Electronic Supplementary Information (ESI) for

Rapid and sensitive detection of nitric oxide by a BODIPY-based fluorescent probe in live cells: Glutathione effects

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I. Photostability of RBA and its sensing product.

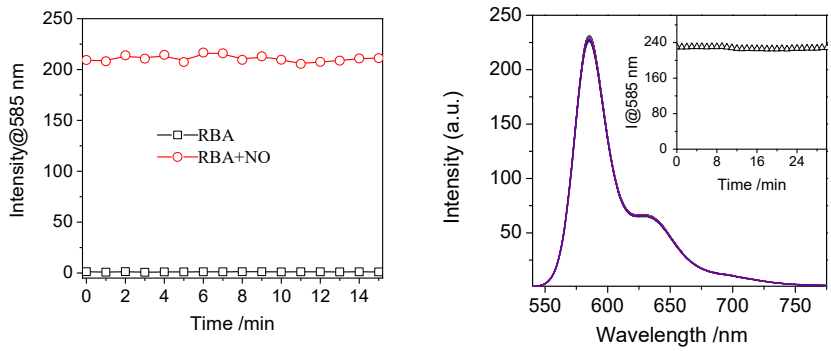


Figure S1. The time courses of fluorescence intensity at 585 nm of 5 μM RBA and its reaction mixture with 40 μM NO (left) or 5 μM RBA-NO (right) in PBS/EtOH (0.1 M, pH 7.4, v/v 65:35), excitation at 530 nm.

II. MS evidence of the sensing products for RBA or GSH with NO

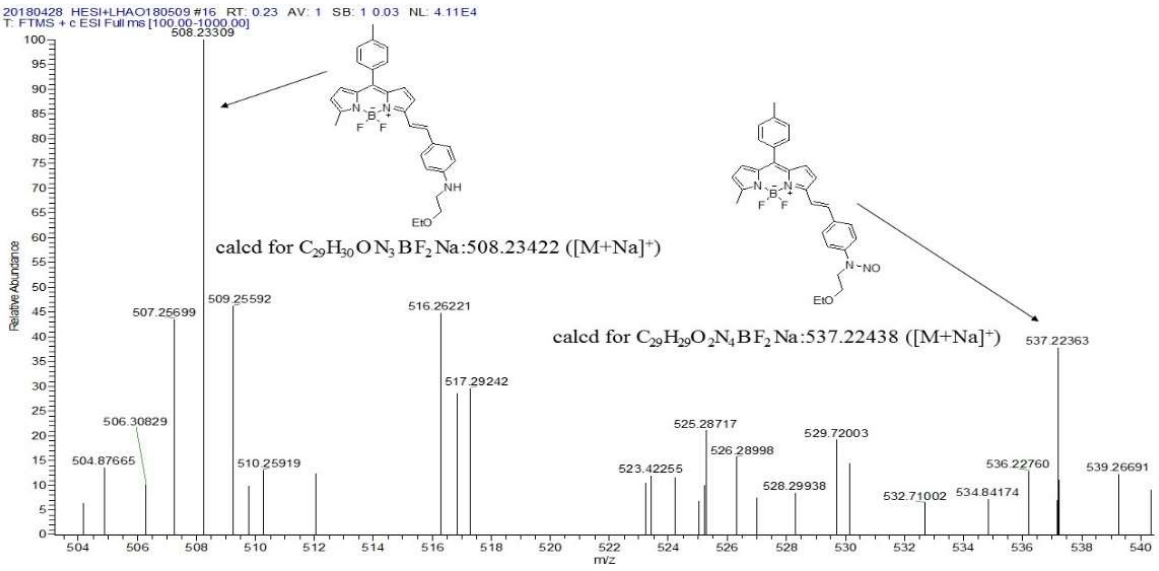


Figure S2. Mass spectrum of the reaction mixture of RBA and NO in the solution (pH 7.4).

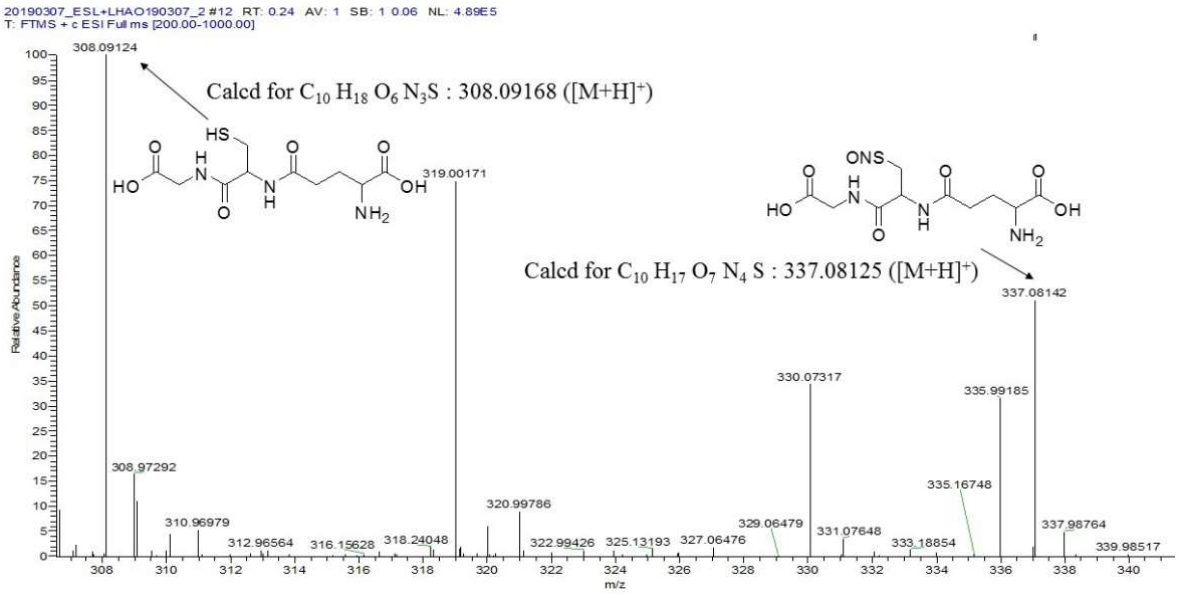


Figure S3. Mass spectrum of GSH and NO mixed in the solution (pH 7.4).

