Supplementary Information

for

Biotinylated Piperazine-rhodol Derivative: a 'Turn-On' Probe for Nitroreductase Triggered Hypoxia Imaging

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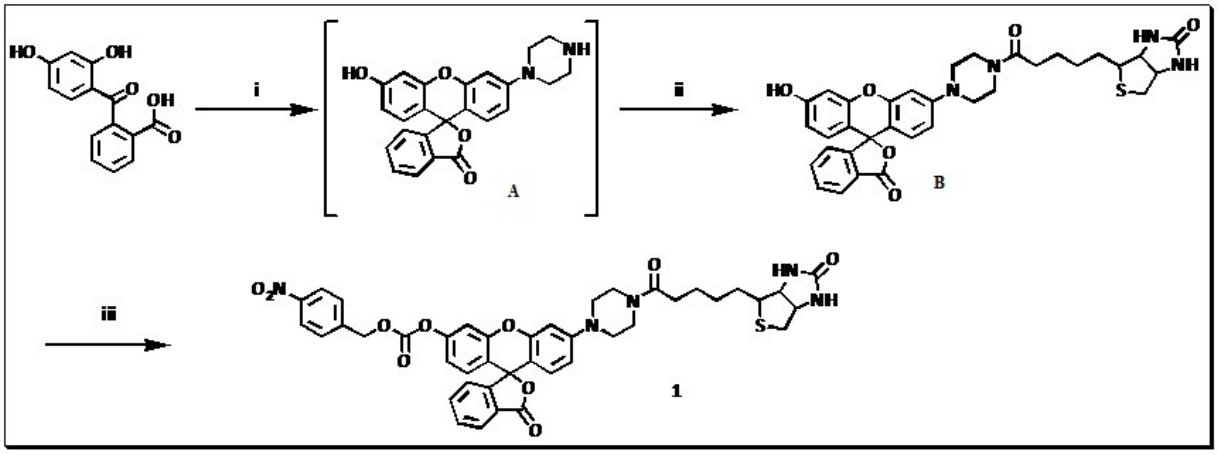
Reaction Scheme:



Scheme S1 Reagents and Reaction conditions: i) 1-(3-hydroxyphenyl)-piperazine, Sealed tube, TFA, 90 °C, 3 h ii) D-Biotin, HATU, DIPEA, RT, 12 h iii) a) 4-nitrophenylchloroformate, DIPEA, 0 °C-RT, 3 h, b) 4-Nitrobenzyl alcohol, Et_3N, DMF, RT, 12 h.

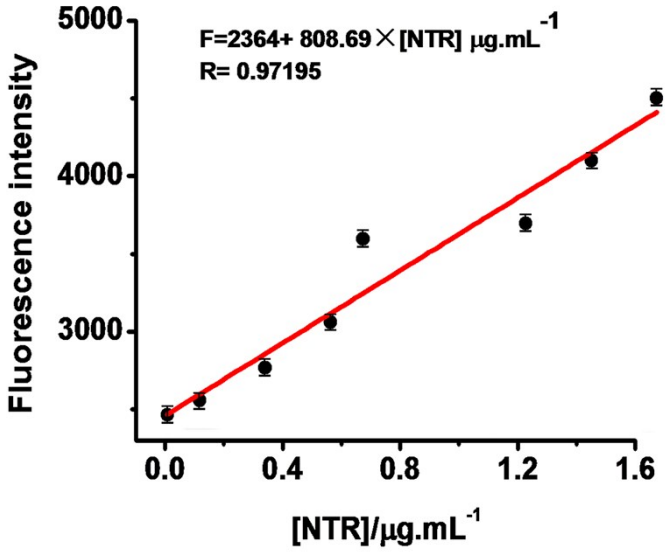


Fig. S1 The changes in the fluorescence intensity of **1** (2 μM) at 550 nm against varied concentrations of NTR from 0 to 1.5 μg/mL in DMSO−PBS buffer (0.01 M, pH 7.4) (V/V= 1:9) with the slit width 10/10 nm. The error bars were obtained from average data of three successive experiments.

