

## Supplementary Information

# Flavin-based Probe for Real-Time Monitoring of Hypochlorous Acid Dynamics in Live Cells

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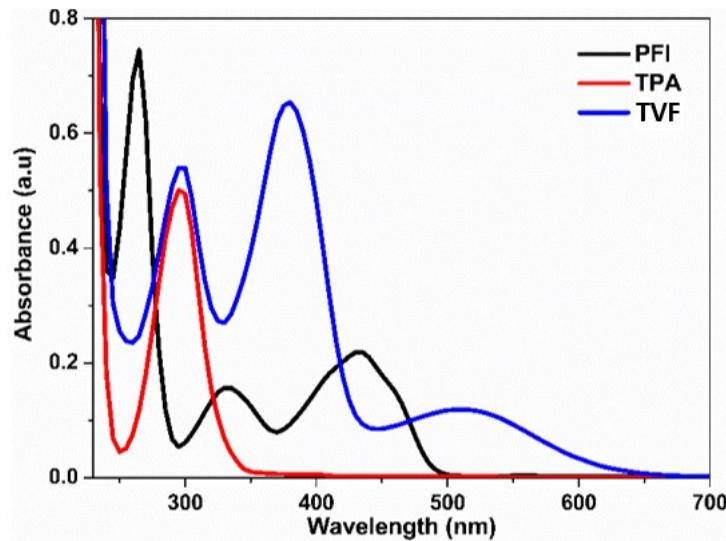
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## 1. Photophysical studies

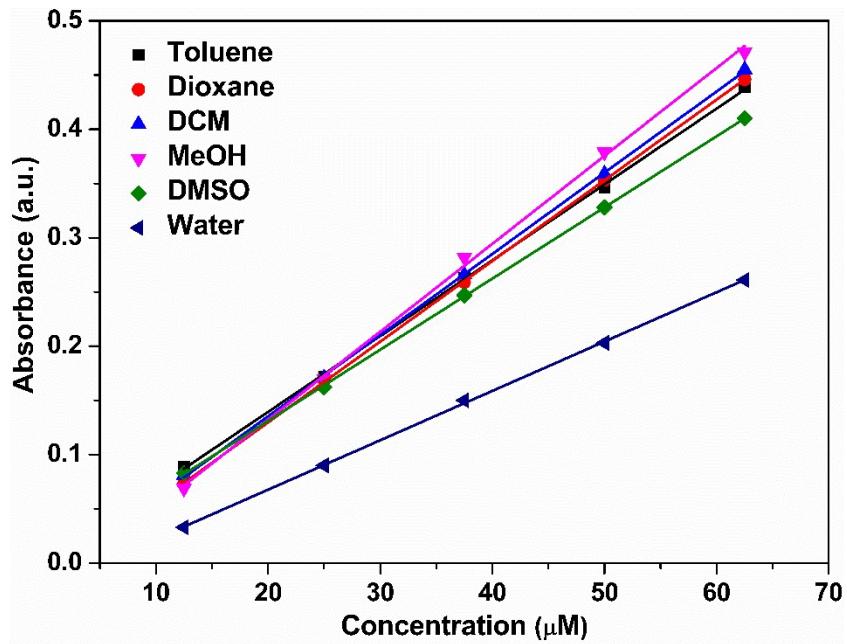


**Figure S1:** UV-visible spectra of Propyl flavin (PFI), TPA, and TPA-vinylene-Flavin (TVF) at 19  $\mu\text{M}$  concentration in methanol.

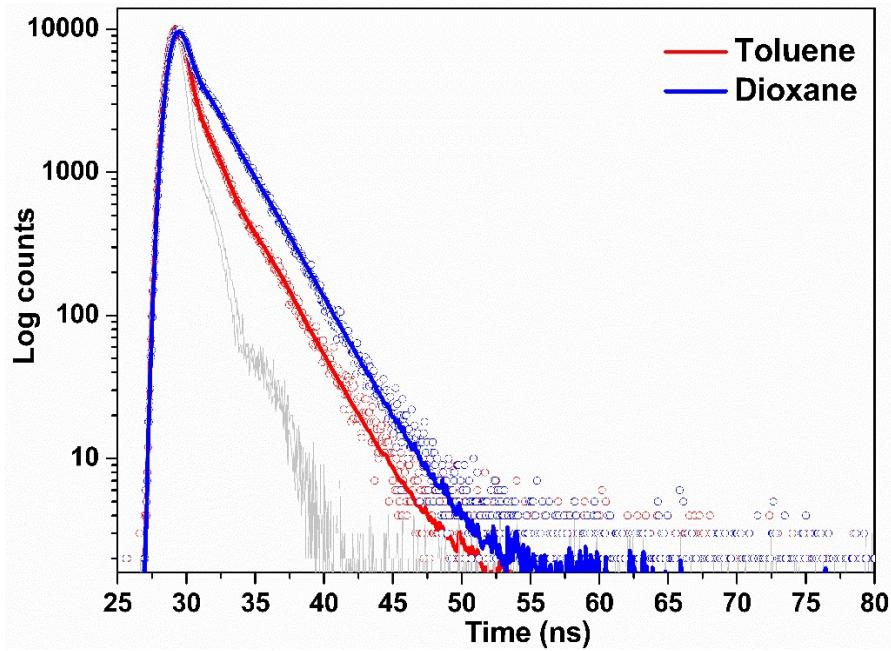
**Table S1.** Summary of photophysical properties of TVF in different polarities of solvent.

