Supplementary material for

Deoxygenation switch embedded red-emitting fluorogenic light-up probe for detection of highly toxic free bilirubin in human blood serum

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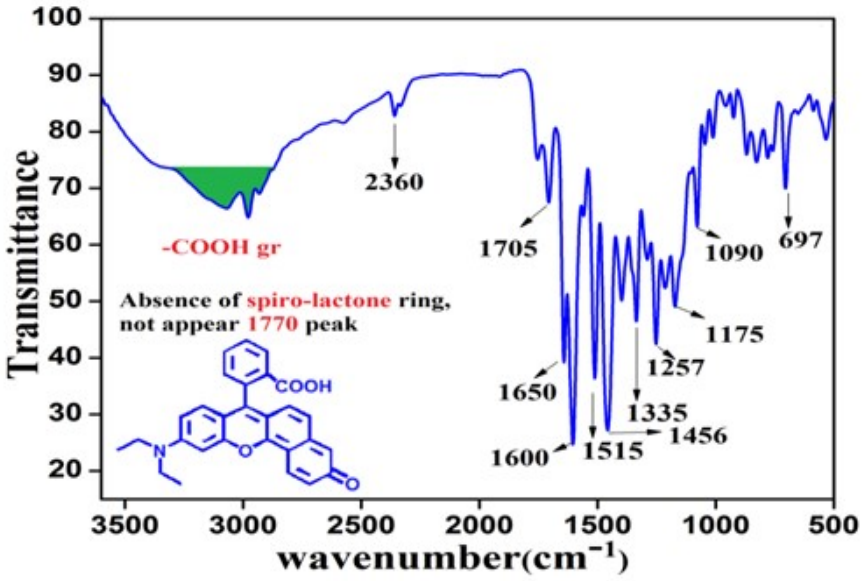

Fig. S1.FT-IR spectrum of DEB.

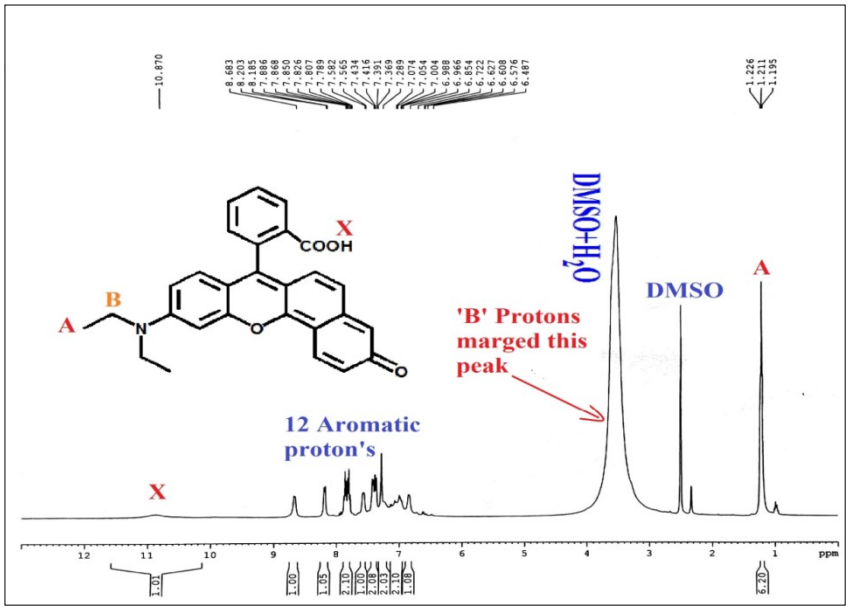

Fig. S2.[1]HNMR spectrum of DEBind_6-DMSO.