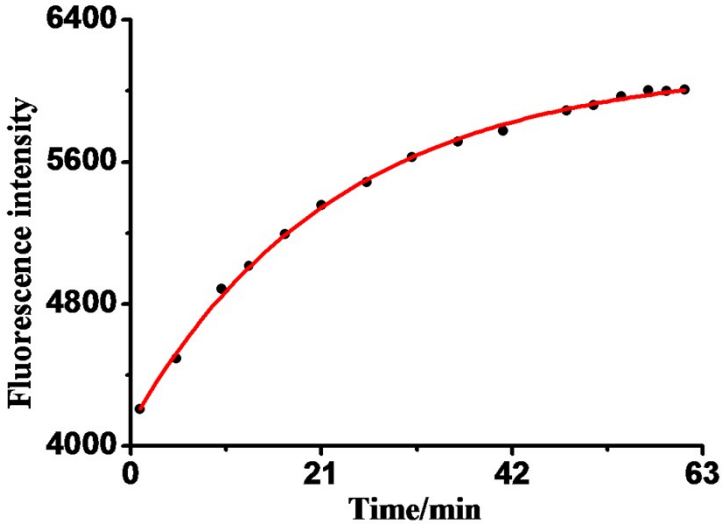


Fig. S2 Kinetic curve of **1** (20 μM) at 550 nm with NTR (15 μg/mL) in DMSO–PBS buffer (0.01 M, pH = 7.4) (V/V = 1:9), containing 0.1 mM NADPH as a coenzyme. The excitation wavelength (λ_{ex}) was 510 nm and the slit widths: 5 nm/5 nm.

