Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information

For

A new metal-free benzorhodol-based photoluminophore selective for carbon monoxide detection applicable in both *in vitro* and *in vivo* bioimaging

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Content

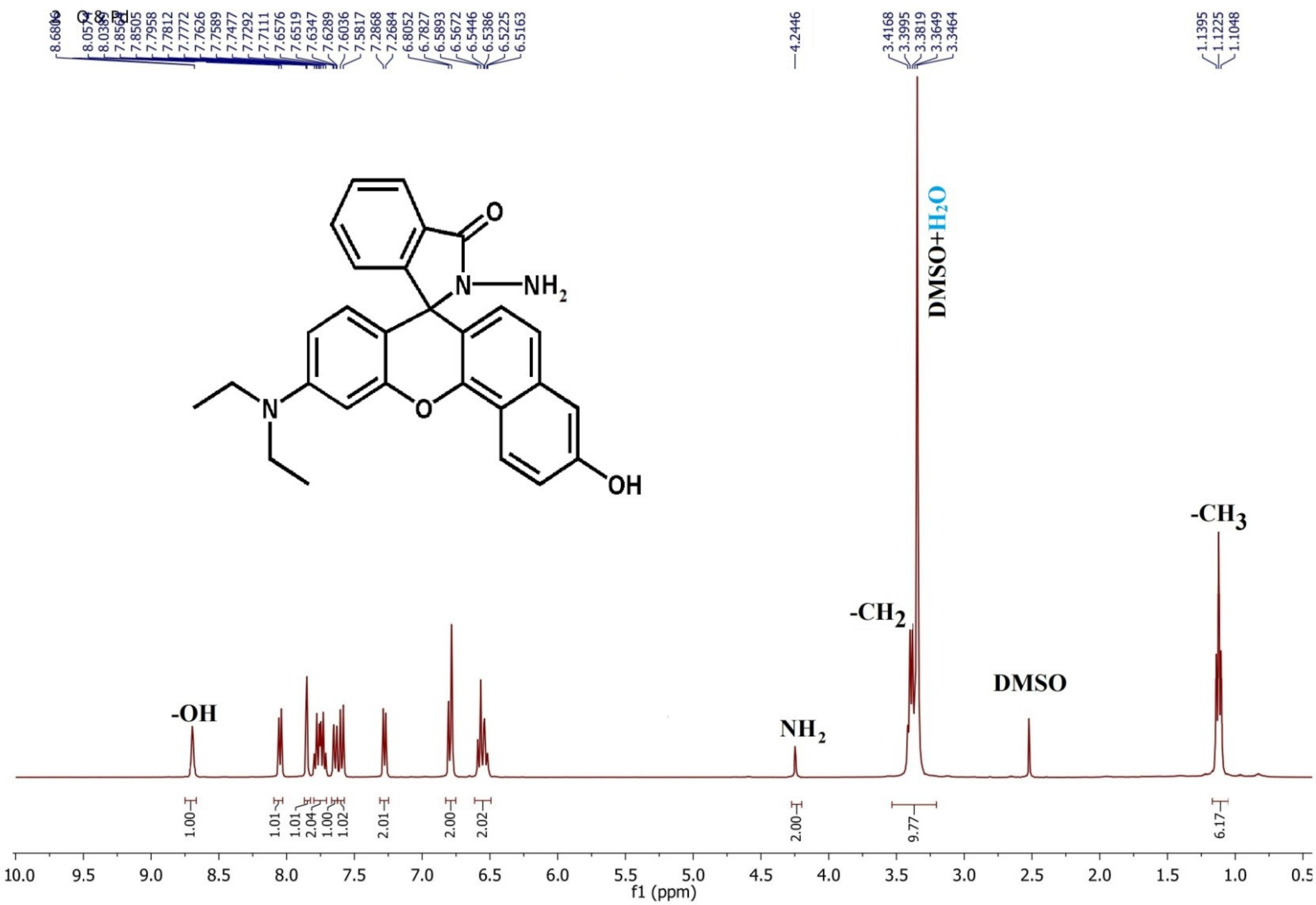


