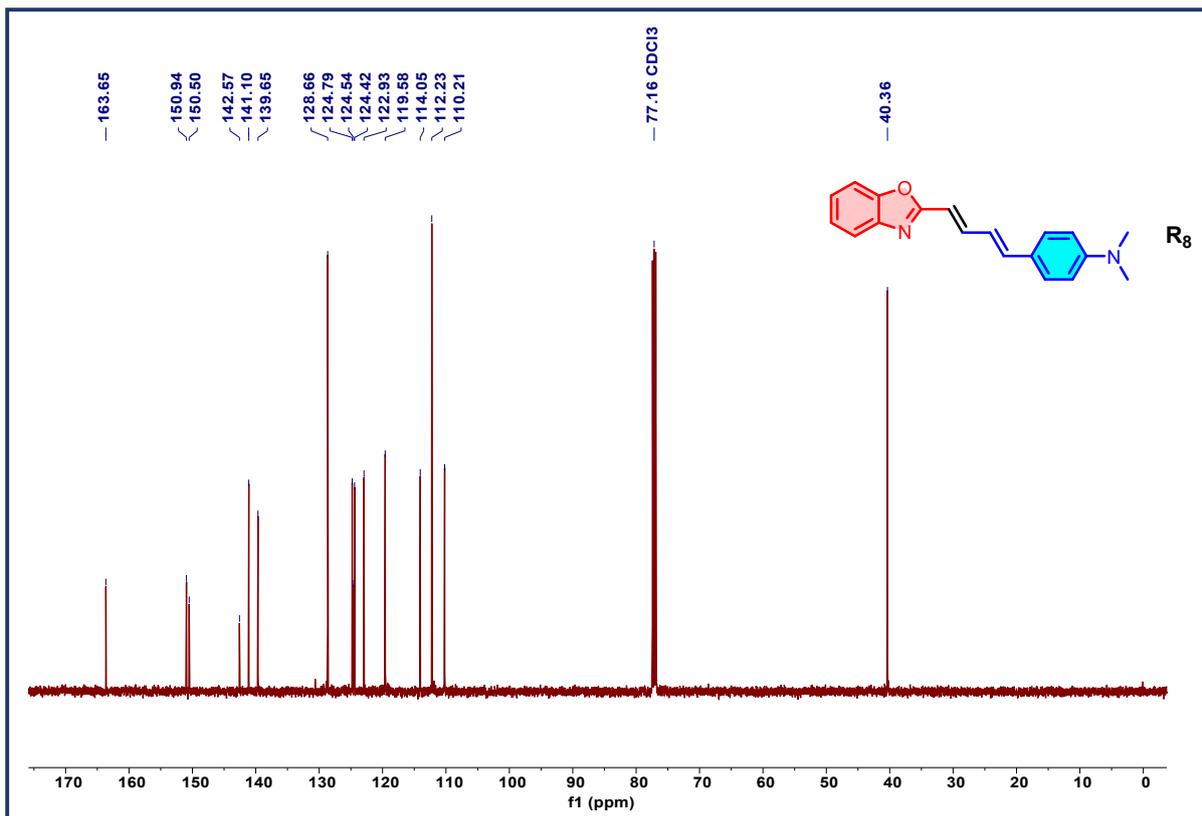


¹H/¹³C NMR Spectra of R7

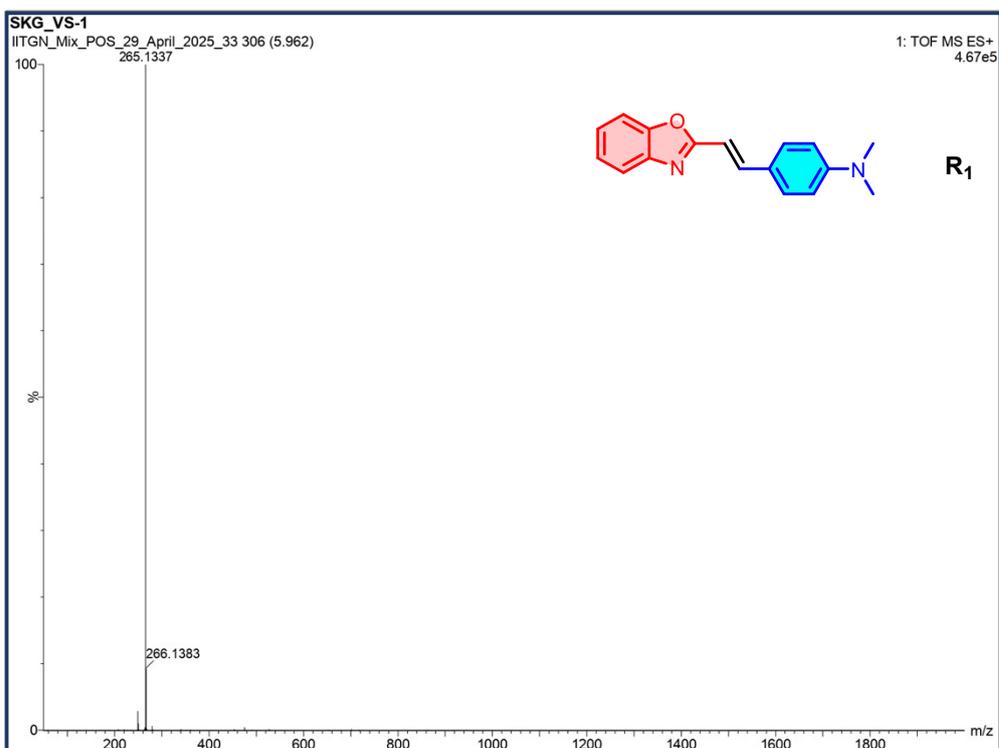