Solvents	$\lambda_{\text{abs}}^{\text{a}}$	$\lambda_{\text{em}}^{\text{a}}$	Stokes shift <sup>b</sup>	$\epsilon^{\text{c}}$	$\Phi_f^{\text{d}}$	$\tau^{\text{e}}$
Toluene	506	656	4519	6990	0.09	0.96
Dioxane	493	636	4561	7440	0.014	1.00
DCM	525	-	-	7490	-	-
Methanol	509	-	-	8100	-	-
DMSO	499	-	-	6560	-	-
Water	531	-	-	4550	-	-

<sup>a</sup>nm <sup>b</sup>cm<sup>-1</sup> <sup>c</sup>Molar extinction coefficient ( $\text{M}^{-1}\text{cm}^{-1}$ ) <sup>d</sup>Quantum yield (reference standard: Ru(bpy)<sup>3+</sup> in Acetonitrile  $\lambda_{\text{exc}} = 450$  nm and  $\Phi = 0.094$ ) <sup>e</sup>Average lifetime (ns).



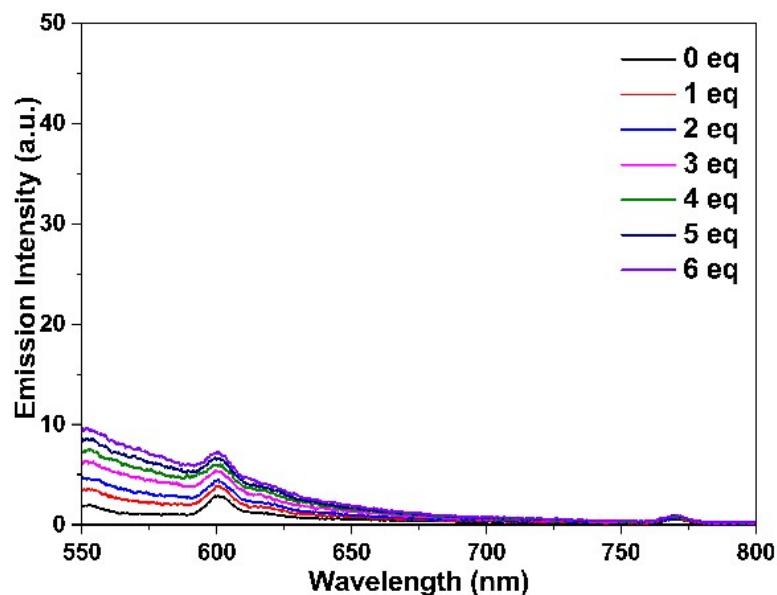
**Figure S2:** Calibration curve for measurement of the extinction coefficient of **TVF** in toluene, dioxane, DCM, Methanol, DMSO, and Water at each solvent absorption maxima.