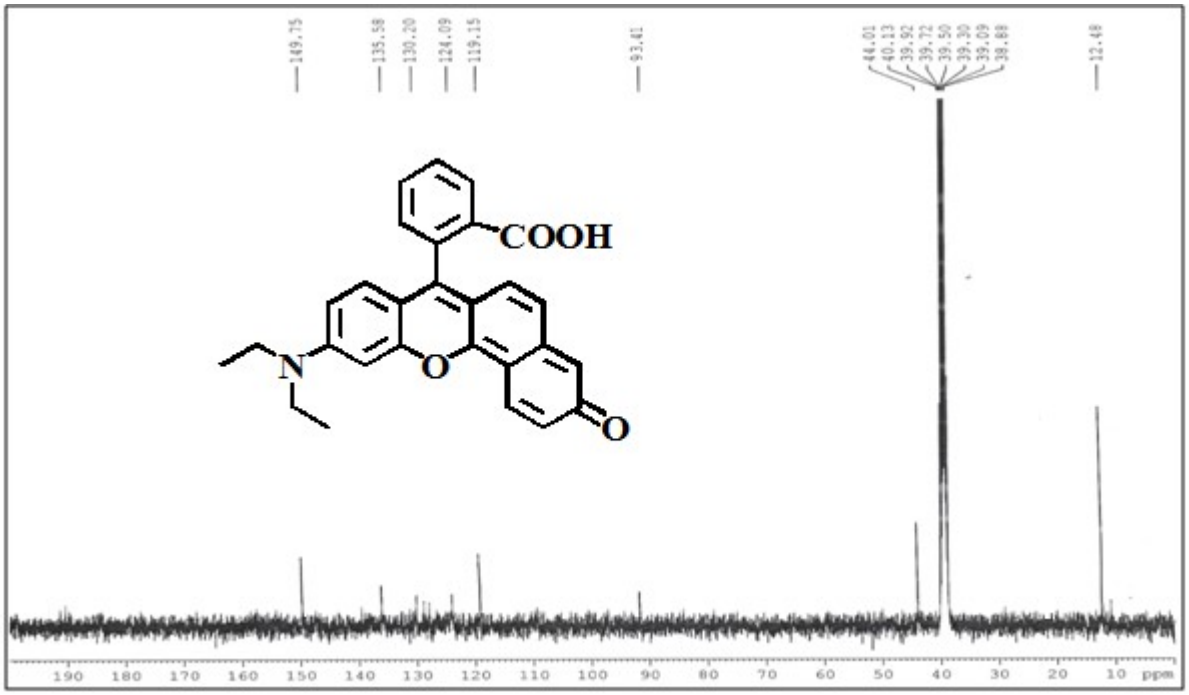

Fig. S3. ^{13}C NMR spectrum of DEB in d_6-DMSO.

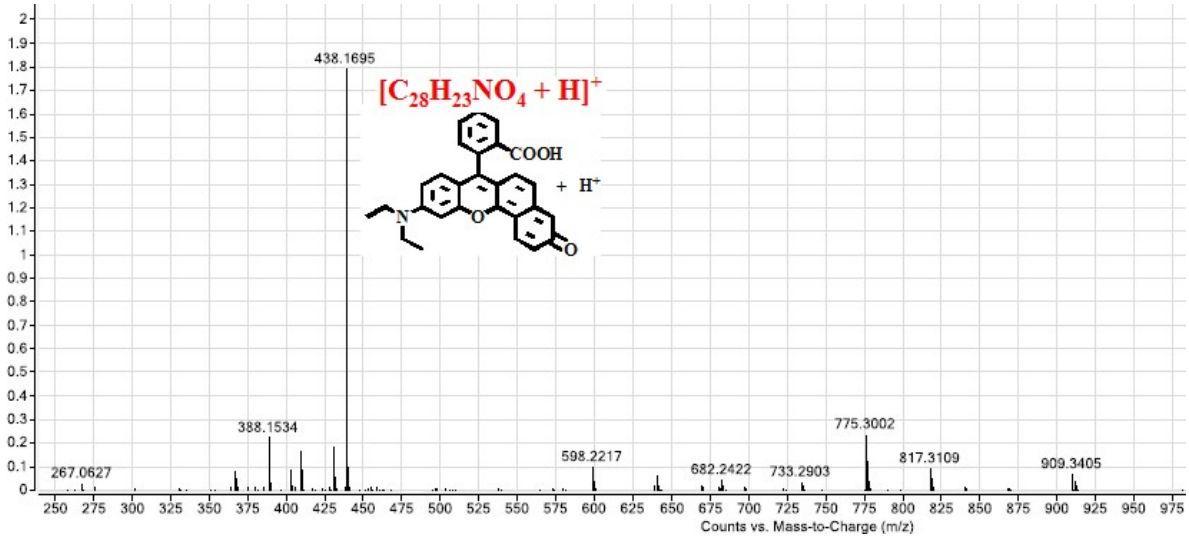

Fig. S4. High resolution mass spectrum of DEB.

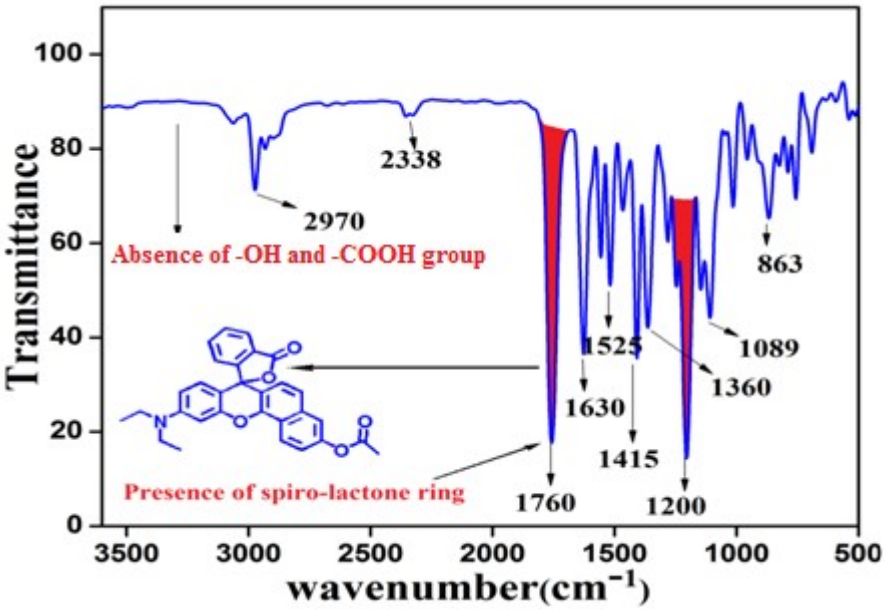

Fig. S5.FT-IR spectrum of Ac-DEB.