$^1\text{H}/^{13}\text{C}$ NMR Spectra of R8



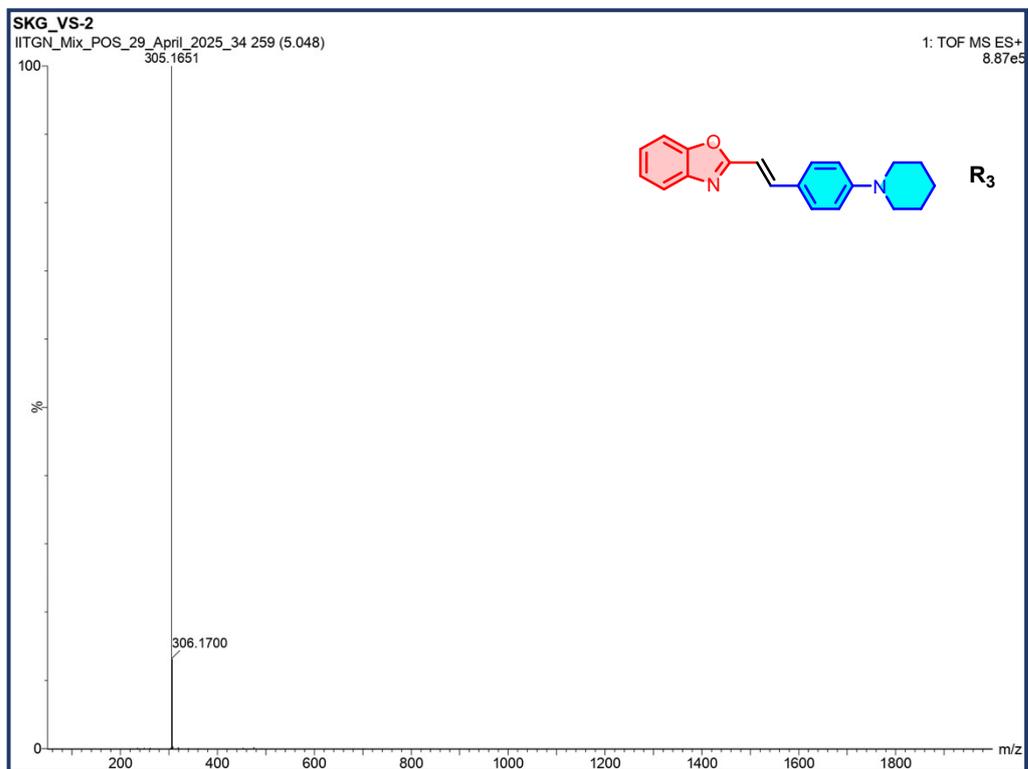


Mass Spectra