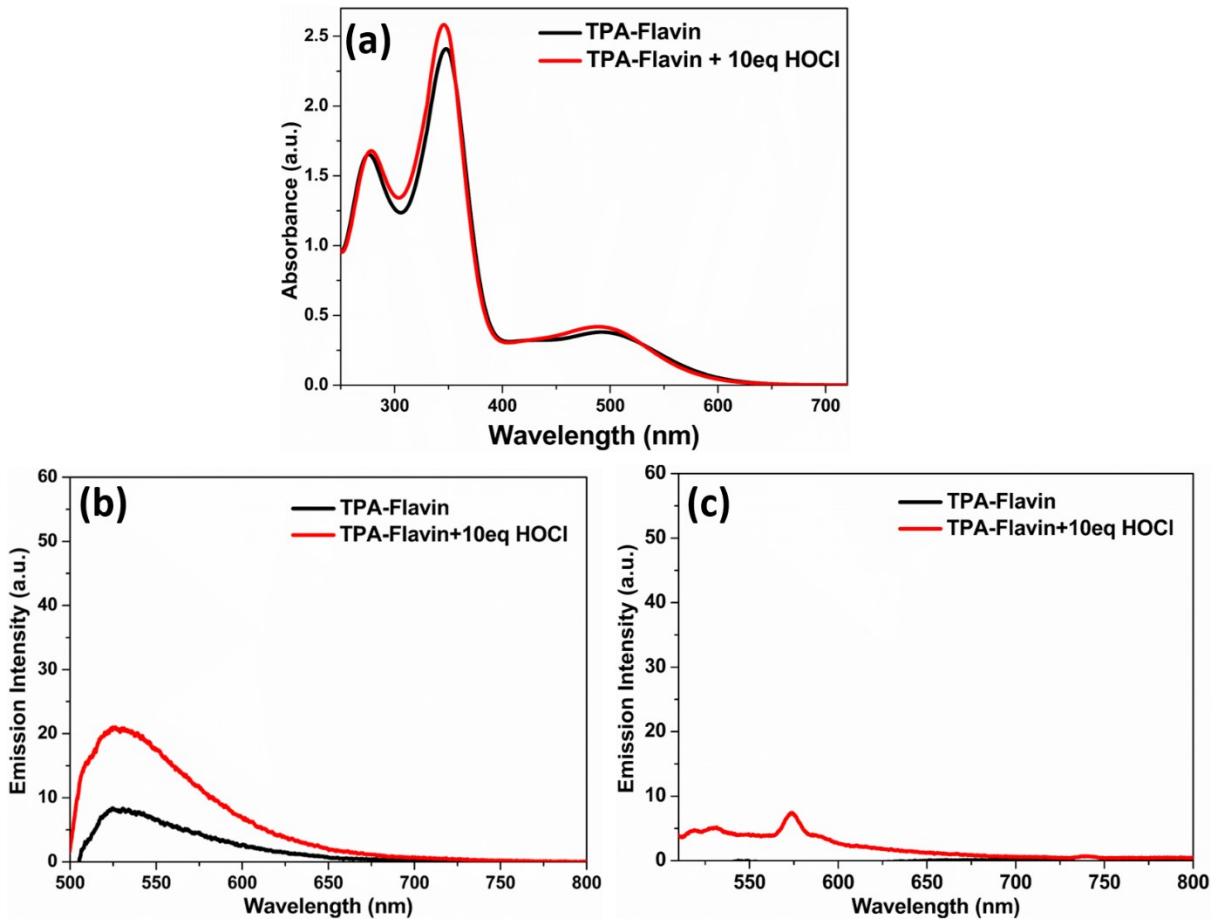
**Figure S3:** Fluorescence lifetime spectra of **TVF** (Bi-exponential decay) in (a) Toluene and (b) Dioxane under air.

**Table S2:** Fluorescence lifetime measurement parameter of TVF.

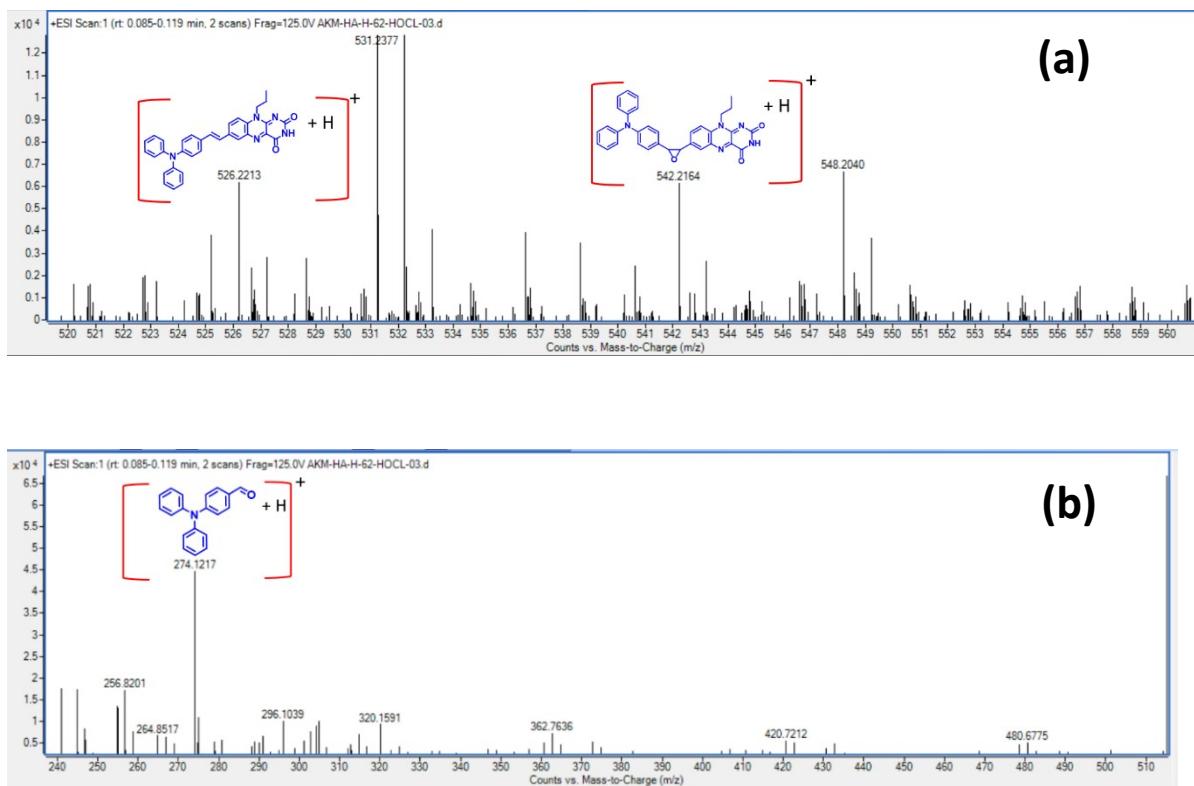
Solvent	T1 (ns)	T2 (ns)	B1	B2	Chi.sq.
Toluene	0.32	2.6	0.17	0.009	1.07
Dioxane	0.04	2.6	2.16	0.02	1.12