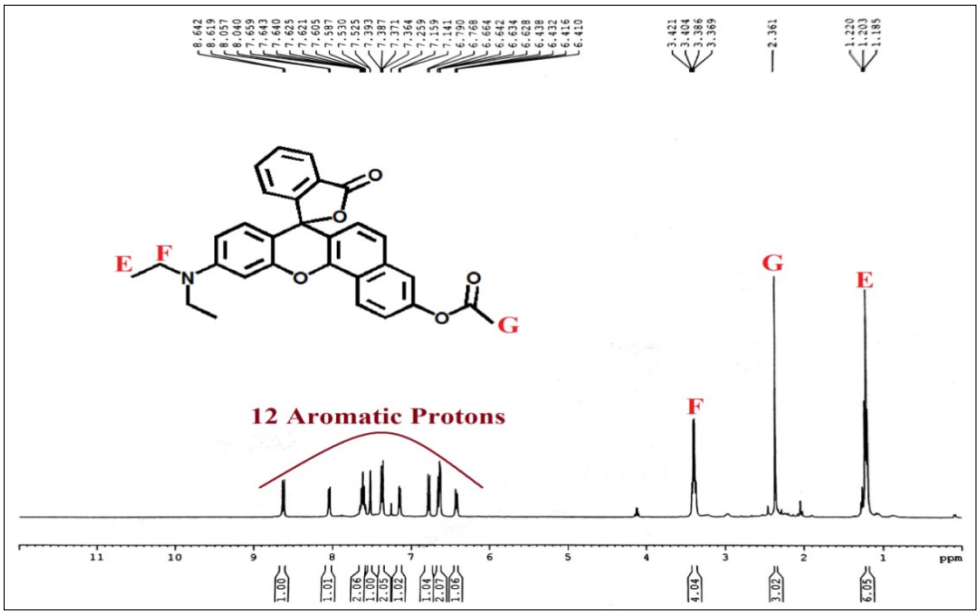

Fig. S6.[1]HNMR spectrum of Ac-DEBinCDCl$_3$.

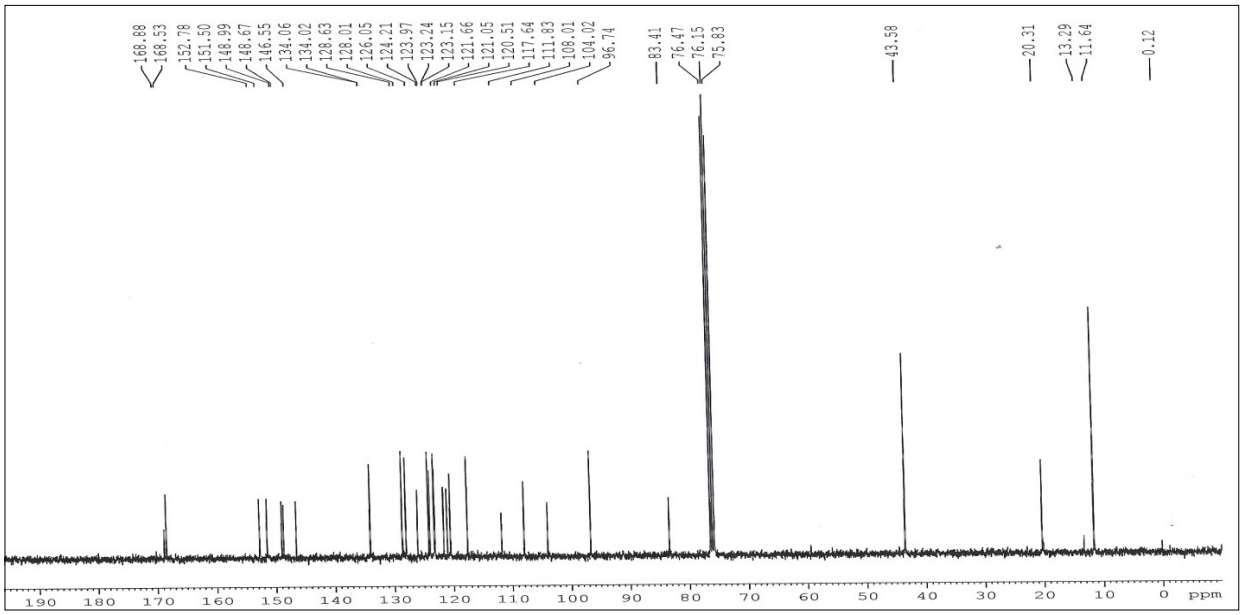

Fig. S7. ^{13}C NMR spectrum of Ac-DEB in $CDCl_3$.