Fig. S1. ^{1}HNMR spectrum of **DEB-HY** in DMSO-d$_6$

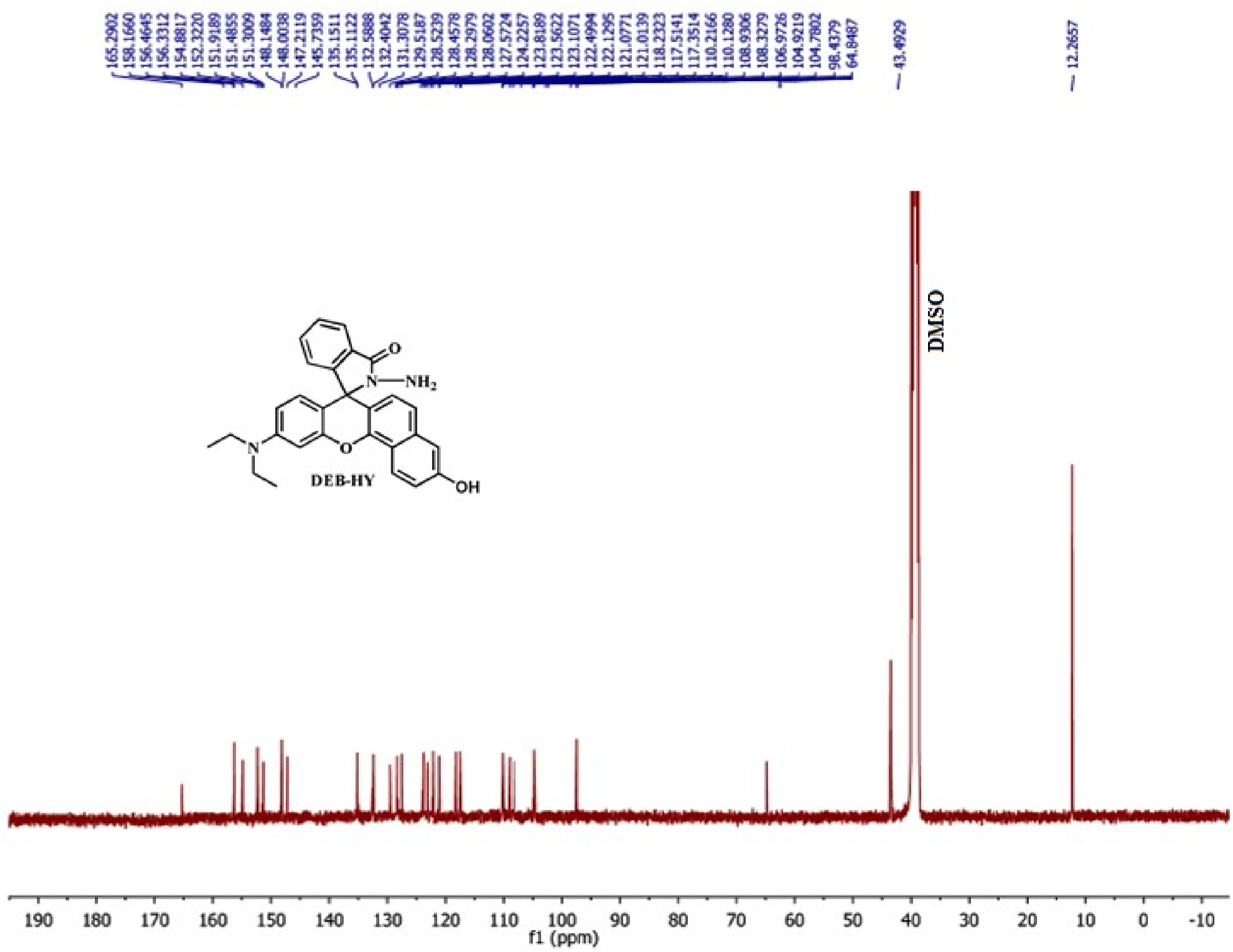


Fig. S2. ^{13}C NMR spectrum of **DEB-HY** in DMSO-d_6

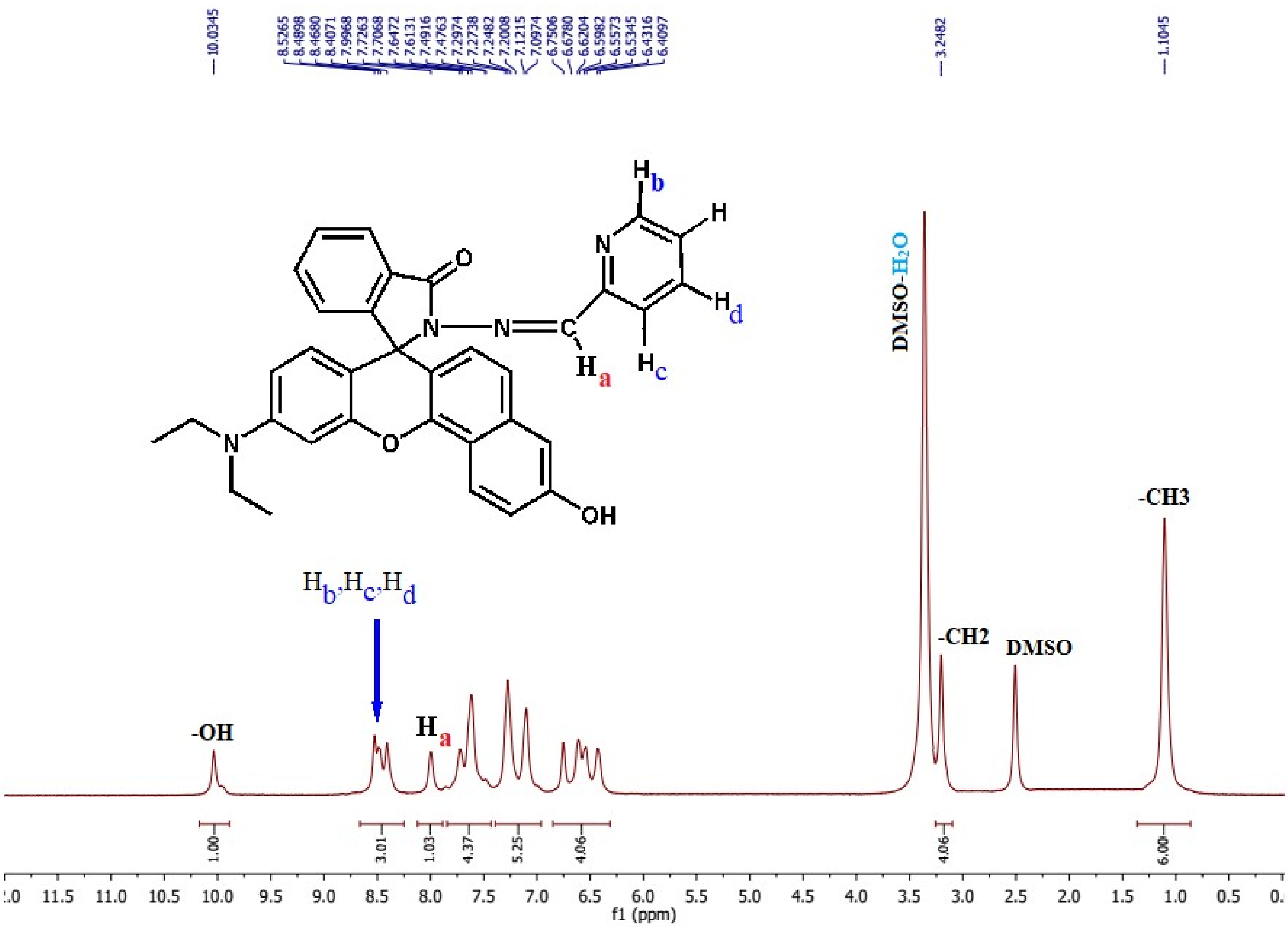


Fig. S3. ^{1}H NMR spectrum of **DEB-CO** in DMSO-d_6

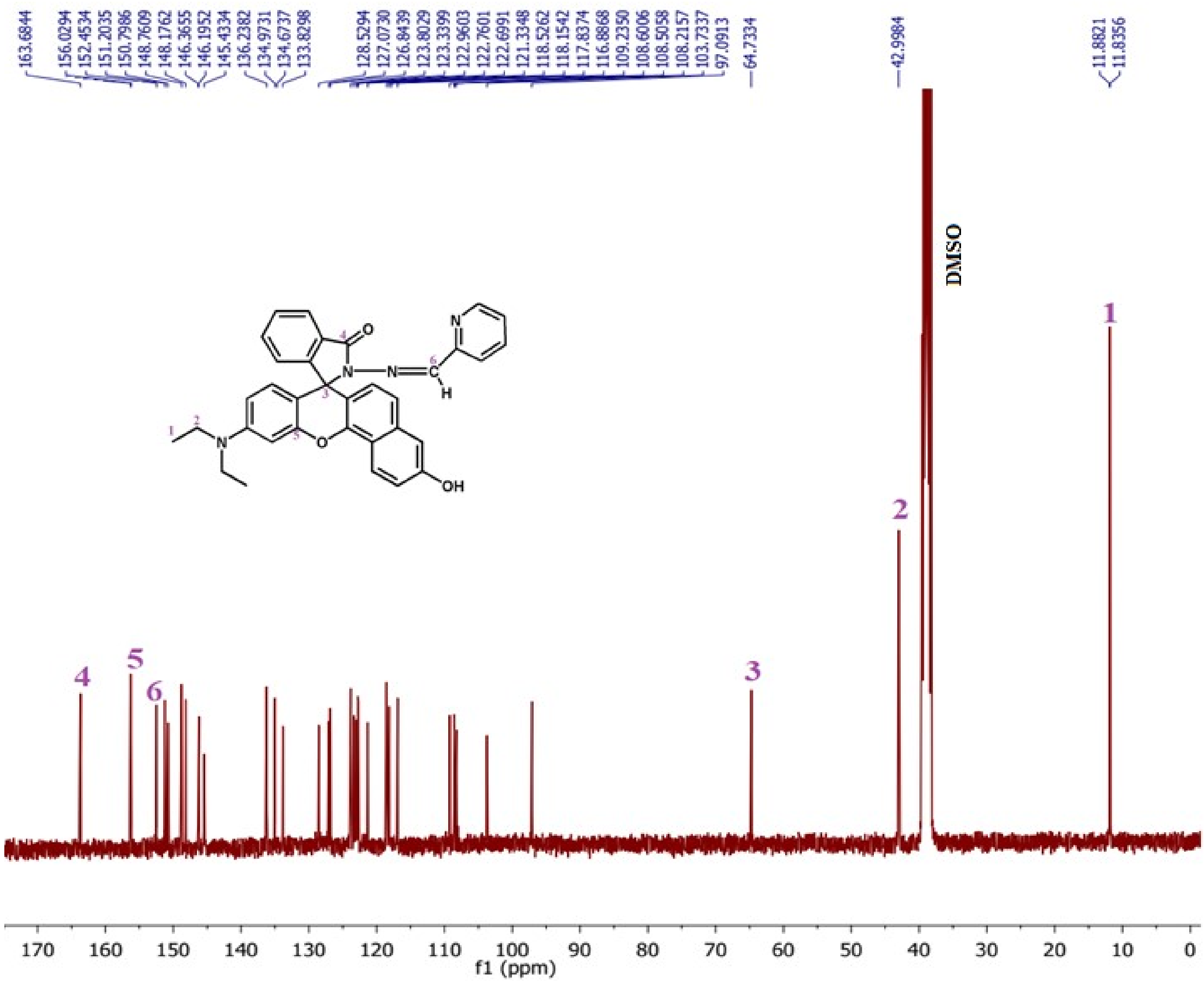
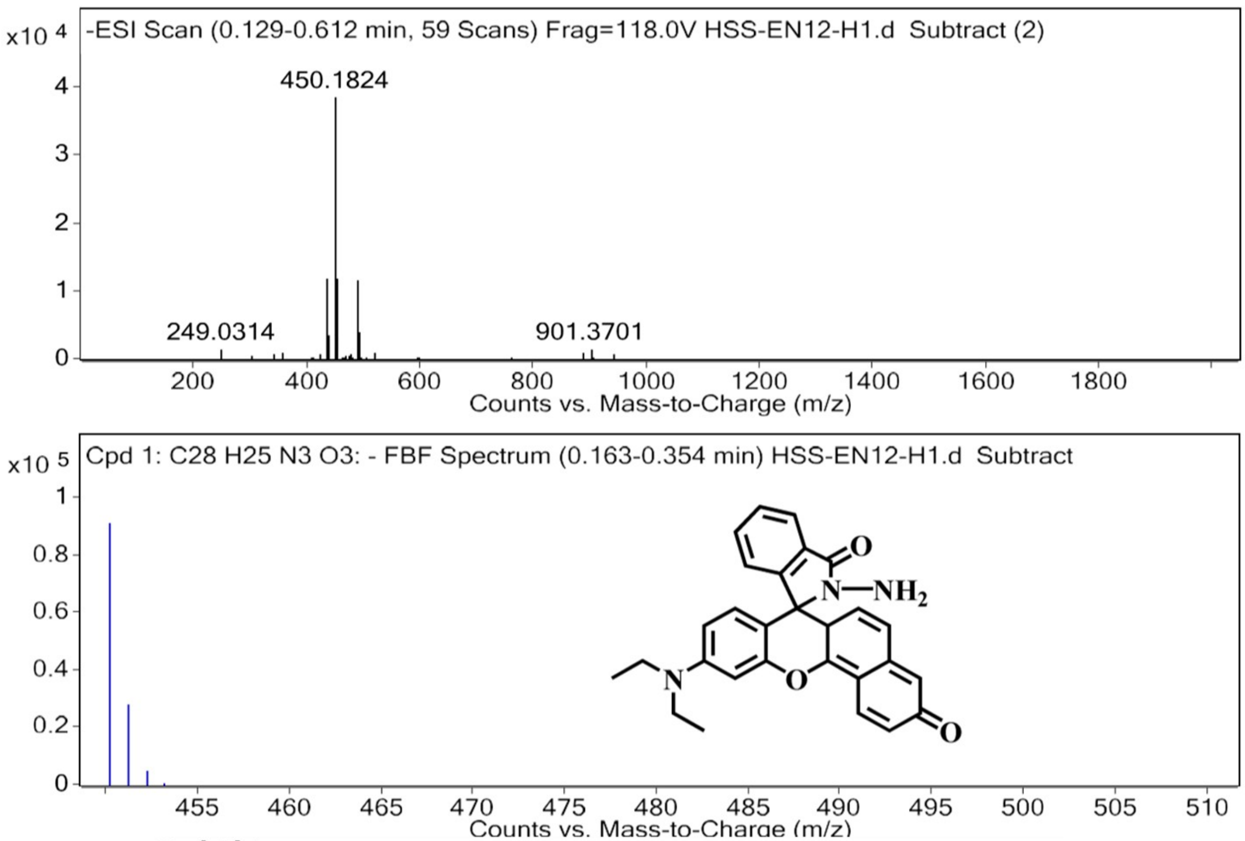