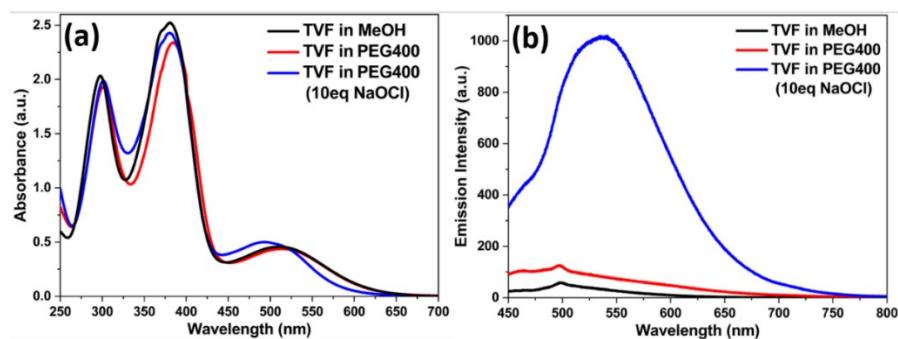
**Figure S4:** Fluorescence spectra of TVF (62  $\mu\text{M}$ ) with the addition of different concentrations of HOCl (0–6 eq) in methanol.  $\lambda_{\text{ex}} = 511 \text{ nm}$ .



**Figure S5:** (a) UV-Visible and (b),(c) Fluorescence spectra of TPA-Flavin ( $62 \mu\text{M}$ ) and TPA-Flavin incubated with 10 equivalents of HOCl in methanol. Fluorescence spectra were recorded with excitation at (b) 435 nm and (c) 491 nm.



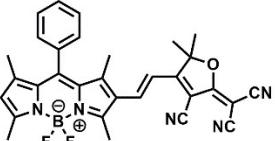
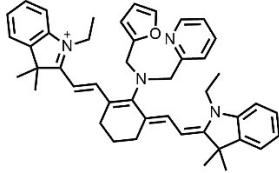
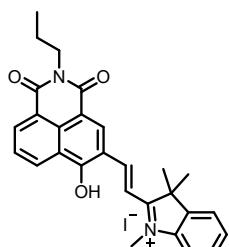
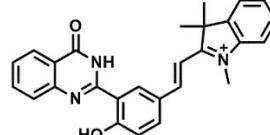
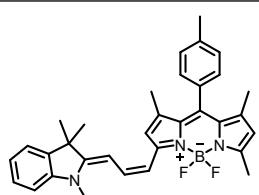
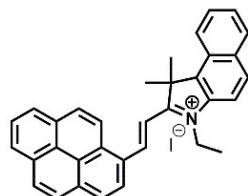
**Figure S6:** Mass spectrum peaks of the product of TVF and  $\text{ClO}^-$ . (a) and (b) is the different zoomed region of the mass spectrum



**Figure S7:** (a) UV-visible and (b) Fluorescence spectra of TVF recorded in different viscosities solvents such as MeOH, PEG400, and addition of HOCl (10 eq) in PEG400.