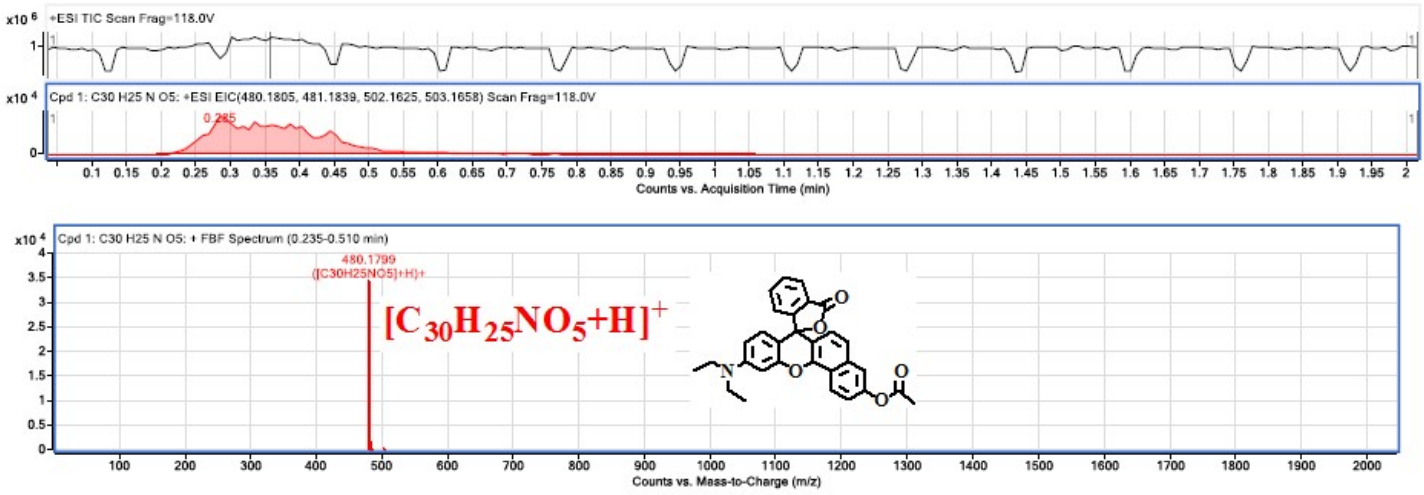

Fig. S8. High resolution mass spectrum of Ac-DEB.

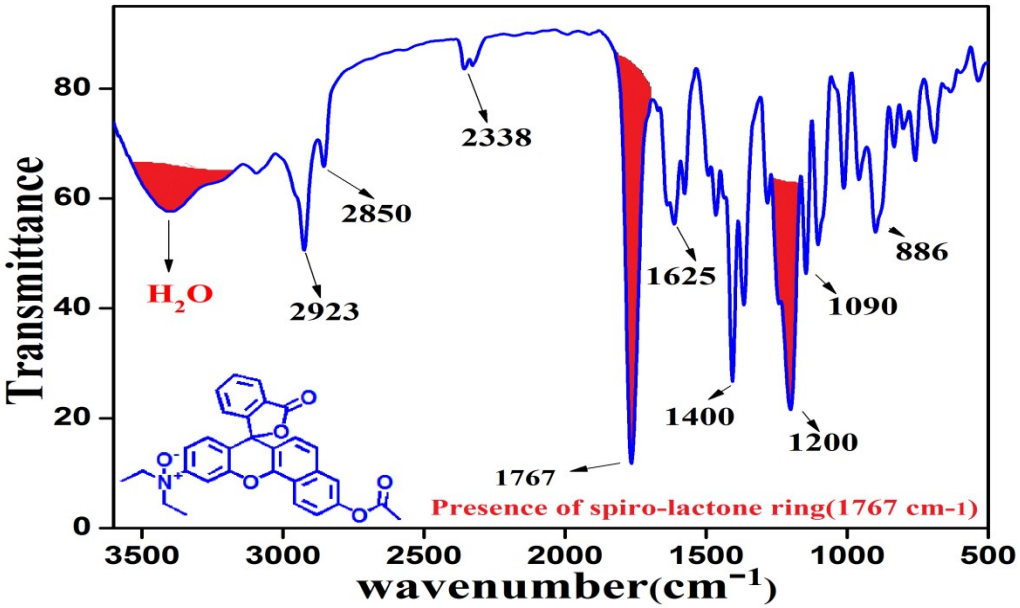

Fig. S9. FT-IR spectrum of Ac-DEBNox.

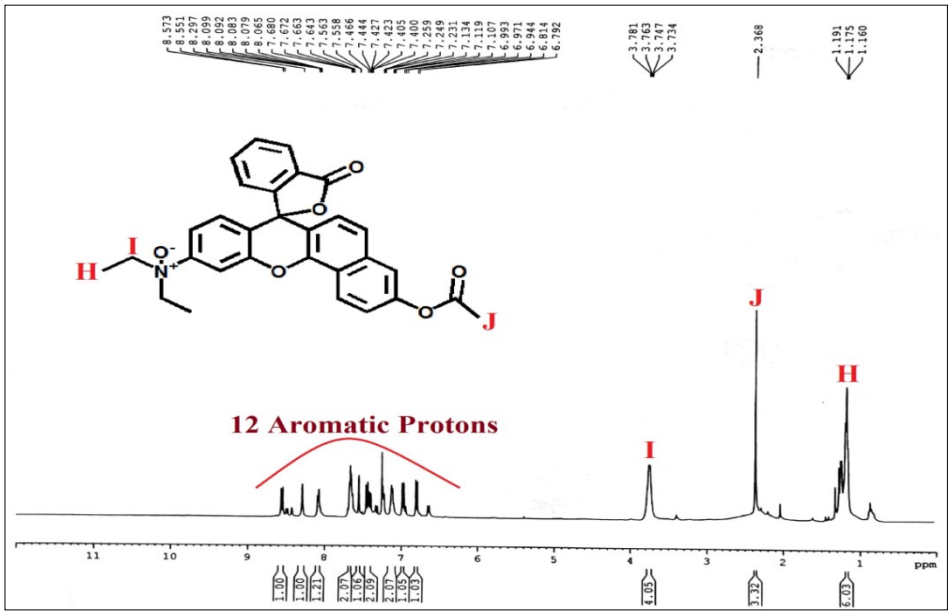

Fig. S10. ^{1}H NMR spectrum of Ac-DEBNox in $CDCl_3$.