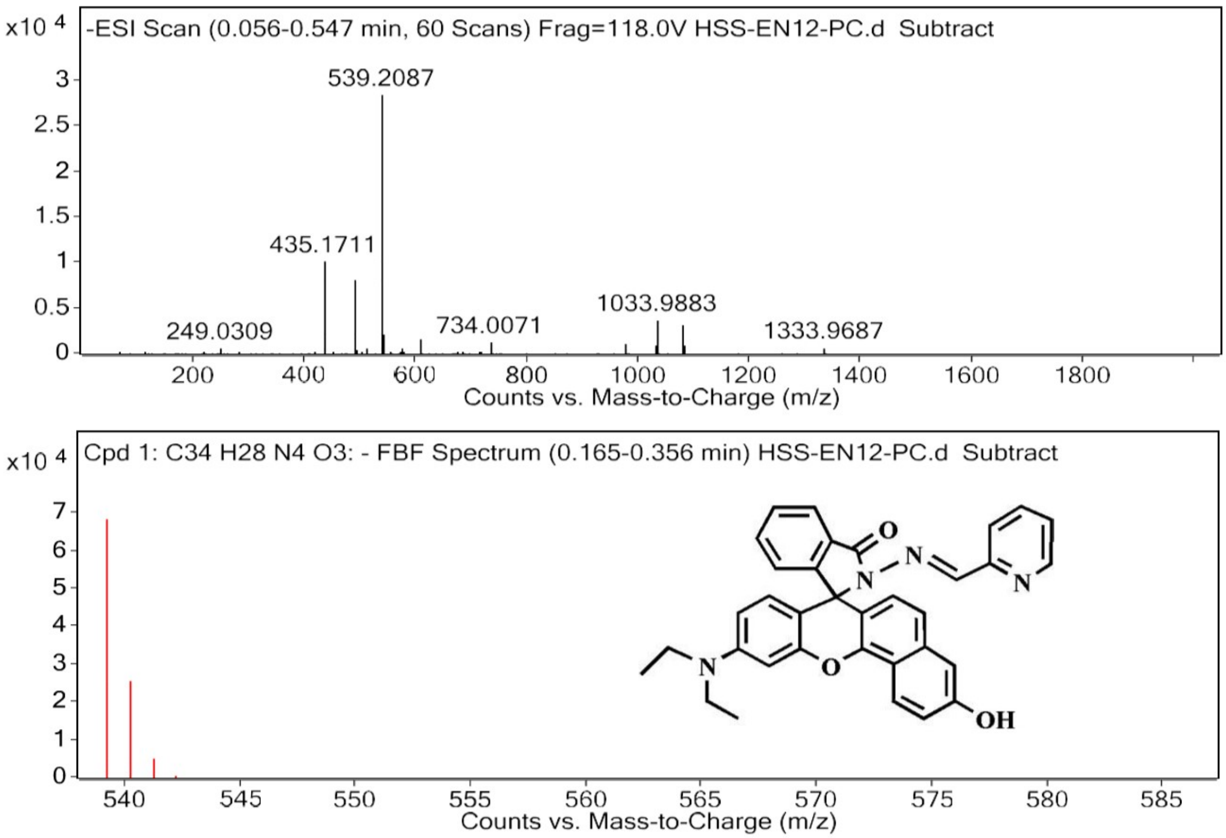
Fig. S4. ^{13}C NMR spectrum of **DEB-CO** in DMSO-d_6



Peak List

m/z	z	Abund	Formula	Ion
450.1825	1	91588.13	C28H24N3O3	(M-H)-
451.1851	1	28445.2	C28H24N3O3	(M-H)-
452.1874	1	5363.49	C28H24N3O3	(M-H)-
453.1872	1	890.68	C28H24N3O3	(M-H)-
454.185	1	140.05	C28H24N3O3	(M-H)-
510.2062	1	84.52	C30H28N3O5	(M+CH3COO)-