## 2. Table for the comparison of TVF with published probes for HOCl sensing

**Table S3:** The comparison of TVF with published probes for HOCl sensing.

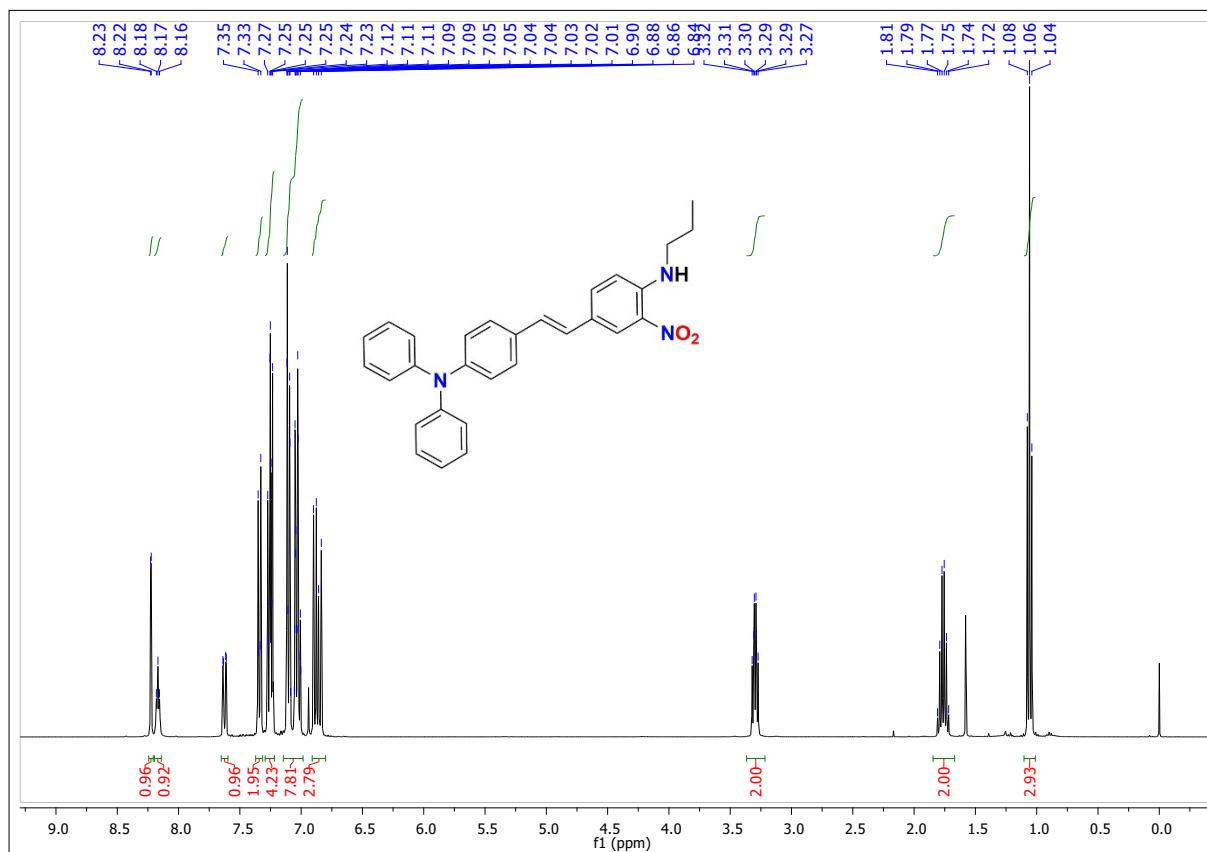
Probe	Emission wavelength	Target Localization	Response type	LOD (nM)	References
	520 nm	Non-specific	Ratiometric	500	1
	774 nm	Non-specific	ON-OFF	700	2
	515 nm	Non-specific	Ratiometric	53	3
	435 nM	Mitochondria	Ratiometric	27	4
	511 nm	Lysosomes	Ratiometric	10.6	5
	455 nm	Mitochondria	Ratiometric	182	6

	530 nm	Non-specific	OFF-ON	68	7
	435 nm and 525 nm	Mitochondria	OFF-ON	12	8
	490 nm	Non-specific	OFF-ON	190	9
	520 nm	Non-specific	OFF-ON	8.3	10
	535 nm	Non-specific	Ratiometric	738	11
	507 nm	Mitochondria	OFF-ON	360	This work