III. DFT calculations for PET process

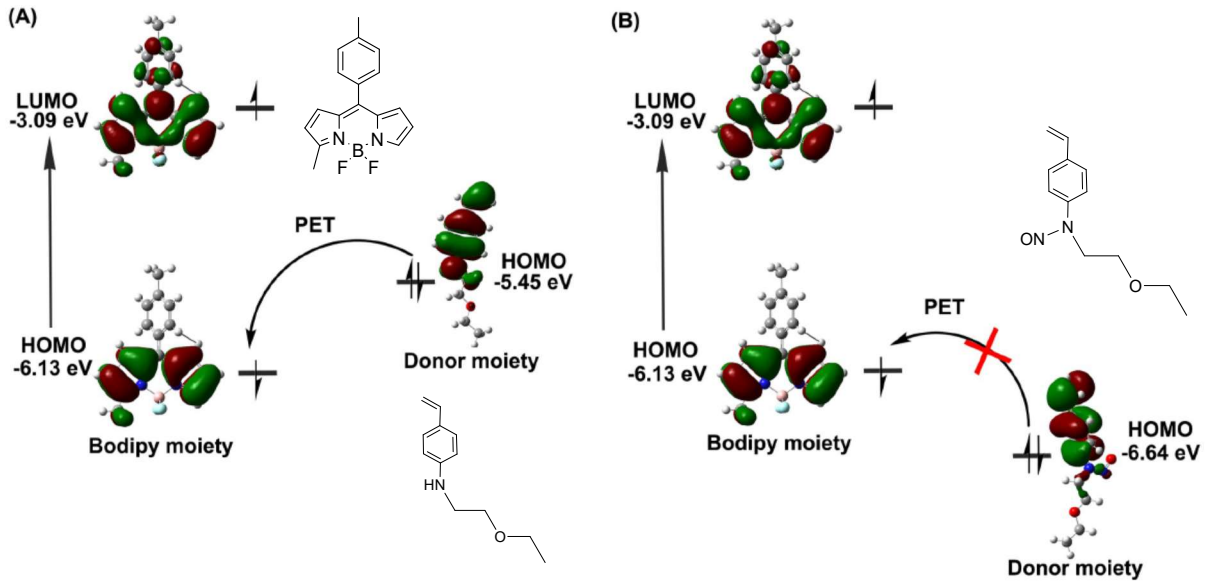
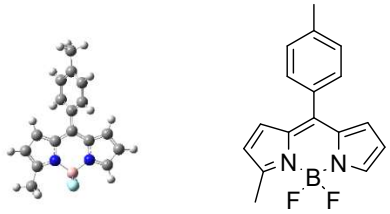


Figure S4. Frontier orbital energy representation of the PET processes in RBA (A) and RBA-NO (B).

Atom coordinates for structures of model compounds



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C	-0.53629012	-1.00401556	-0.04488916
N	-1.93410544	-0.89168526	-0.01801581
B	-2.71173673	0.45065579	0.00544009
N	-1.65946904	1.58299128	0.04910397
C	-0.27554065	1.41311101	0.06270803
C	0.29774383	0.12303552	0.00489031
C	-1.91961733	2.90485801	0.15008873
C	-0.71819887	3.62765166	0.23304711
C	0.31806262	2.69343424	0.18638195
C	-0.21591163	-2.38298637	-0.16254921
C	-1.41335823	-3.08229602	-0.18851751
C	-2.46292168	-2.13625022	-0.10182612
C	-3.93101192	-2.40789835	-0.10751723
F	-3.51345427	0.57737490	-1.15035789
F	-3.54731394	0.52341645	1.14092626
C	1.77110992	-0.03478629	0.00163248
C	2.41171787	-0.85035310	0.95124187
C	3.80005948	-0.98519787	0.95025415
C	4.59490083	-0.33109661	-0.00360091
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H	1.82420562	-1.35429227	1.71203861
H	4.27239642	-1.60626381	1.70671334
H	4.53922164	0.99726600	-1.70356434
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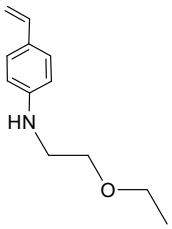
H	6.47986506	-0.76787804	0.96393007



Donor moiety in RBA

	X	Y	Z
C	-5.70526175	-1.43505460	-0.00288308
C	-5.06857438	-0.25205085	0.06583188
C	-3.62131808	0.00219930	0.02620815
C	-3.14947114	1.33002963	0.08579064
C	-1.79340699	1.63108949	0.04938278
C	-0.82774467	0.60546843	-0.05089384
C	-1.28808867	-0.72924609	-0.10001236
C	-2.65111917	-1.01319673	-0.06552199
N	0.51871452	0.93361628	-0.13357464
C	1.57105874	-0.05432394	0.06530025
C	2.93690922	0.59603152	-0.12925721
O	3.92684868	-0.40027560	0.07856694
C	5.25974747	0.09120464	-0.08713246
C	6.23573199	-1.04836111	0.14741532
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H	-5.18173417	-2.38216804	-0.10211147
H	-5.67952659	0.64601767	0.16111300
H	-3.86613275	2.14445617	0.16315682
H	-1.46850461	2.66770995	0.09336877
H	-0.58262511	-1.54985526	-0.16325704
H	-2.95865515	-2.05425057	-0.10402839
H	0.73811738	1.85810234	0.21716934
H	1.52473477	-0.51333198	1.06561254
H	1.46018160	-0.85688454	-0.67121144
H	3.01658767	1.01283219	-1.14483690
H	3.07520617	1.42222829	0.58735753
H	5.37493709	0.50053517	-1.10243396
H	5.43862860	0.91081302	0.62562466
H	7.26303230	-0.69020515	0.02502976
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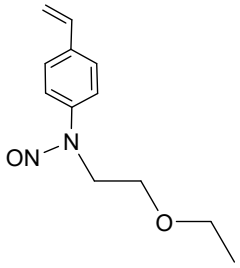