Fig. S5. ESI-Mass spectrum of **DEB-HY**



Peak List

m/z	z	Abund	Formula	Ion
539.2086	1	68522.02	C34H27N4O3	(M-H)-
540.2115	1	25849.61	C34H27N4O3	(M-H)-
541.2143	1	5344.32	C34H27N4O3	(M-H)-
542.2167	1	931.74	C34H27N4O3	(M-H)-
543.2052	1	68.61	C34H27N4O3	(M-H)-
585.2207	1	101.48	C35H29N4O5	(M+HCOO)-
586.208	1	23.83	C35H29N4O5	(M+HCOO)-

Fig. S6. ESI-Mass spectrum of **DEB-CO**

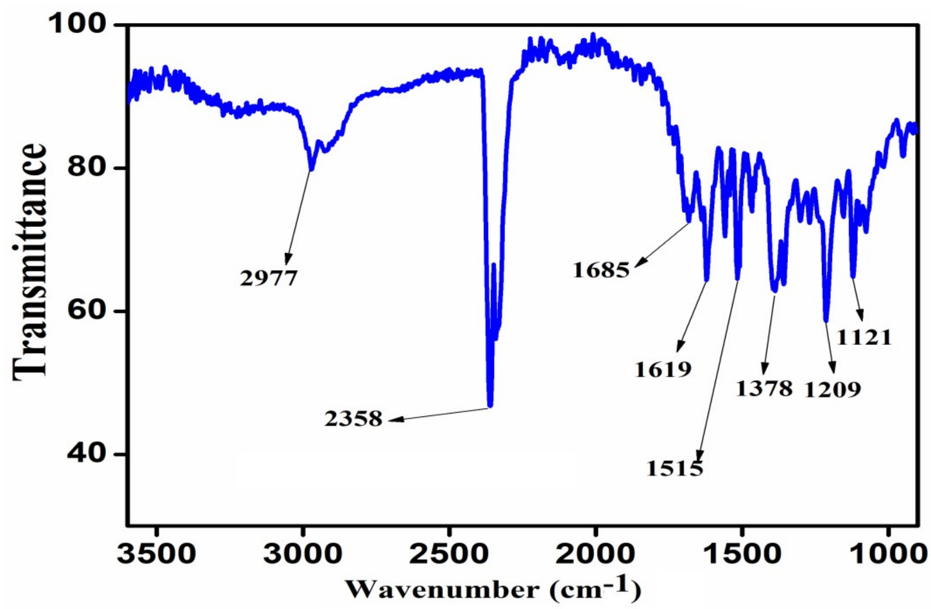


Fig. S7: FT-IR (cm^{-1}) spectra of **DEB-HY** in solid state.

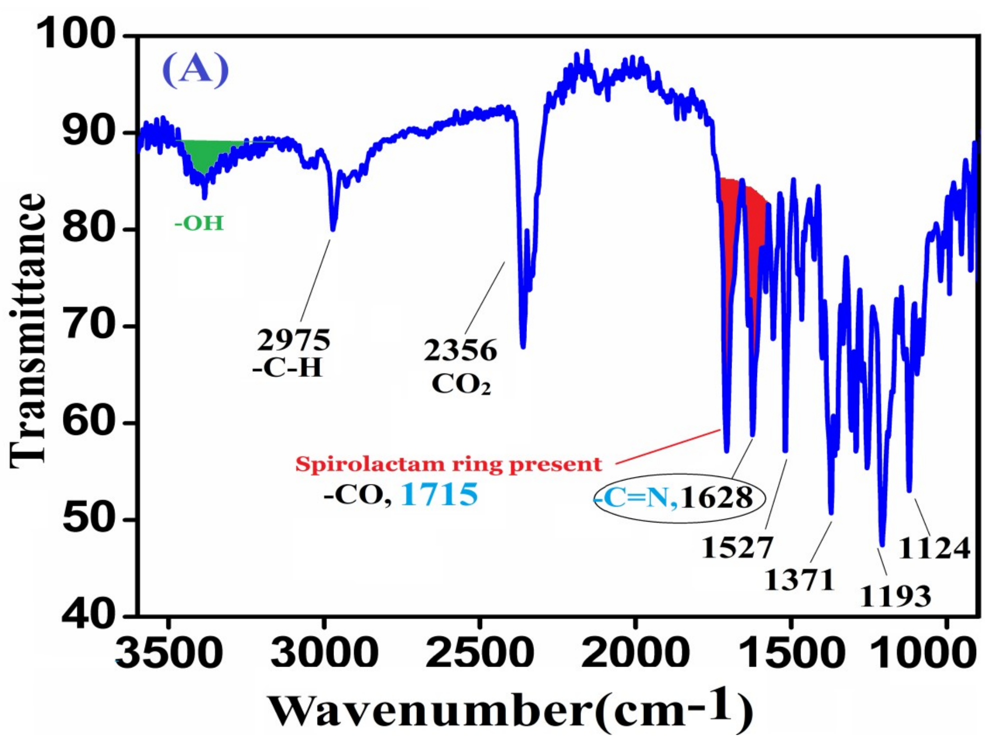
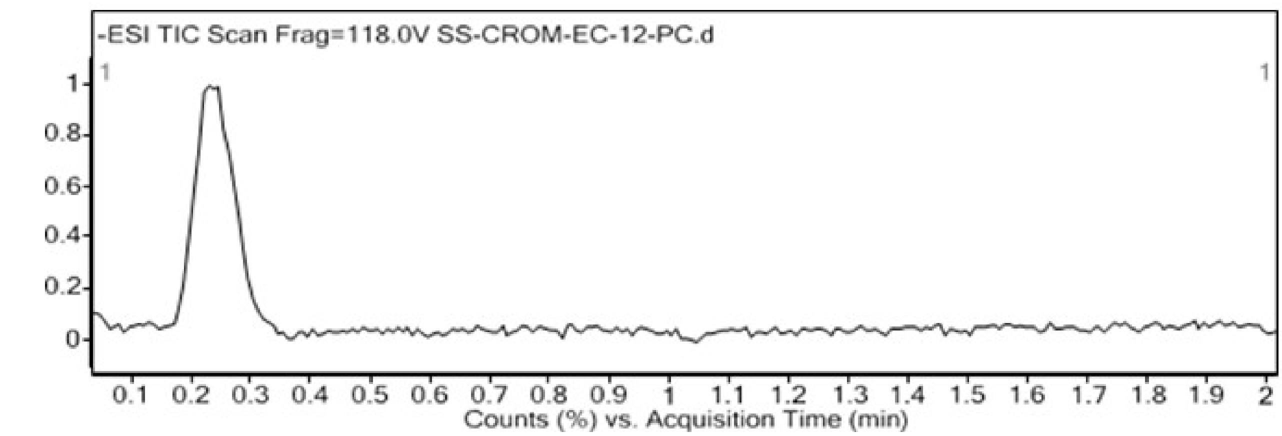
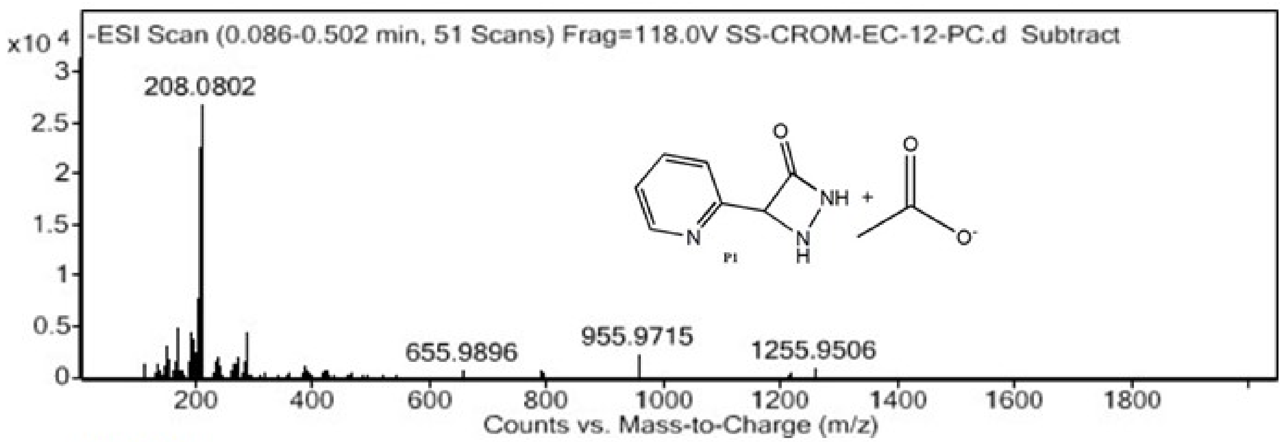