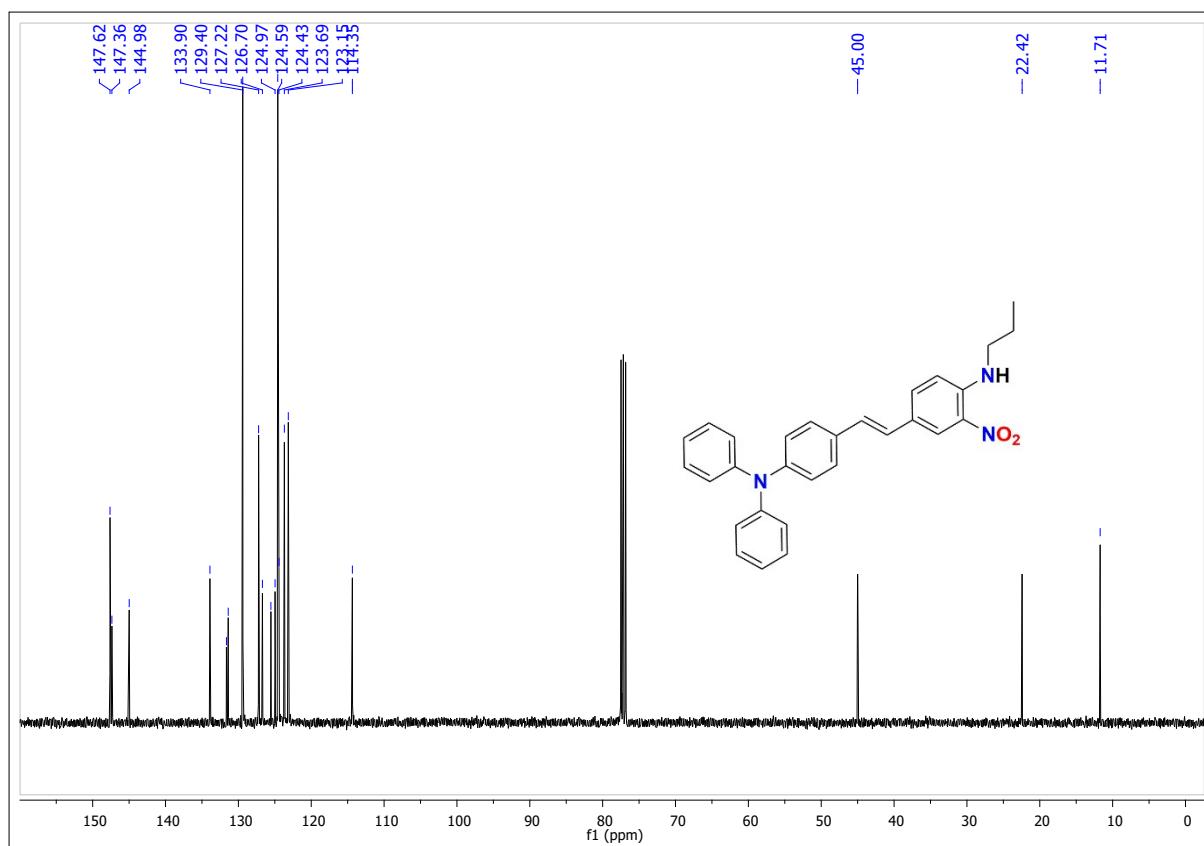
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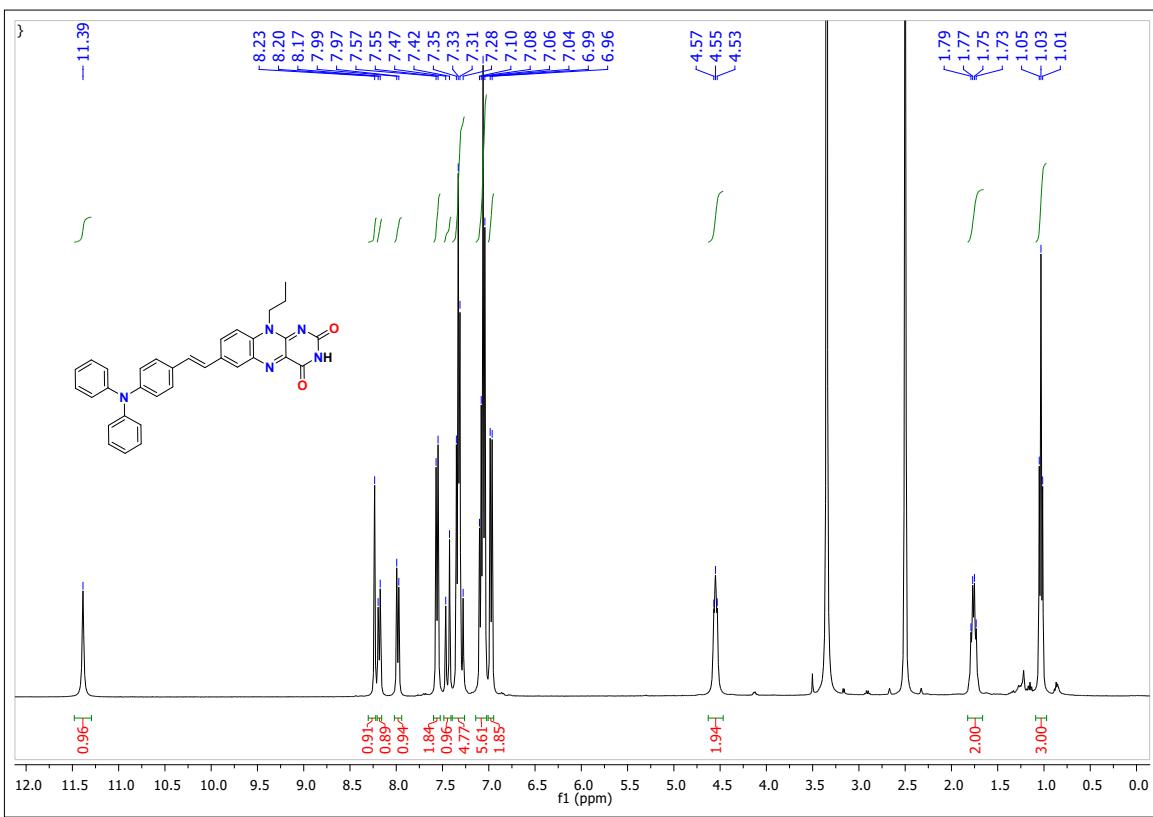
### 3. List of NMR