	X	Y	Z
C	5.82400378	-1.28419794	-0.61988518
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C	2.86397128	-0.12687665	1.37657210
C	1.53619011	0.29565639	1.30555698
C	0.90636135	0.36709888	0.06255028
C	1.59688779	0.01956626	-1.10366135
C	2.92160105	-0.40019361	-1.02253813
N	-0.47410574	0.78296148	-0.02119630
C	-1.53352848	-0.23329692	0.07035840
C	-2.94395204	0.33625223	-0.05927141
O	-3.81784512	-0.78097796	0.03651402
C	-5.19819483	-0.41695900	-0.06759021
C	-6.04419491	-1.67399973	0.03132446
N	-0.83207084	2.04699930	-0.17802456
O	0.09187336	2.87144471	-0.25068248
H	6.83939438	-1.59051716	-0.38967165
H	5.54224341	-1.29114407	-1.66896286
H	5.34943815	-0.94176873	1.38631218
H	3.35227373	-0.18304140	2.34539463
H	0.99504130	0.56726835	2.20634582
H	1.09741900	0.07835328	-2.06560657
H	3.44164990	-0.66750506	-1.93640916
H	-1.36378258	-0.97161267	-0.72037965
H	-1.43206214	-0.74620094	1.03270085
H	-3.15297373	1.05990758	0.74030331
H	-3.07533711	0.84775004	-1.02258656
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H	-7.10510062	-1.41547725	-0.04738503
H	-5.80007761	-2.37254667	-0.77536323
H	-5.88397067	-2.17810269	0.98977295

IV. GSH effects

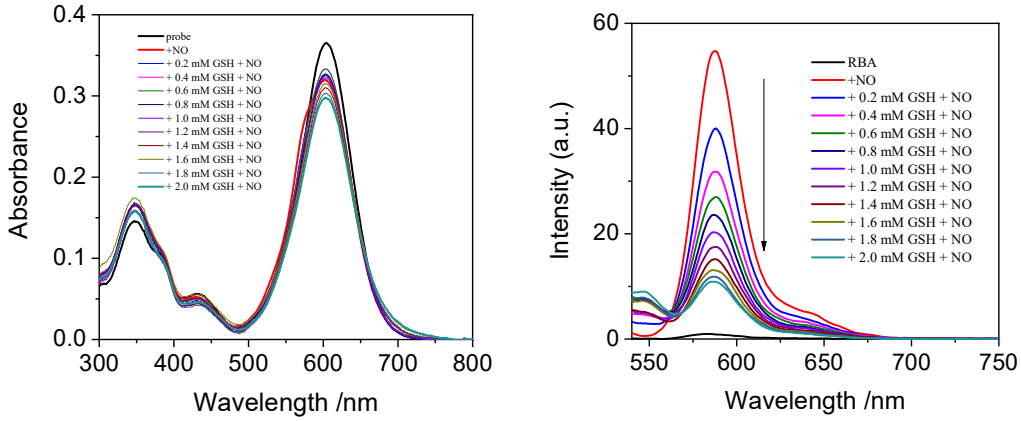


Figure S5. UV/Vis absorption (left) and fluorescence (right, λ_{ex} = 530 nm) spectra of RBA (5 μM) solution upon additions of different amounts of GSH (0-2 mM), and then treated with NO (40 μM) for 5 min.

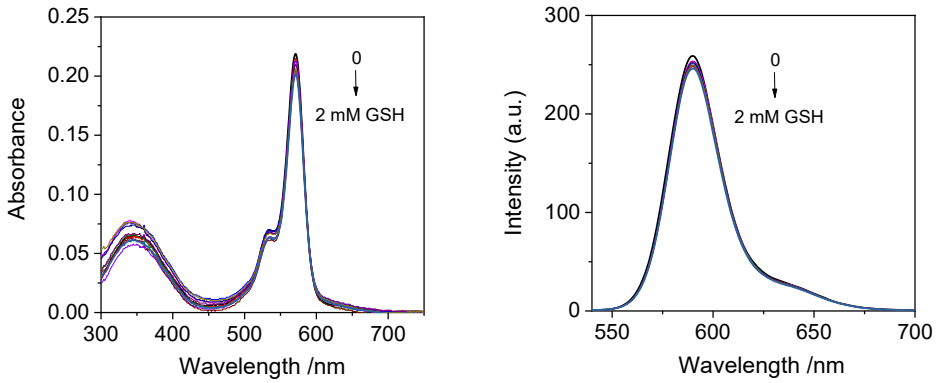


Figure S6. UV/Vis absorption (left) and fluorescence (right, λ_{ex} = 530 nm) spectra of RBA-NO (2 μM) solution upon additions of different amounts of GSH (0-2 mM) for 5 min.

V. MTT assay for cytotoxicity

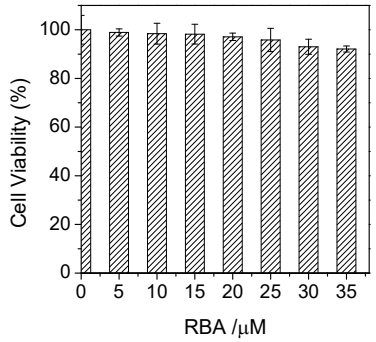


Figure S7. Viability of HeLa cells incubated with various RBA concentrations (0–35 μM) for 24 h measured by MTT assay.

VI. Fluorescence intensity from imaging of NO in live cells

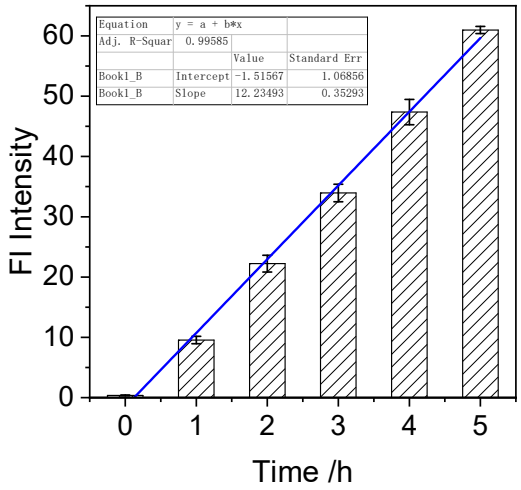


Figure S8. Plots of fluorescence intensity of 5.0 μM RBA to endogenous NO in RAW 264.7 cells with *L*-Arg (0.5 mg/mL), LPS (20 μg/mL) and IFN-γ (400 U/mL) for different times (0–5 h). The mean values of intensity was analyzed by Image J software.