Fig. S11. ^{13}C NMR spectrum of Ac-DEBNox in d_6-DMSO.

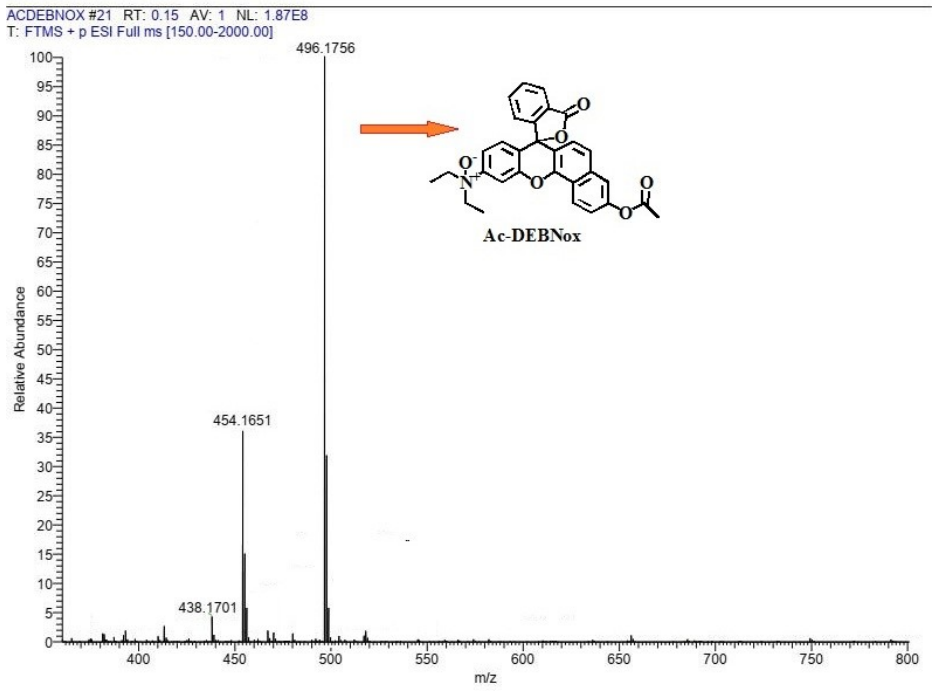

Fig. S12. High resolution mass spectrum of Ac-DEBNox.

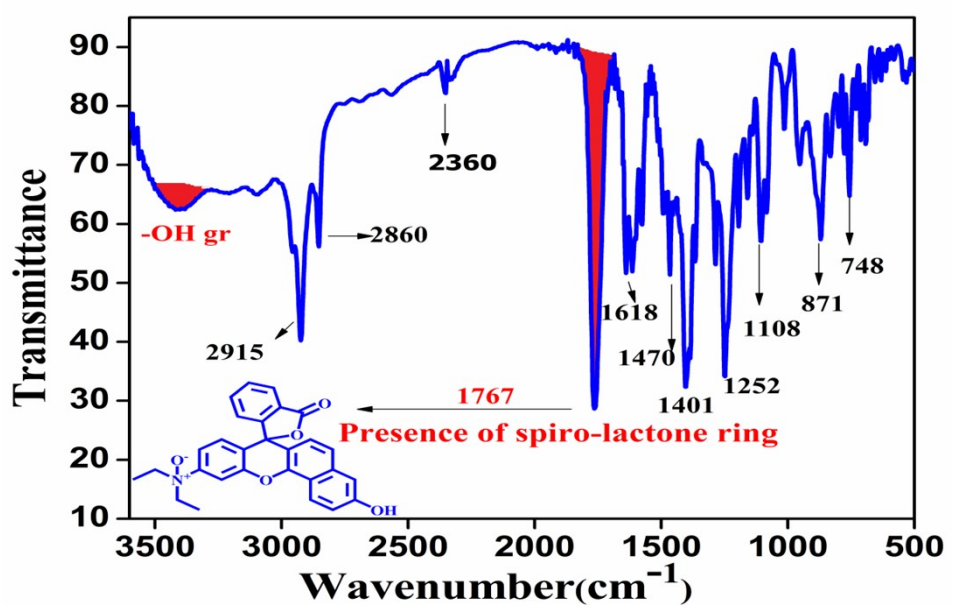

Fig. S13. FT-IR spectrum of DEBNox.