Fig. S8. FT-IR (cm^{-1}) spectra of **DEB-CO** in solid state.





Peak List

m/z	z	Abund	Formula	Ion
208.0802	1	27008.64	C7H7N3O	(M+CH3COO) -
210.0805	1	14233.18	C7H7N3O	(M+CH3COO) -

Fig. S9. ESI-Mass (Negative mode) spectrum of **DEB-CO** with **CORM-3**

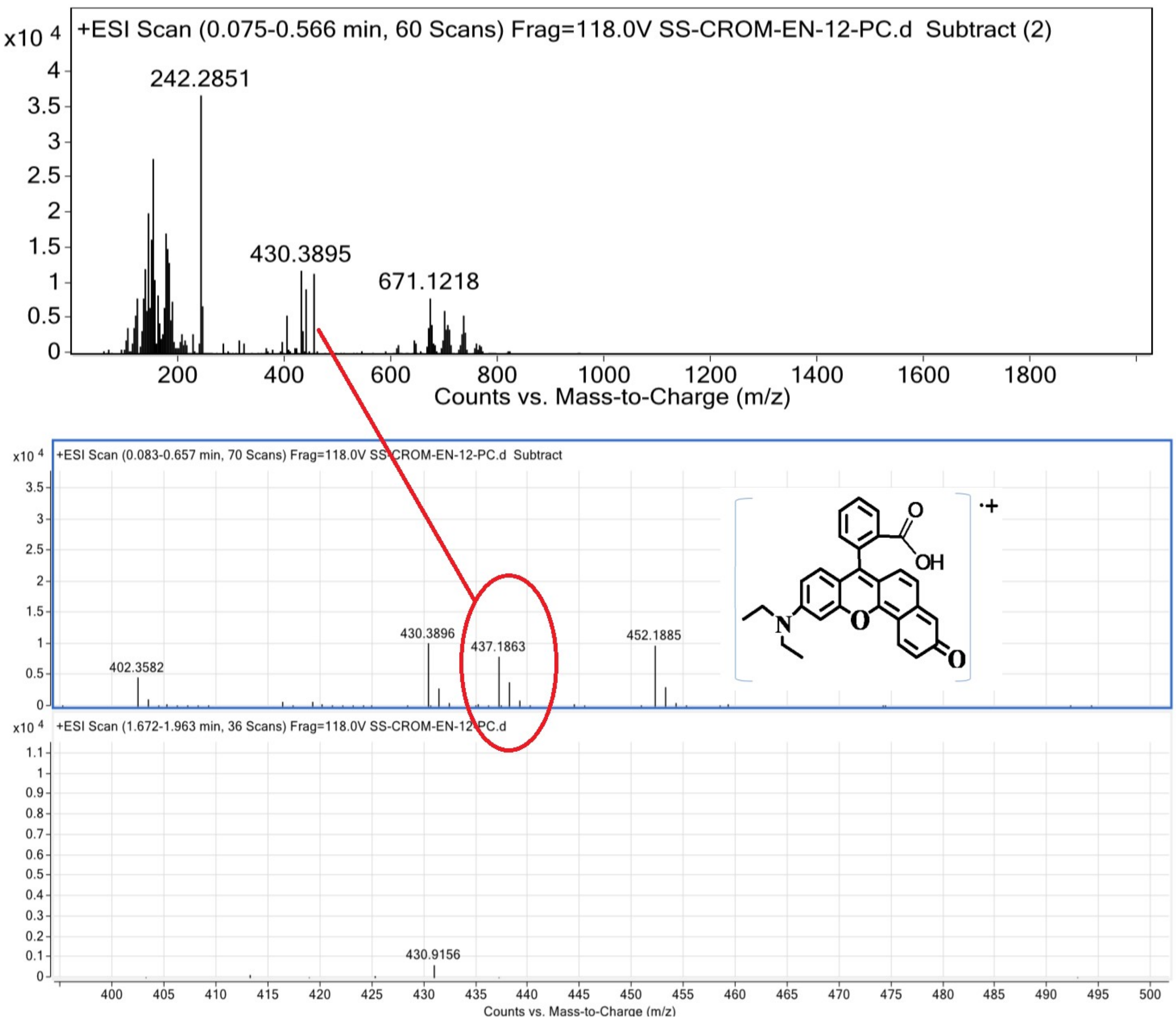


Fig. S10. ESI-Mass (Positive Mode) spectrum of **DEB-CO** with **CORM-3**

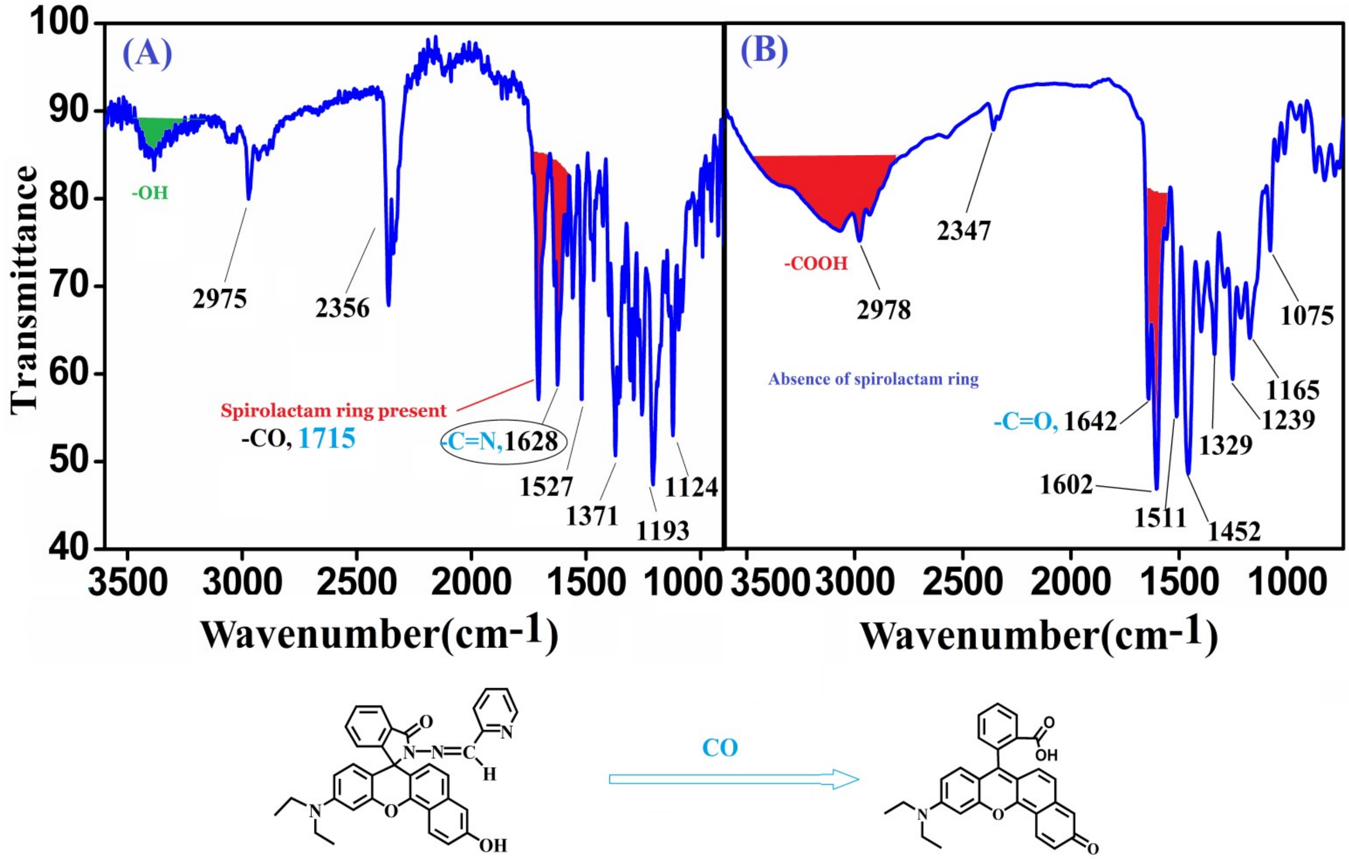


Fig. S11. IR spectra (A) **DEB-CO** and (B) **DEB-CO** with **CORM-3**

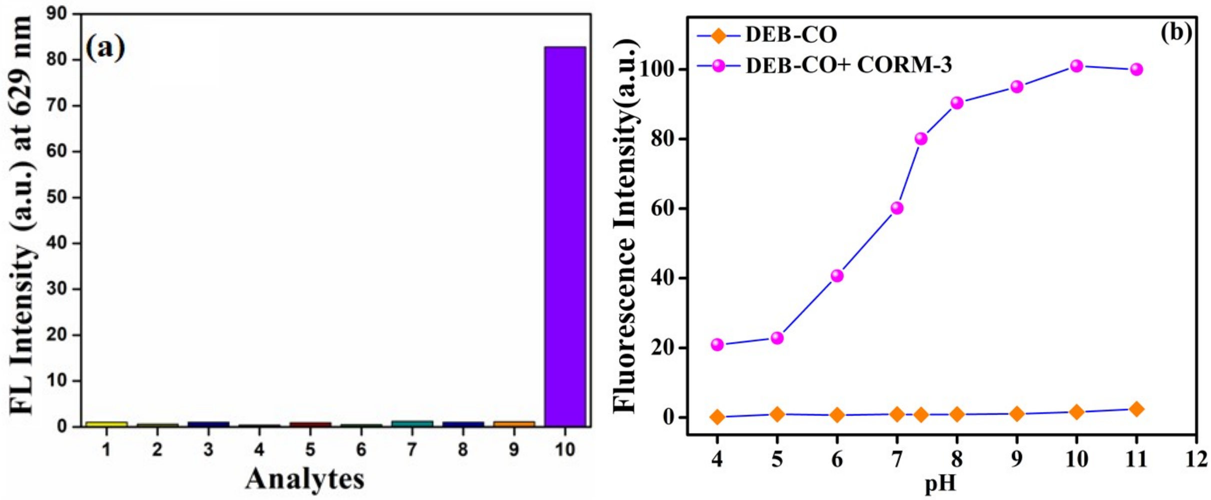


Fig. S12. Fluorescence intensity of DEB-CO toward (a) various biomolecules (1) none, (2) H_2S (source: NaHS), (3) NO (gas), (4) O^-_2 (source: KO2), (5) ^tBuOOH, (6) NaOCl, (7) H_2O_2, (8) HNO, (9) GSH and (10) 100 μM CORM-3. (b) Effects of pH on the fluorescence of DEB-CO (10 μM) with 20 μM CORM-3 at λ_{em} ~ 629 nm.

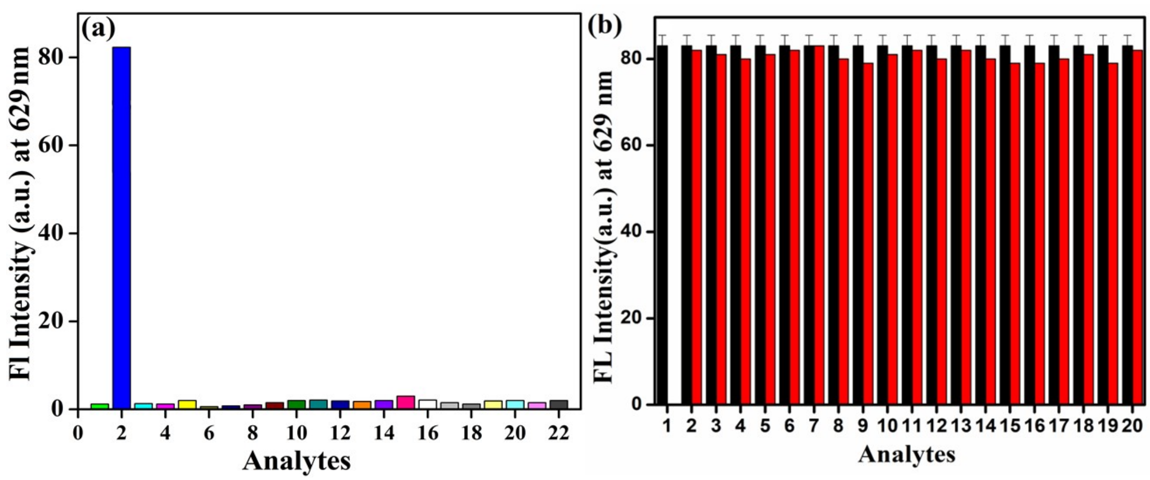