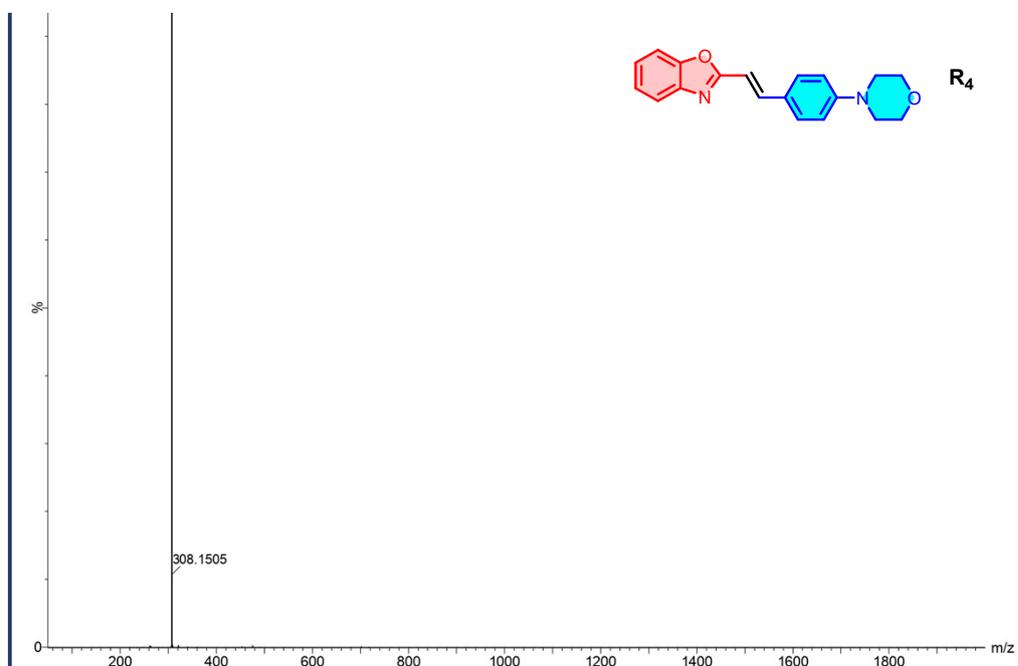
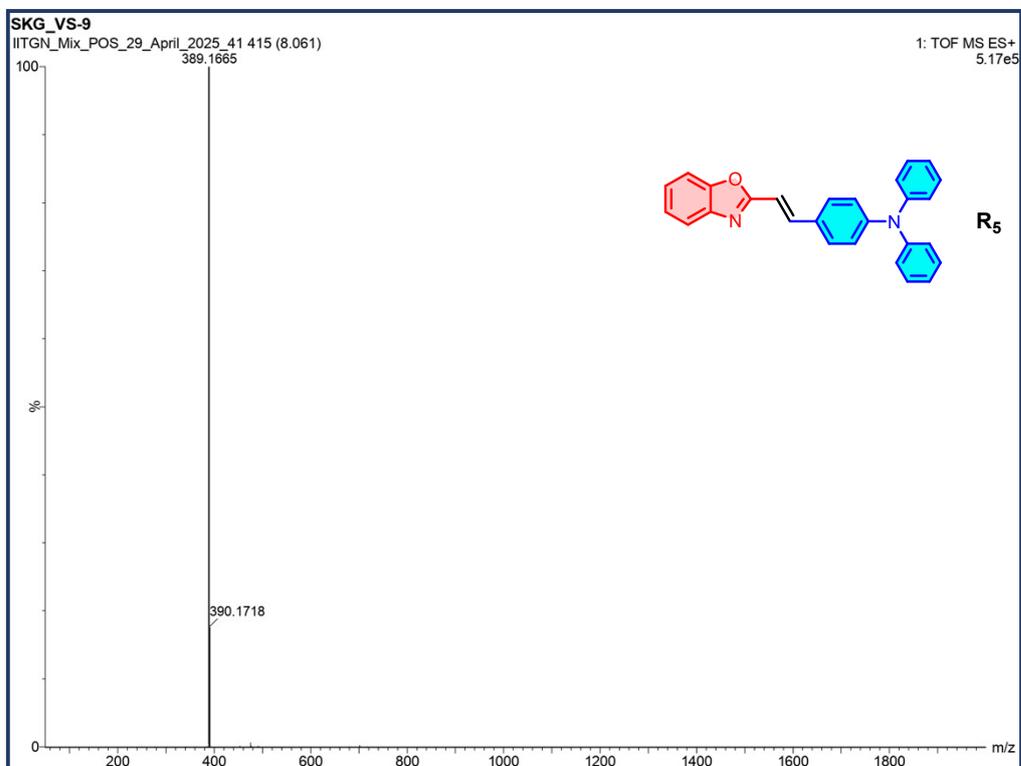
Mass Spectra of R1