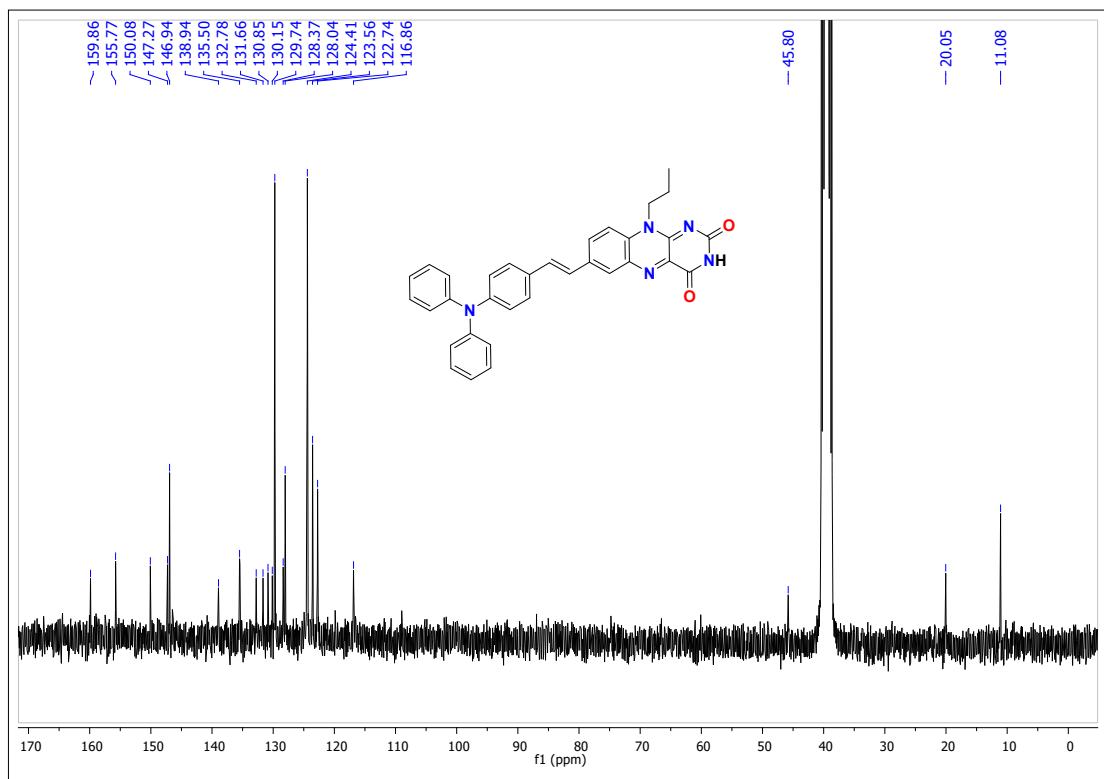


**Figure S8.** <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) of compound 3.



**Figure S9.**  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) of compound 3.





**Figure S11.