Fig. S13. (a) Fluorescence emission intensities of DEB-CO in the presence of various amino acids and inorganic salt ions in HEPES buffer (10 mM, pH = 7.4 at 37 °C) respectively (1. only DEB-CO, 2. CORM-3, 3. Ala, 4. Asp, 5. Arg, 6. Cys, 7. Gly, 8. Glu, 9. His, 10. Hcy, 11. Lys, 12. Pro, 13. Tyr, 14. Na^+, 15. Al^{3+}, 16. Ca^{2+}, 17. Cr^{3+}, 18. Fe^{2+}, 19. Fe^{3+}, 20. Cu^{2+}, 21. Hg^{2+}, 22. Cd^{2+} (b) Change in fluorescence intensity of DEB-CO (10 μM) in the presence of both CORM-3 (100 μM) and foreign species (100 μM) in HEPES buffer (10 mM, pH = 7.4 at 37 °C) respectively (1. blank, 2. CORM-3, 3. NaOCl, 4. H_2O_2, 5. NO, 6. GSH, 7. Gly, 8. Glu, 9. His, 10. Cys, 11. Lys, 12. Pro, 13. Tyr, 14. Cr^{3+}, 15. Al^{3+}, 16. Fe^{2+}, 17. Fe^{3+}, 18. Cu^{2+}, 19. Hg^{2+}, 20. Cd^{2+}

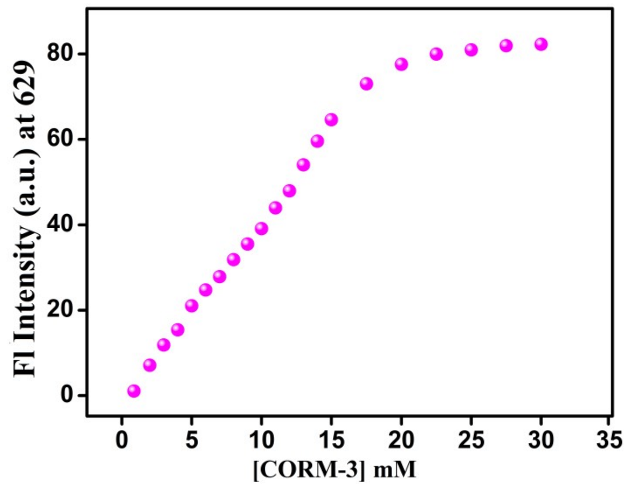


Fig. S14: Fluorescence emission intensities of DEB-CO (10 μM) at 629 nm vs. CORM-3 concentration.