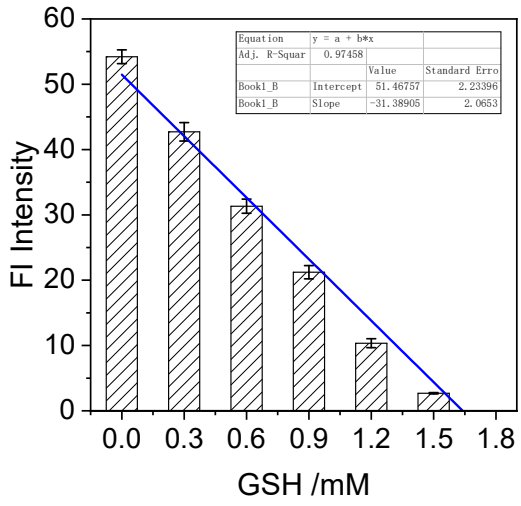
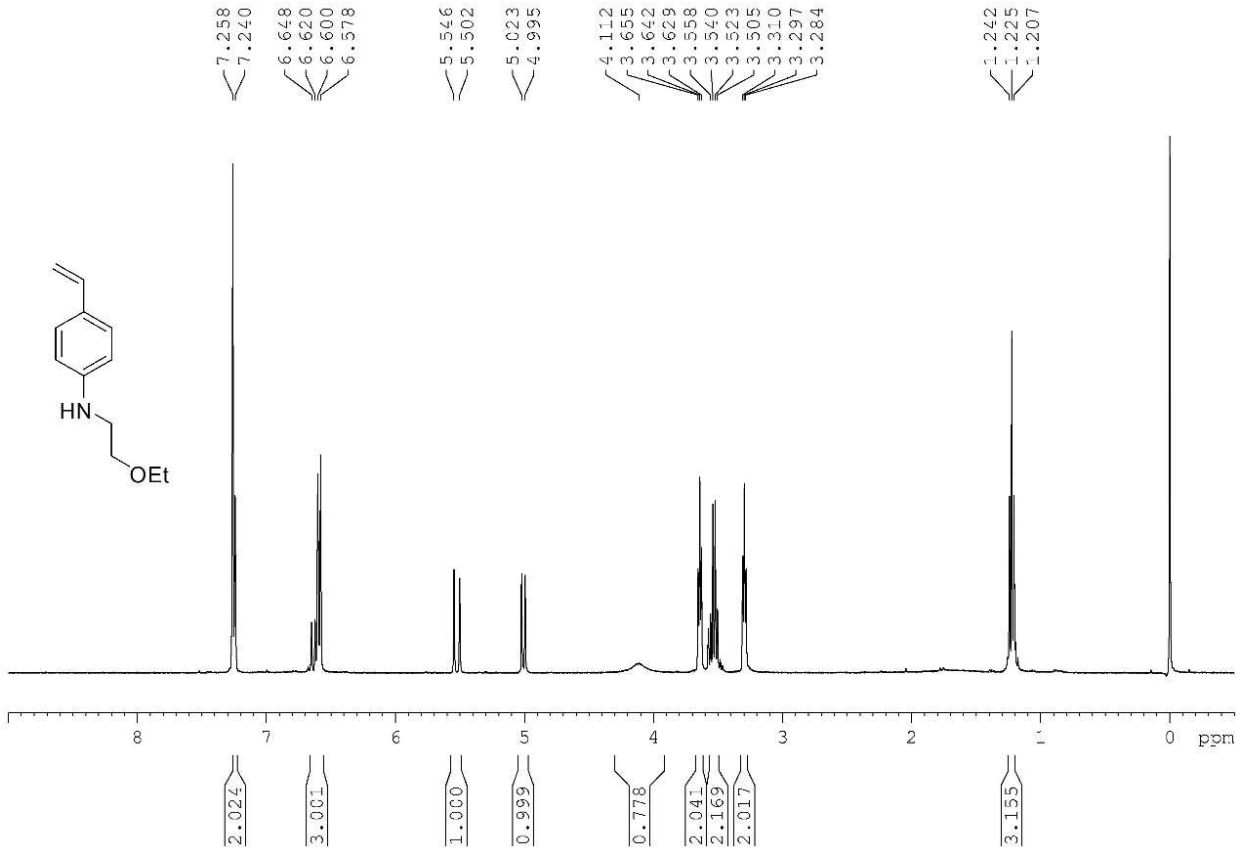


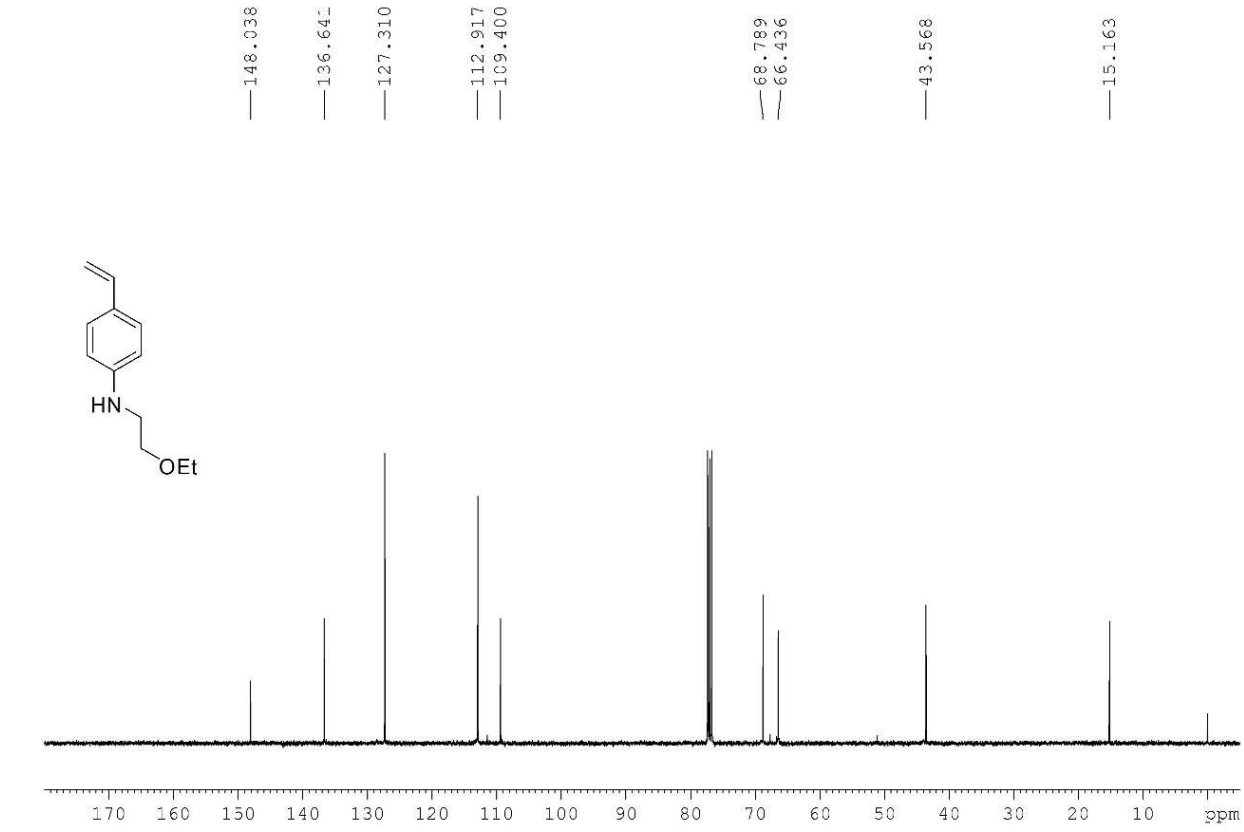
Figure S9. Plots of fluorescence intensity of HepG2 cells stained with 1.0 mM NEM for 1 h, and additions of various amounts of GSH (0-1.8 mM) for 30 min, then 5.0 μM RBA for another 1h and 40 μM NO for 10 min, The mean values of intensity was analyzed by Image J software.