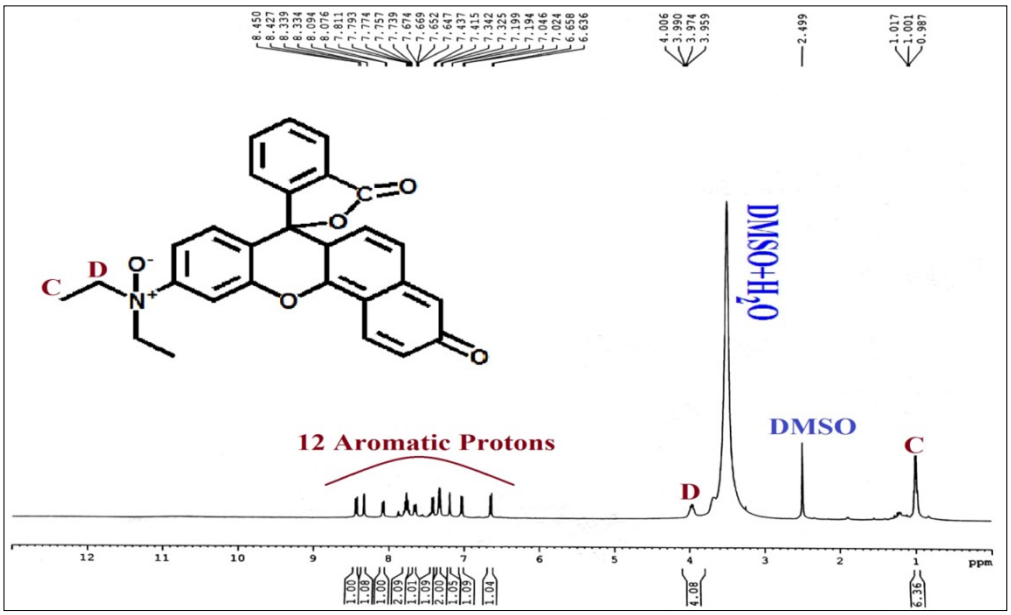

Fig. S14. ^{1}HNMR spectrum of DEBNox in$CDCl_3$

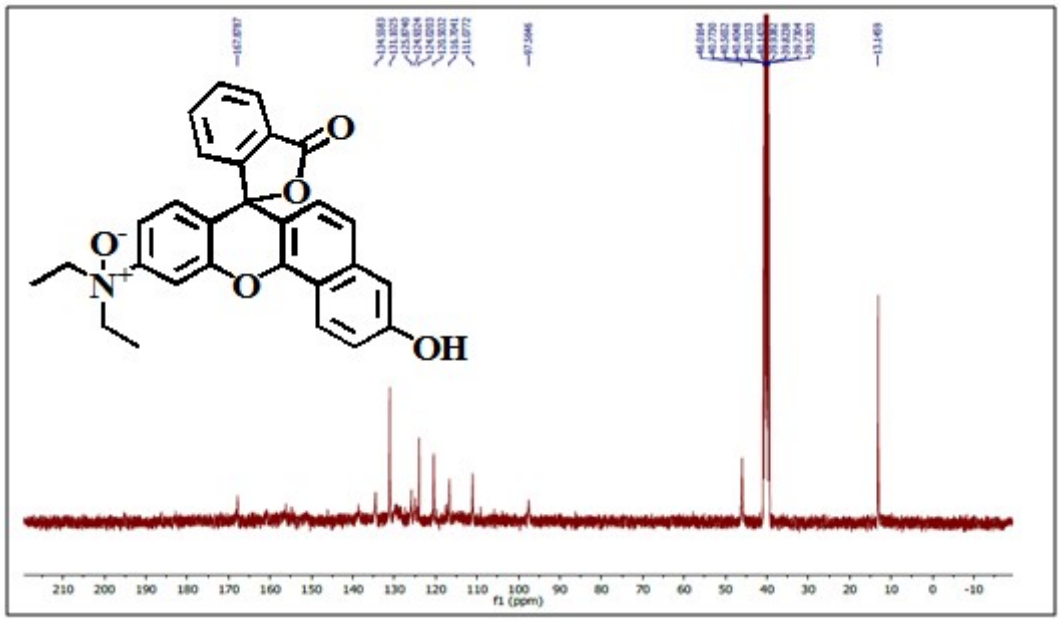

Fig. S15.[13]CNMR spectrum of DEBNox in d_6-DMSO.

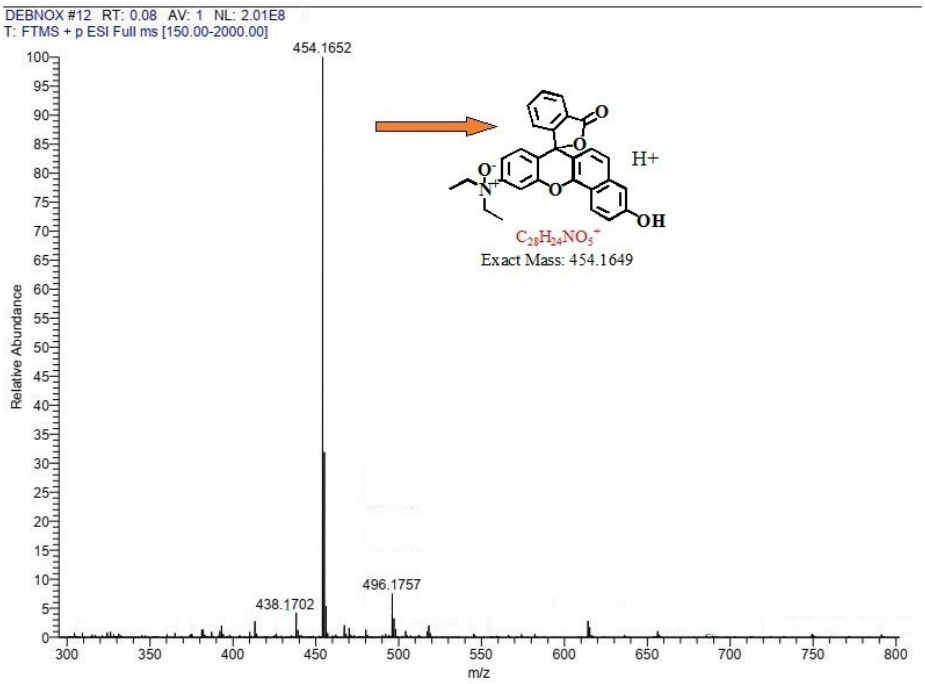

Fig. S16. High resolution mass spectrum of DEBNox.