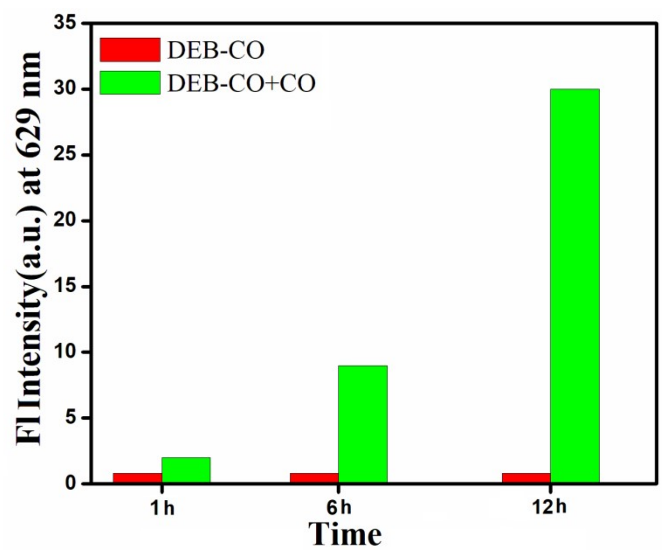


Fig. S15**.** Fluorescence intensity changes of **DEB-CO** (10 μM) solution after continuous CO gas was ventilation. $\lambda_{ex/em}$ = 580/629 nm.

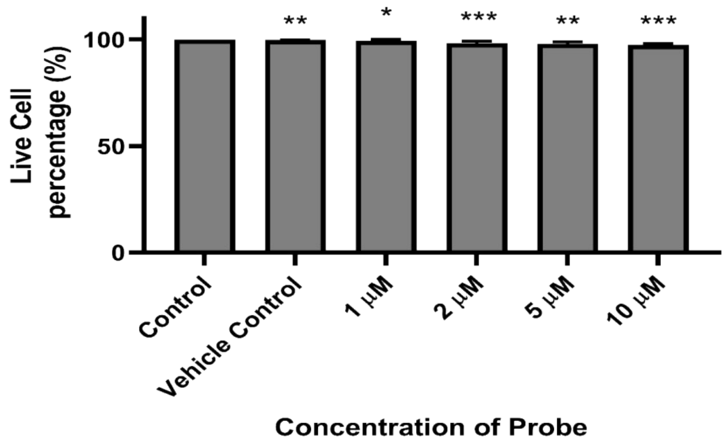


Fig. S16**:** ***Cytotoxicity measurement of the Probe against MCF7 cells.*** Live cell percentage was calculated using MTT assay of mcf7 cells. mcf7 cells were treated with different concentrations of drug (1µM, 2µM, 5µM, and 10µM) and incubated for 24 hr. Graph showing the live cell percentage of PBMC cells treated with drug compared to control*, P<0.05, significantly different from the vehicle group

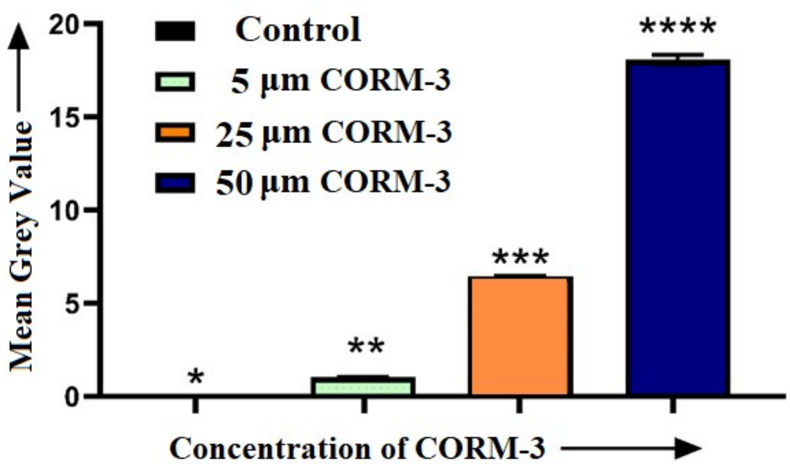
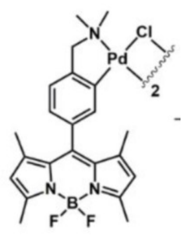
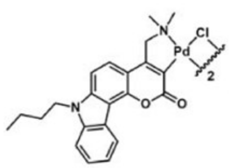
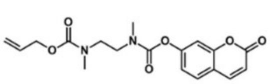
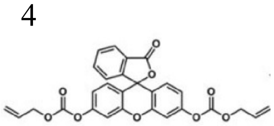
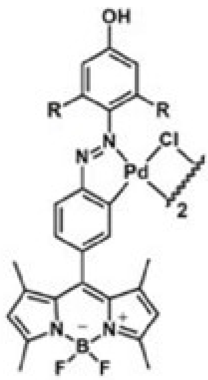
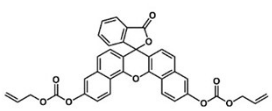
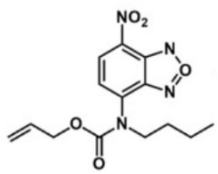
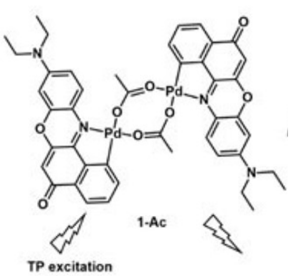
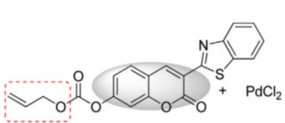
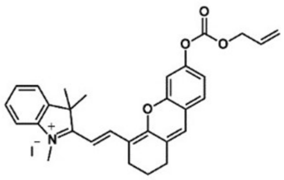
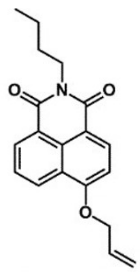
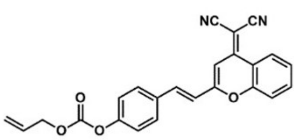
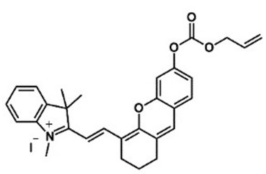
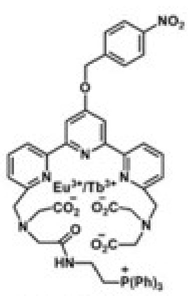
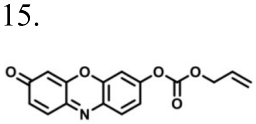


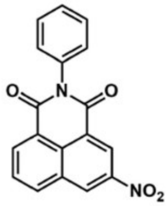
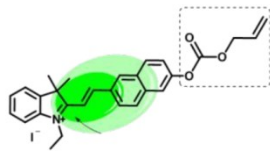
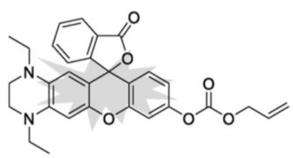
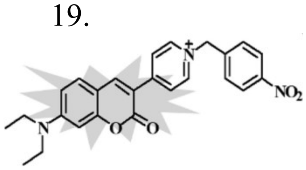
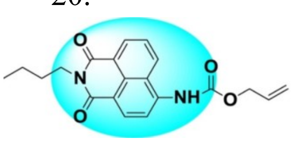
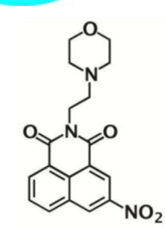
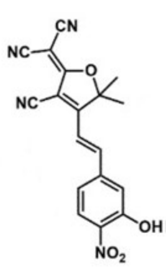
Fig. S17**:** The mean grey value of the probe at different concentrations.