VII. Copies of NMR spectra of new compounds

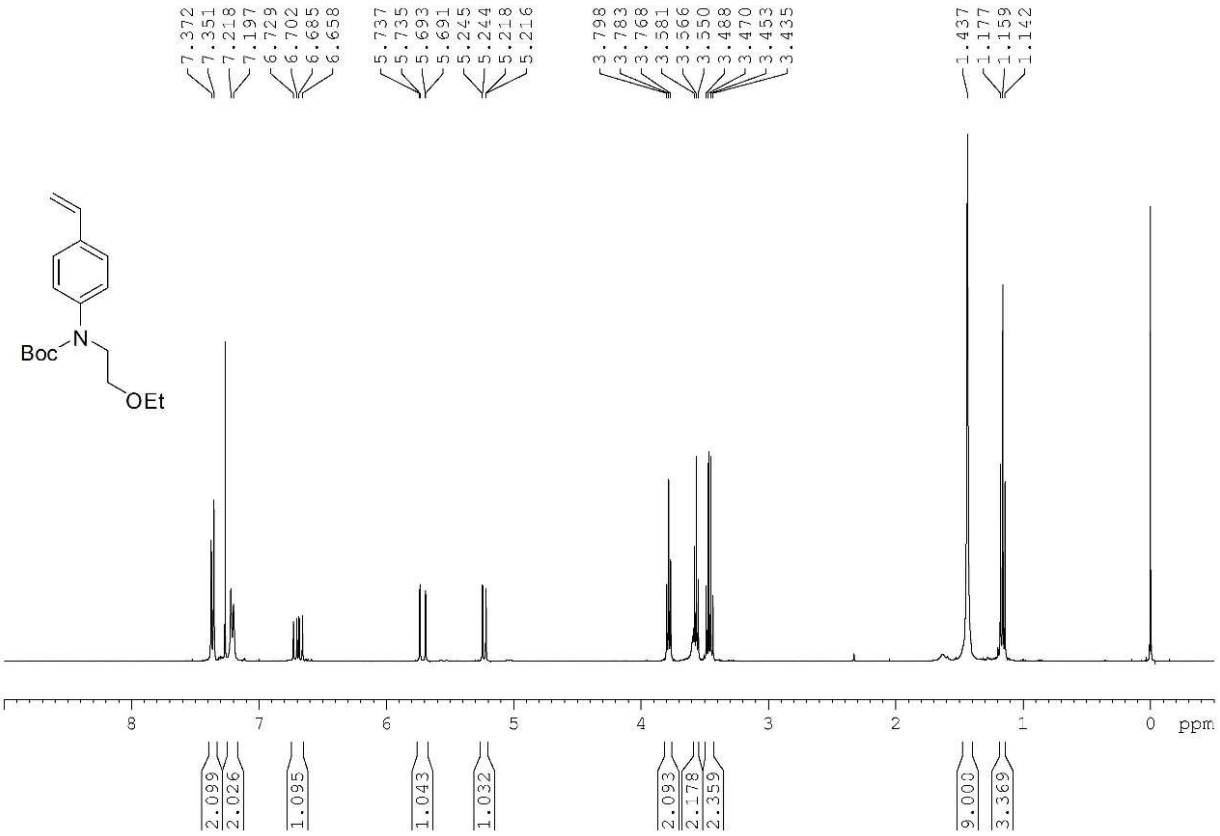
^{1}H NMR of **1** in $CDCl_3$



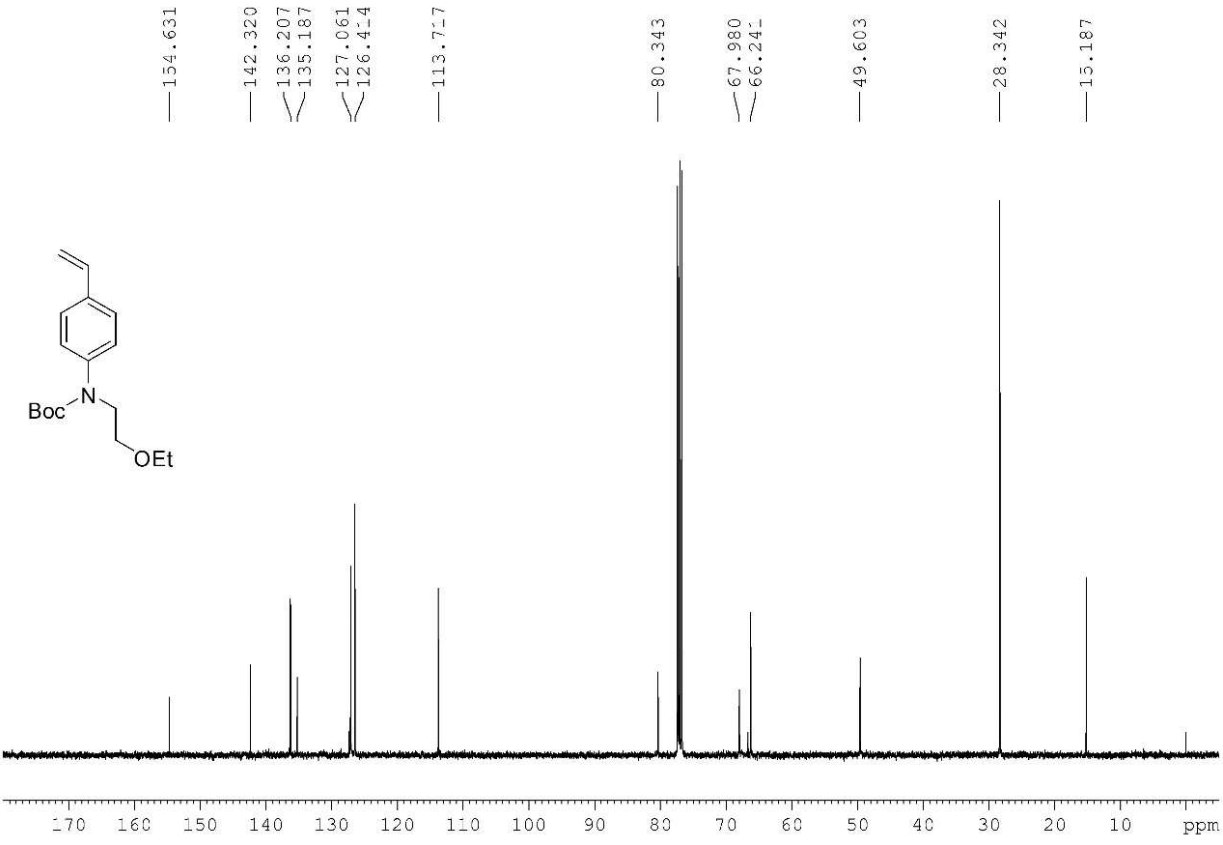