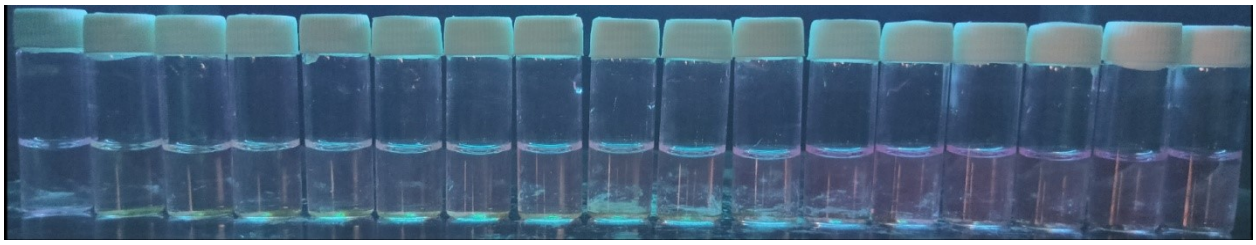

Fig. S17. Naked eye color change of DEBNox (10 μM) towards increasing concentration of bilirubin in reaction buffer (50 mM HEPES, 5% DMSO, pH 7.4) at 27 °C in presence of 100 μM Fe^{3+}.

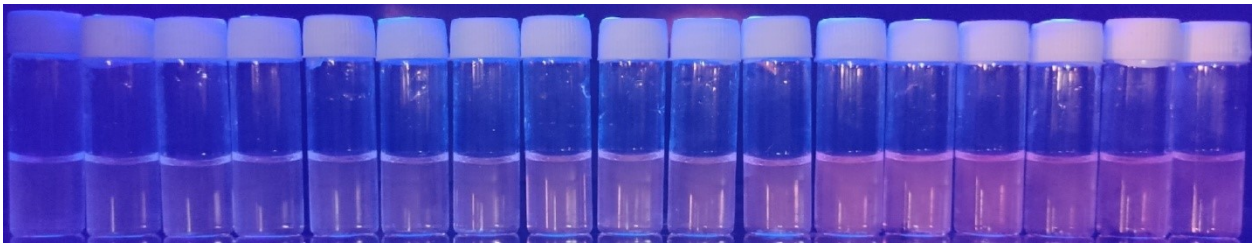

Fig. S18. The fluorescence enhancement behavior of DEBNox (10 μM) towards the increasing concentration of bilirubin (0 - 10 μM) in reaction buffer (50 mM HEPES, 5% DMSO, pH- 7.4) at 27 °C in presence of 100 μM Fe^{3+}.

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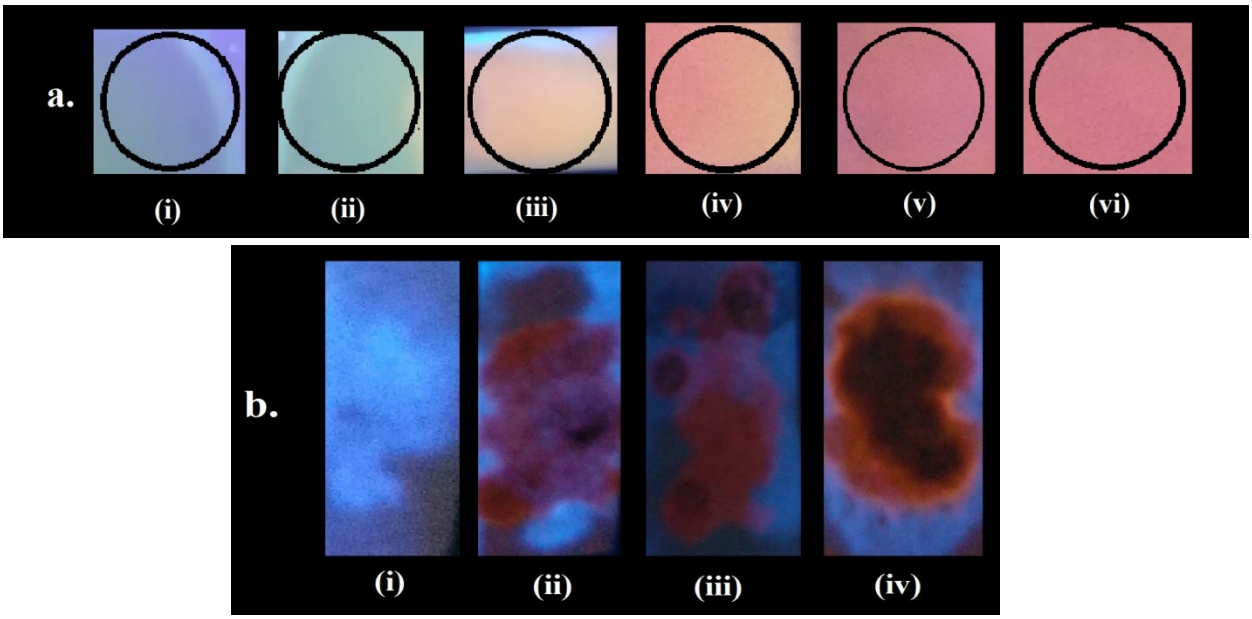