**  $^{13}\text{C}$  NMR (75 MHz,  $\text{DMSO}-d_6$ ) of compound TVF.

#### 4. List of HRMS

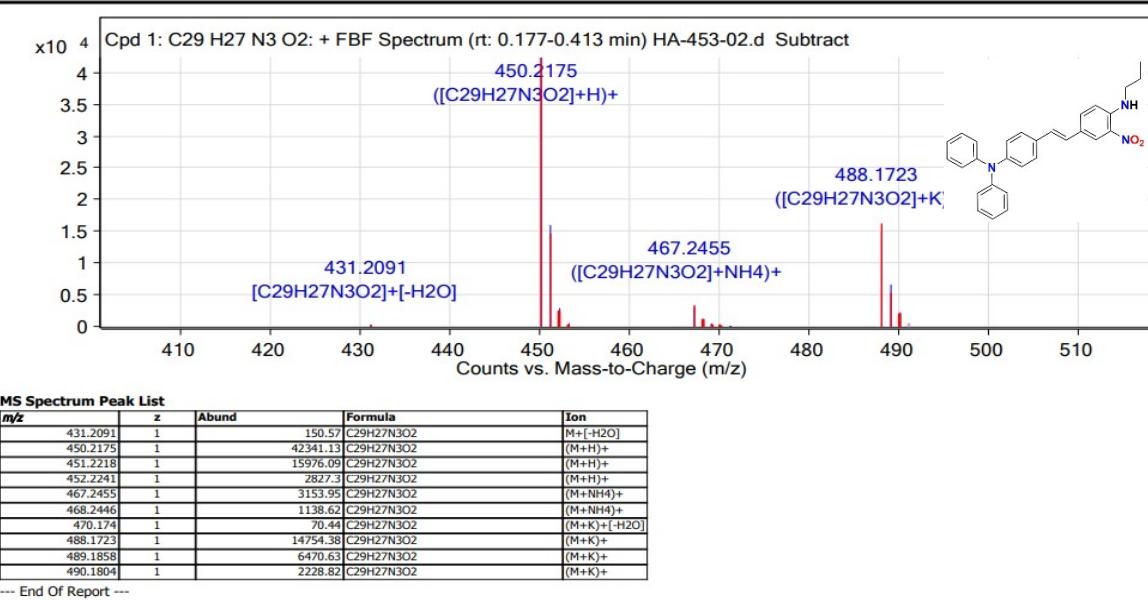


Figure S12. HRMS spectra of compound 3.

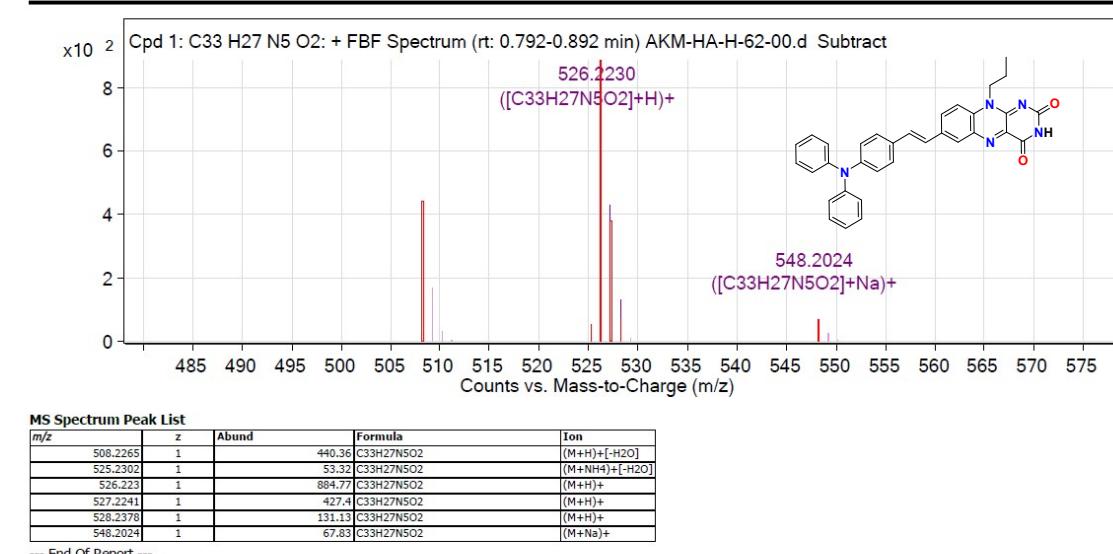


Figure S13. HRMS spectra of compound TVF.