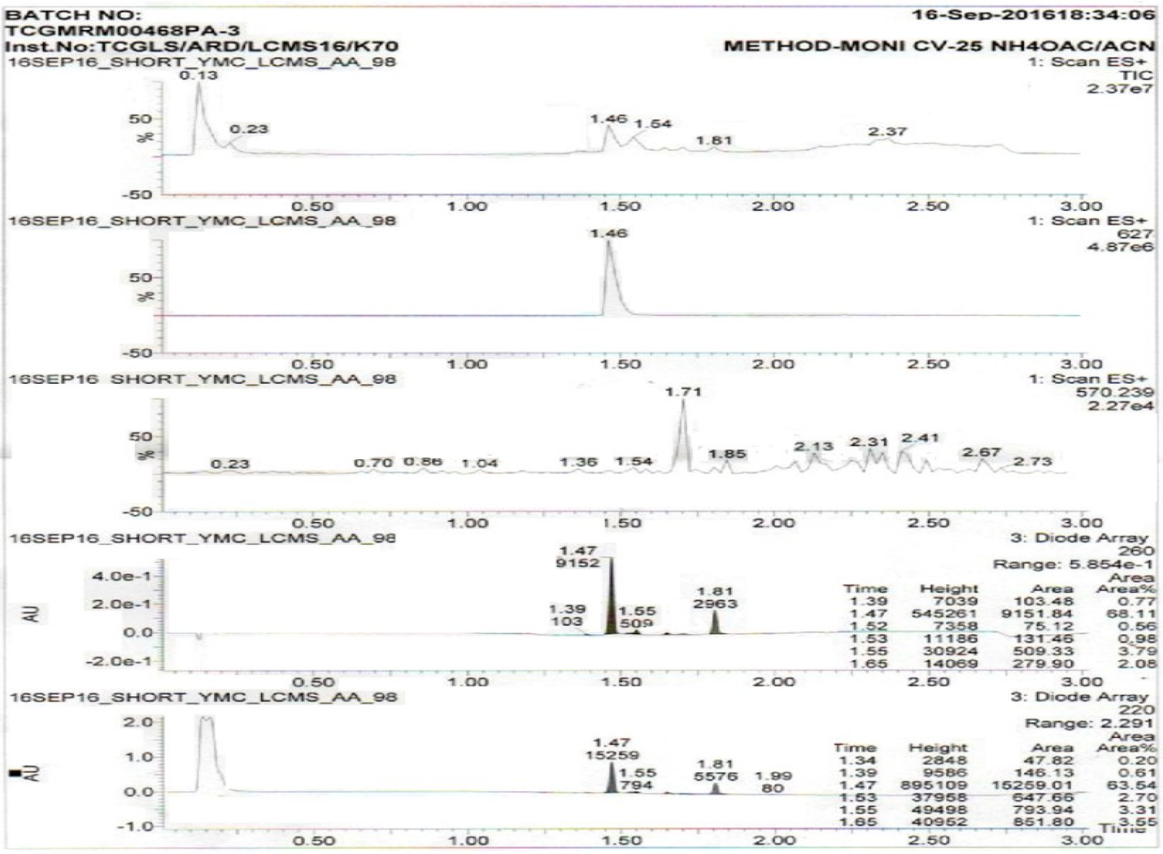
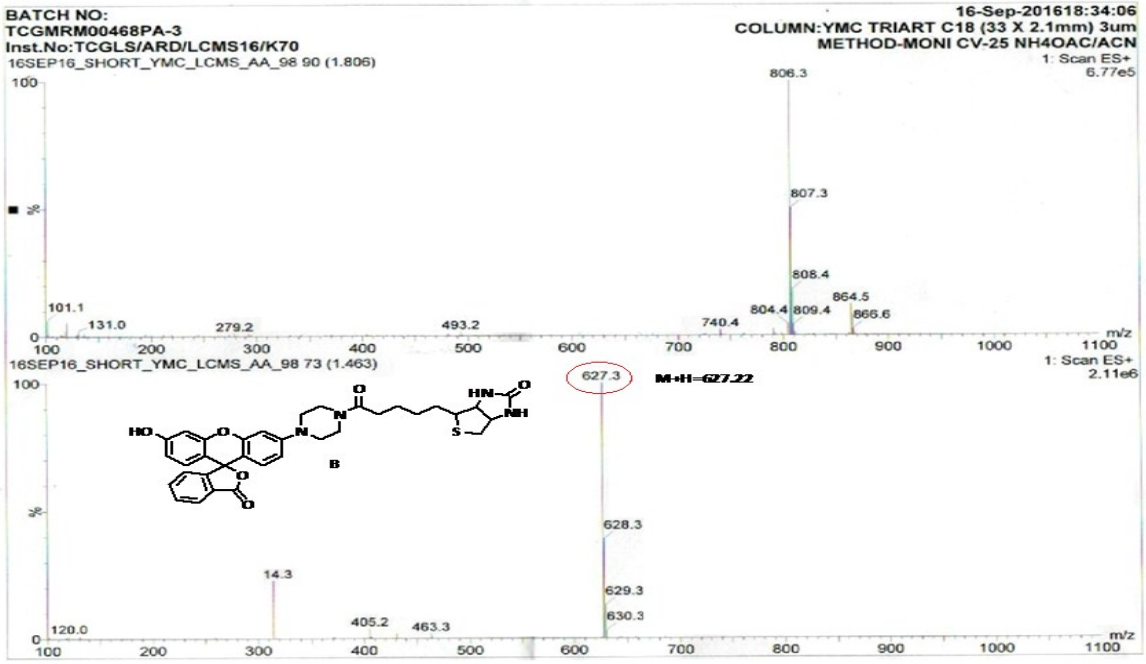


Fig.



S3. LC-MS data of the products after **1** was stirred with NTR and NADPH for 90 min in DMSO-PBS buffer (0.01 M, pH= 7.4) (V/V= 1:9).