^{13}C NMR of **1** in $CDCl_3$



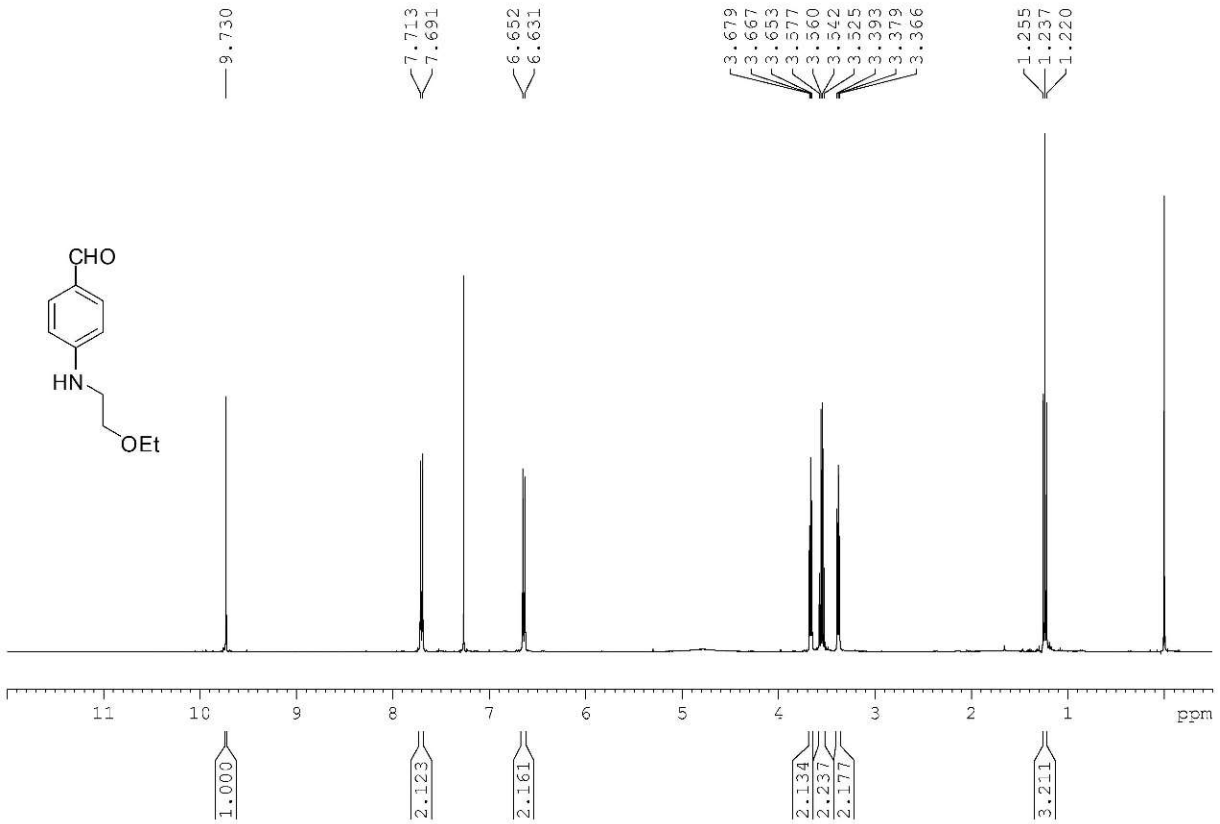
^{1}H NMR of **2** in $CDCl_3$



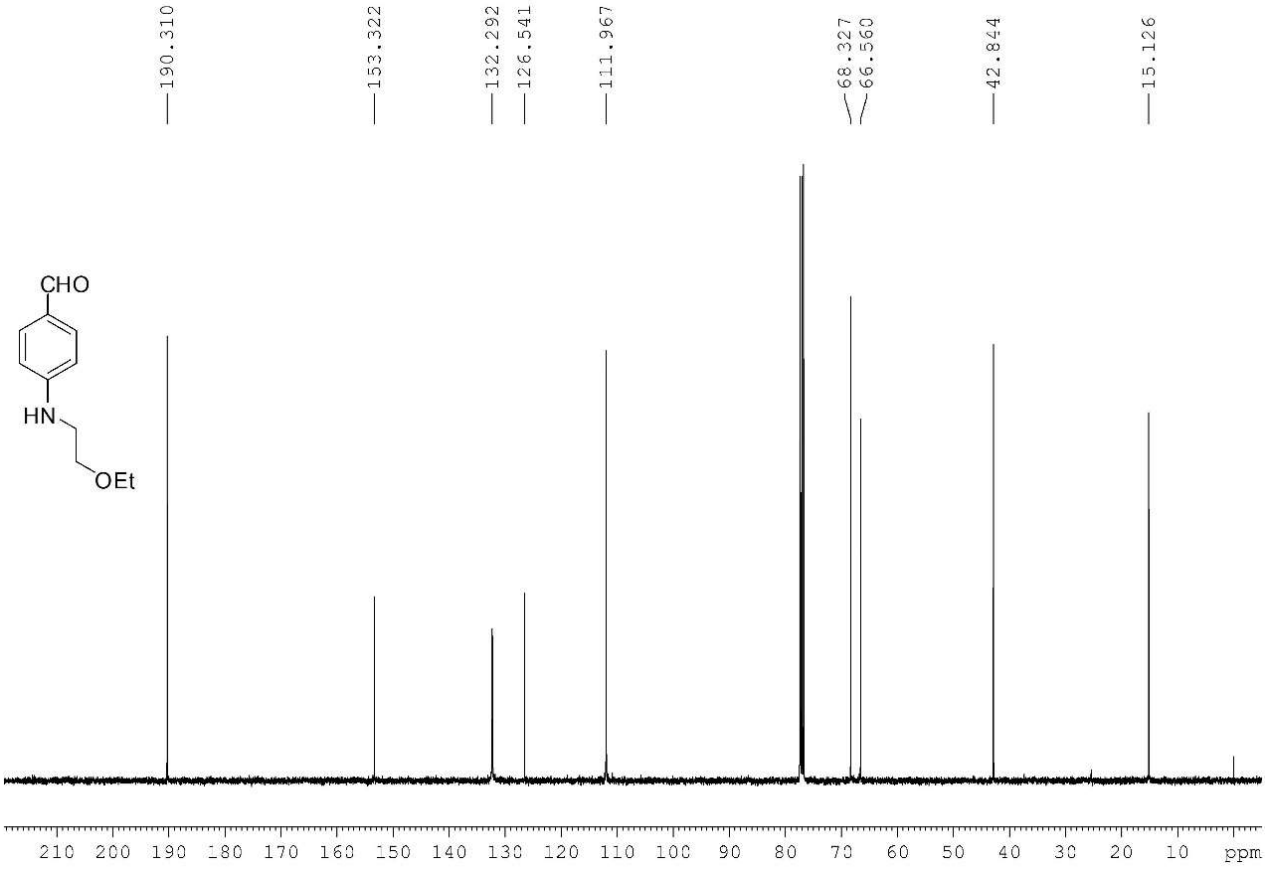
^{13}C NMR of **2** in $CDCl_3$