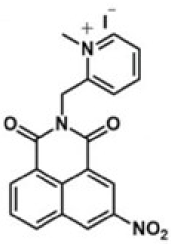
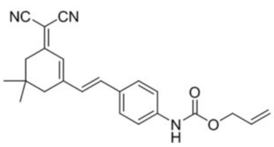
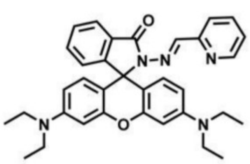
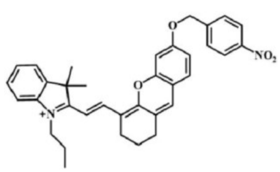
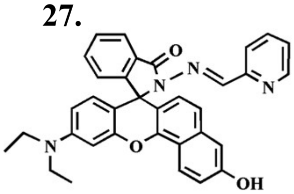
Table S1. Comparison of 'analytical figure of merit' with the other previous works

Entry	$\lambda_{ex}/\lambda_{em}$ (nm)	**Whether metal-freeYes/ No**	**Wheter CO source other then CORM Yes/No**	**Detection limit (nM)**	**Whether applicable in living cells Yes/No and about *in vitro*/*in vivo***	**Ref.**
1  COP-1	475/503	NO	NO	Not given	Yes, but only *in vitro*	1
2  CC-CO	370/477	NO	NO	653	Yes, but only *in vitro*	2
3  PCO-1	340/460	NO	NO	7.77	No	3
4  FL-CO-1	490/520	NO	NO	37	Yes, but only *in vitro*	4

5	 ACP-1: R=H ACP-2: R=-CH_3	498/512	NO	NO	720	Yes, but only *in vitro*	5
6	 NF-APC	620/670	NO	NO	127	Yes, but only *in vitro*	6
7	 NBD-APC	480/549	NO	NO	26.3	Yes, but only *in vitro*	7
8		580/660	NO	NO	50	Yes, both *in vitro* and *in vivo*	8
9	 BTHC-CO	462/490	NO	NO	25	Yes, but only *in vitro*	9

10  Probe 1	670/714	NO	NO	3.2	Yes, both *in vitro* and *in vivo*	10
11  Ratio-CO	430/(545 *vs* 455)	NO	NO	17.9	Yes, but only *in vitro*	11
12  Probe 1	565/685	NO	NO	57	Yes, but only *in vitro*	12
13  Probe 1	690/736	NO	NO	170	Yes, both *in vitro* and *in vivo*	13
14  Mito-NBTTA-Tb^{3+}/Eu^{3+}	500/540 *vs* 610	NO	NO	444	Yes, both *in vitro* and *in vivo*	14
15.  P1	410/ (515*vs* 600)	NO	NO	380	Yes, both *in vitro* and *in vivo*	15

16. 	440/522	NO	NO	123	Yes, but only *in vitro*	16
17. 	530/ 585	NO	NO	62	Yes, but only *in vitro*	17
18. 	541/676	NO	NO	37	Yes, both *in vitro* and *in vivo*	18
19. 	420/500	No	No	4	Yes, both *in vitro* and *in vivo*	19
20. 	420/(545 *vs* 472)	NO	Yes	58	Yes, both *in vitro* and *in vivo*	20
21. 	440/525	Yes	NO	600	Yes, but only *in vitro*	21
22. 	580/665	YES	NO	6.1	Yes, both *in vitro* and *in vivo*	22

23.  NucFP-NO_2	440/520	Yes	No	180	Yes, but only *in vitro*	23
24. 	440/655, 592	Yes	No	61	Yes, both *in vitro* and *in vivo*	24
25.  RCO	558/578	Yes	Yes	10	Yes, but only *in vitro*	25
26. 	620/712	Yes	No	103	Yes, both *in vitro* and *in vivo*	26
27. 	**580/623**	Yes	Yes	64.29	**Yes, both *in vitro* and *in vivo***	**Our Work**

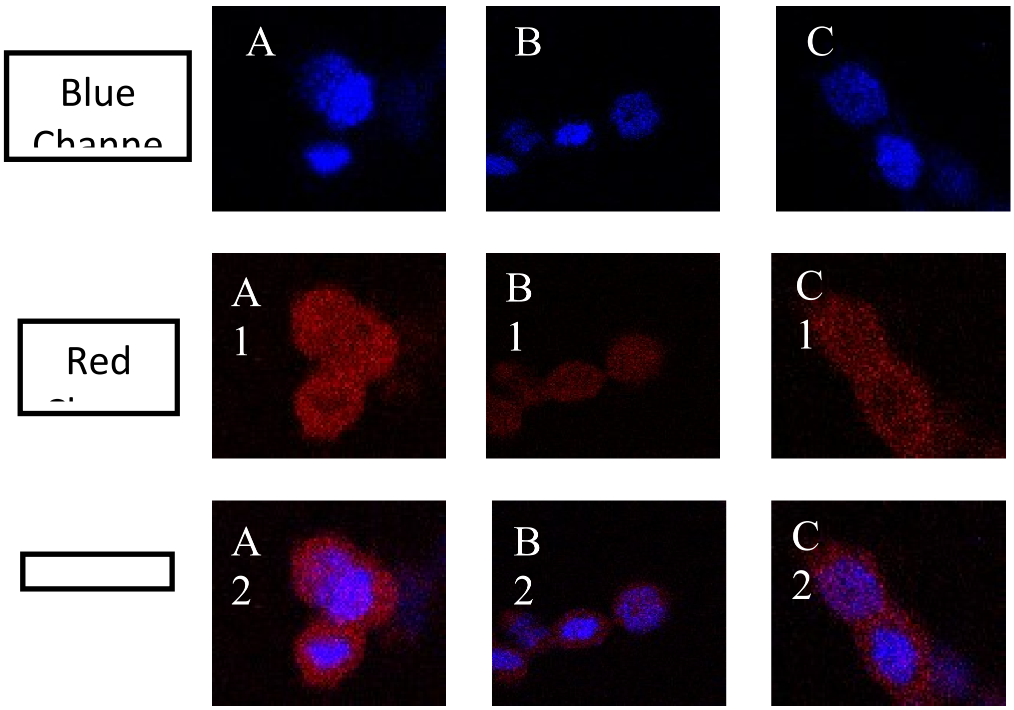


Fig. S18**:** Fluorescent images of CO in the living different single MCF7 cells coincubated with **DEB-CO** probe (10 μM), DAPI (for staining) and CORM-3(50 μM).

References

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9 W. Feng, D. Liu, Q. Zhai, G. Feng, Lighting up carbon monoxide in living cells by a readily available and highly sensitive colorimetric and fluorescent probe, *Sens. Actuators B*., 2017, **240,** 625–630.

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