Mass Spectra of R2



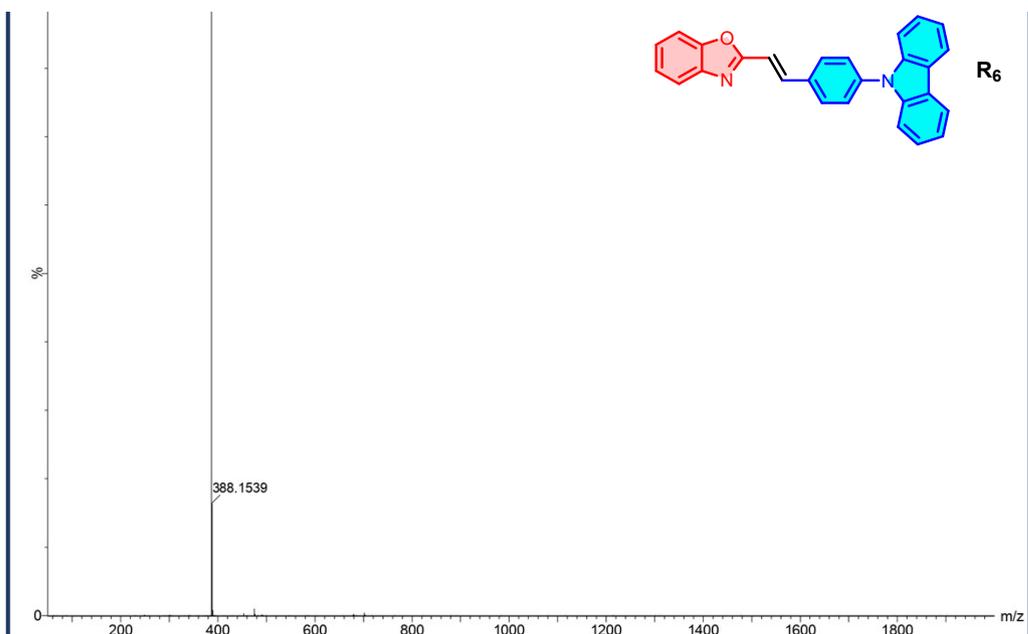
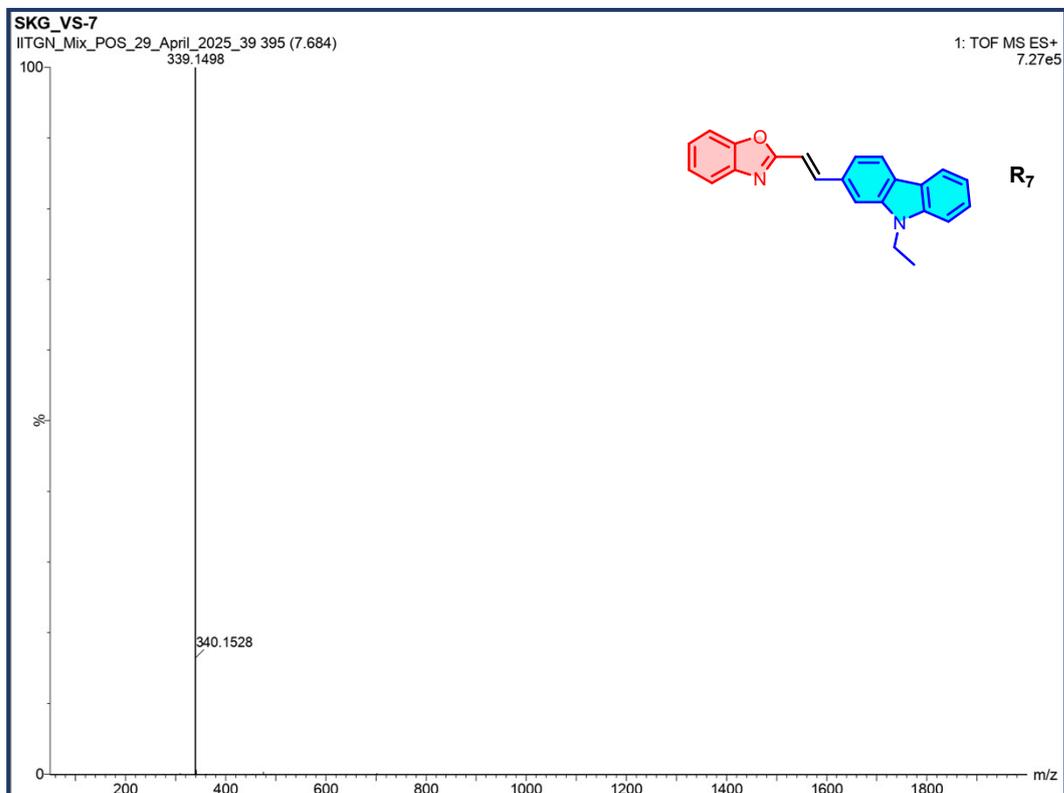
Mass Spectra of R3

Mass Spectra of R4



Mass Spectra of R5

Mass Spectra of R6



Mass Spectra of R7

Mass Spectra of R8