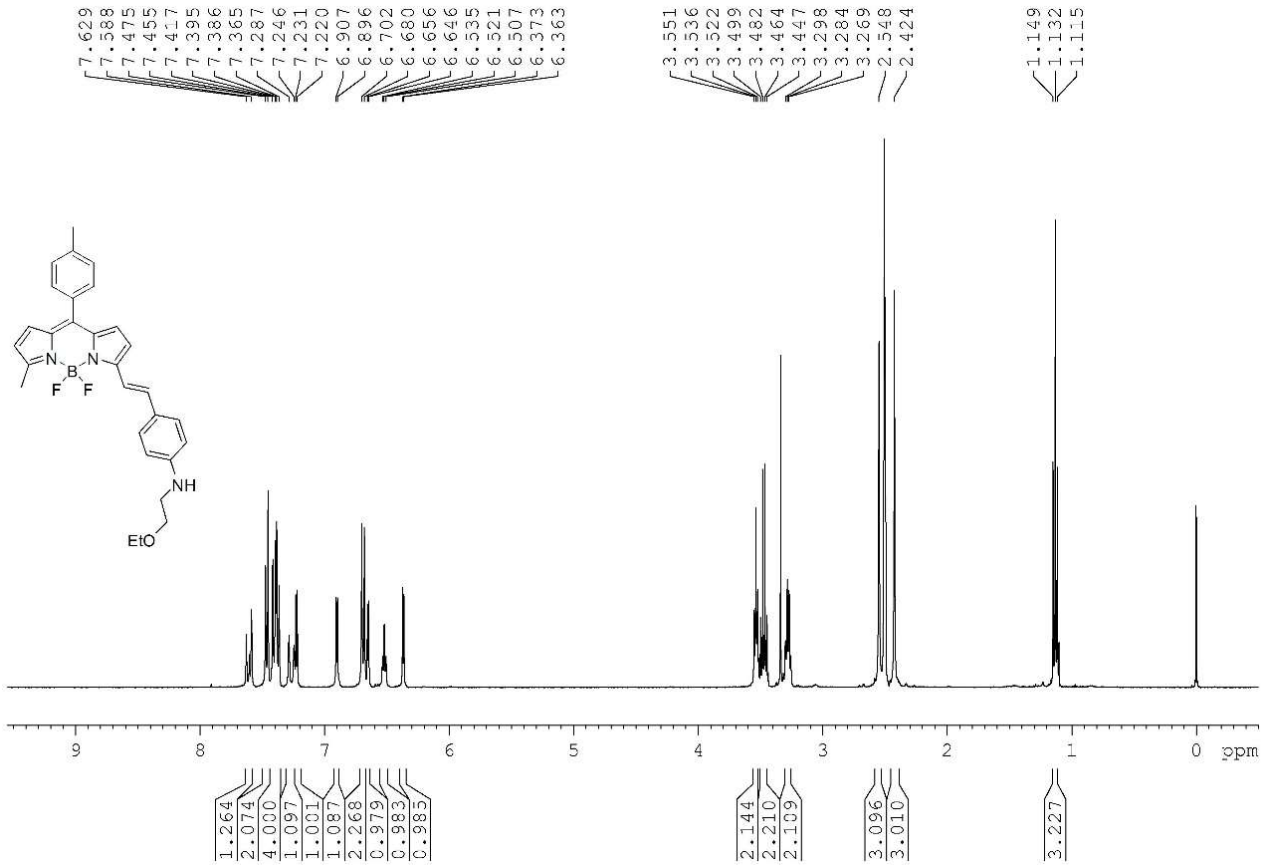
^{1}H NMR of **3** in $CDCl_3$



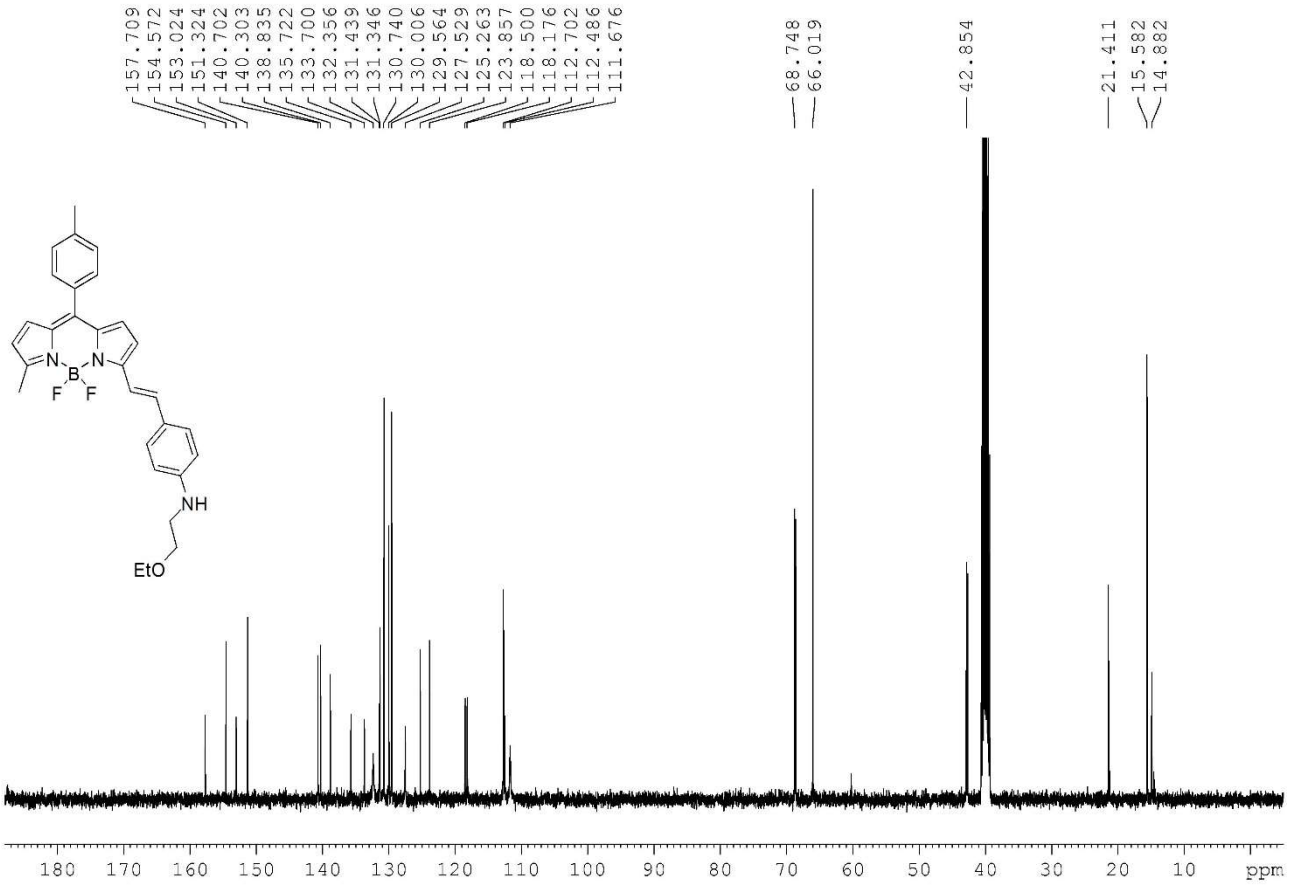
^{13}C NMR of **3** in $CDCl_3$