Fig. S19. (a) Paper-strip test with 20 μM DEBNox and 100 μM Fe^{3+} with different concentrations of bilirubin: (i) DEBNox, (ii) DEBNox + 100 μM Fe^{3+}, (iii) DEBNox + Fe^{3+} + 2.5 μM bilirubin, (iv) DEBNox + Fe^{3+} + 5 μM bilirubin, (v) DEBNox + Fe^{3+} + 7.5 μM bilirubin, (vi) DEBNox + Fe^{3+} +10 μM bilirubin. (b) different human blood serum sample with high bilirubin concentration (images were taken under UV lamp).

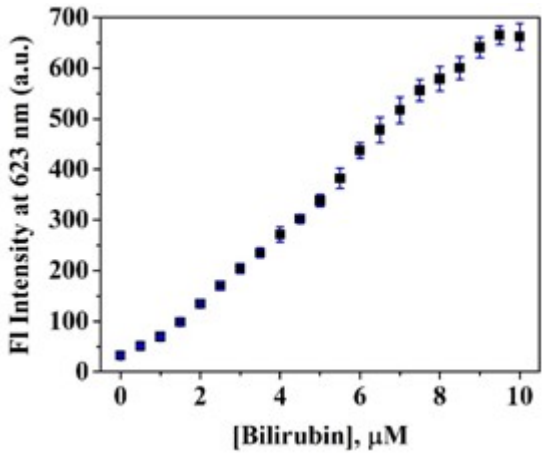

Fig. S20. Fluorescence intensity of DEBNox (10 μM) at 623 nm *vs* bilirubin (0 μM to 10 μM) concentration in the reaction buffer (50 mM HEPES, 5% DMSO, pH 7.4) using 100 μM Fe^{3+} (ex: 580 nm).

Table S1. (a) Determination of free bilirubin in different human blood serum specimens using DEBNox incubated at 27 °C for 1 hr. and existing colorimetric diazo-method.

Serum specimen	Free bilirubin estimated using DEBNox (mg/dl)	Free bilirubin estimated using existing colorimetric diazo-method (mg/dl)
Sample-01	0.64	0.74
Sample-02	0.60	0.71
Sample-03	0.72	0.81
Sample-04	0.99	1.10
Sample-05	1.20	1.32

[a]Average of three replicate experiments UNIT CONVERSION: mg/dl x 16.95 = µmol/l

(b)Recovery of free bilirubin by adding standard amount of 10 µM bilirubin to different human blood serum specimens using two methods.

Serum specimen	Free bilirubin estimated using DEBNox (mg/dl)		Free bilirubin estimated using existing colorimetric diazo-method (mg/dl)	
	Total bilirubin after addition[a]	Recovery (%)	Total bilirubin after addition[a]	Recovery (%)
Sample-01	1.18 ± 0.03	96.11	1.54 ± 0.04	115.88
Sample-02	1.14 ± 0.04	96.23	1.51 ± 0.04	116.02
Sample-03	1.26 ± 0.03	95.99	1.63 ± 0.03	116.18
Sample-04	1.52 ± 0.02	96.30	1.88 ± 0.03	111.21
Sample-05	1.74 ± 0.04	97.13	2.11 ± 0.03	110.53

Table S2. Comparative study of fluorescence probe for the detection of bilirubin with their sensing mechanism, method, excitation wavelength and LOD.

Sl. No.	Method of detection	Principle	LOD	Wavelength (λex/λem) in nm	References in main manuscript
1	Fluorimetry (turn off)	MOF (UIO-66-PSM) Quenching	0.59 μM	340/470	[22]
2	Fluorimetry (turn off)	Polyfluorenes, polymer Quenching	0.15 μM	325/~450	[23]
3	Fluorimetry (turn off)	Gold nanoclusters Quenching	248 nM	295/380	[24]
4	Fluorimetry (turn off)	MOF(1-NH2@THB) Quenching	1.26 pM	325/425	[25]
5	Fluorimetry (turn off)	MoS2 quantumdots Quenching	2.10 nM	310/392	[26]
6	Fluorimetry (turn off)	Nanosheets Quenching	41.0 nM	274,356,367/ 550-570	[27]
7	Fluorimetry (turn off)	Gold Nanoclusters Quenching	0.61 μM	487/597	[28]
8	Fluorimetry (turn off)	MOFs, Zr(IV)-Based Quenching	0.45 μM	254,320/614	[29]
9	Fluorimetry (turn off)	Quantum Dots Quenching	1.8 0 μM	325/ 410,470,590	[30]
10	Fluorimetry (on-off-on)	Gold Nanoclusters (2fold)	3.42 μM	~350 /~570	[31]
11	Fluorimetry (on-off-on)	S,N-doped carbon dots (3fold)	0.12 nM	375/452	[32]
12	Fluorimetry (on-off-on)	Copper Nanocluster (2fold)	6.62 nM	320/405	[33]
13	Fluorimetry (turn on)	Coumarin-fluorophores (12 Fold)	76.0 nM	400/515	Our previous work [34]
14	Fluorimetry (turn on)	Benzorhdolfluorophores (20 Fold)	33.0 nM	580 /623	Present work