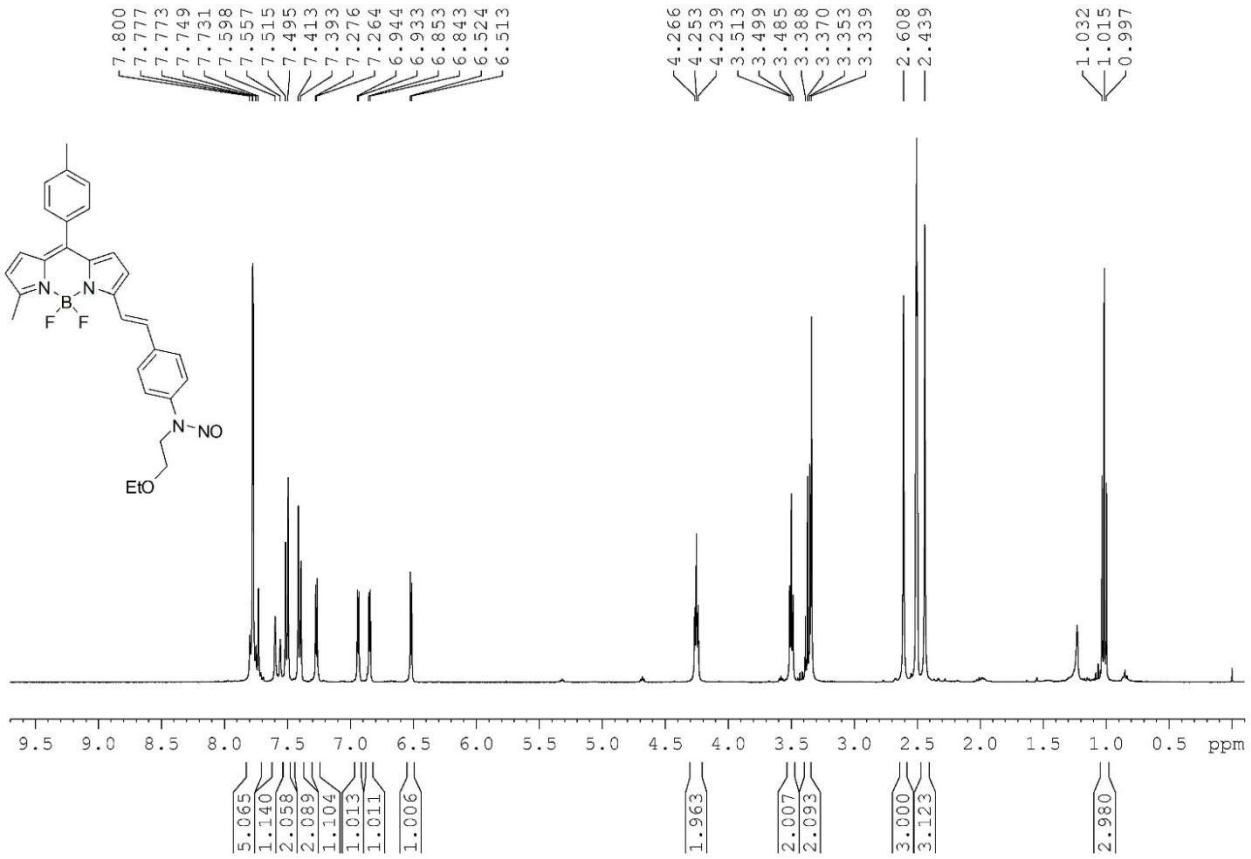
^{1}H NMR of **RBA** in DMSO-d_6



^{13}C NMR of **RBA** in DMSO-d_6



^{1}H NMR of **RBA-NO** in DMSO-d_6



^{13}C NMR of **RBA-NO** in DMSO-d_6