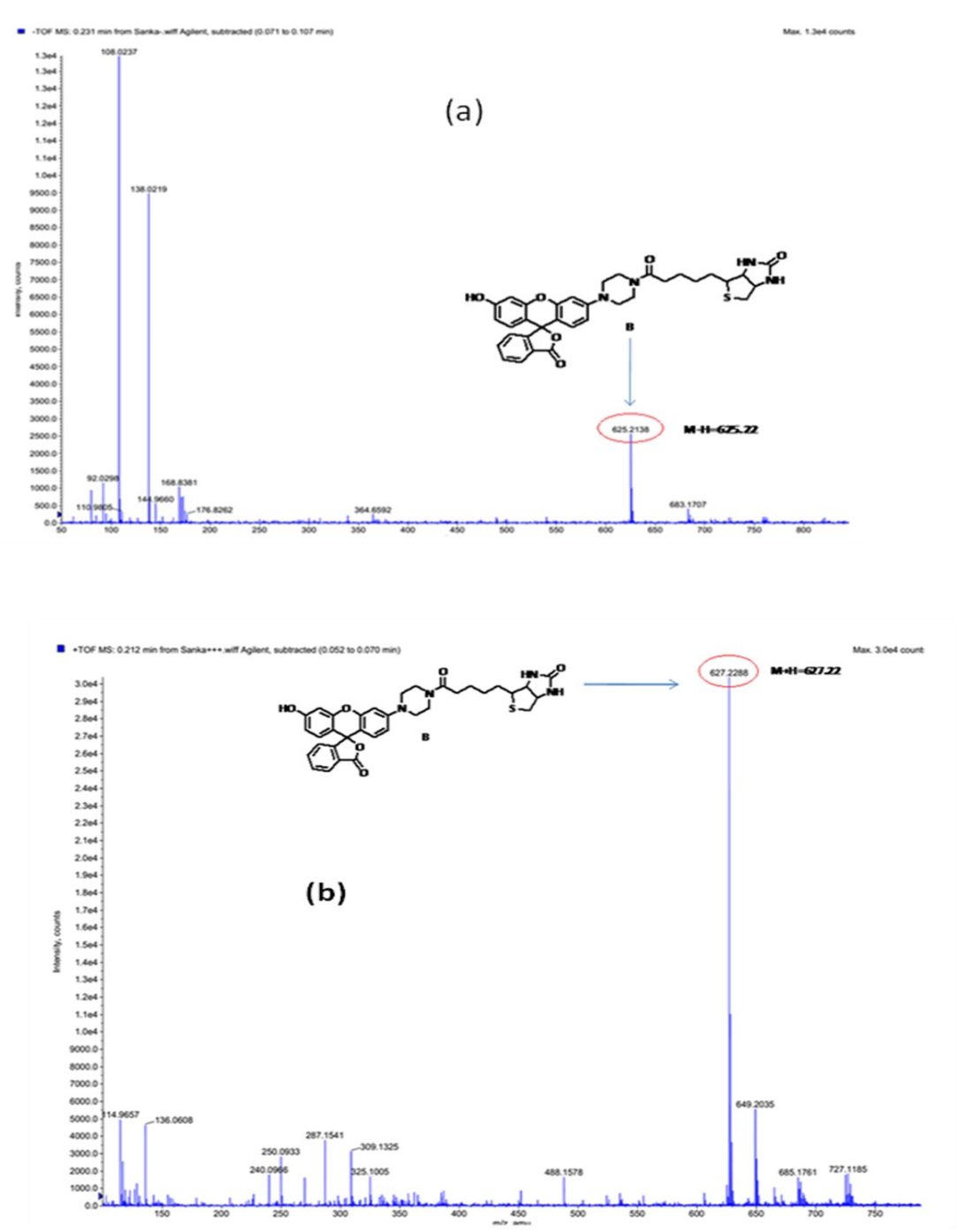


Fig. S4 (a) The TOF^{-} and (b) TOF^{+} tests of the products after **1** was stirred with NTR and NADPH for 90 min in DMSO-PBS buffer (0.01 M, pH= 7.4) (V/V= 1:9).

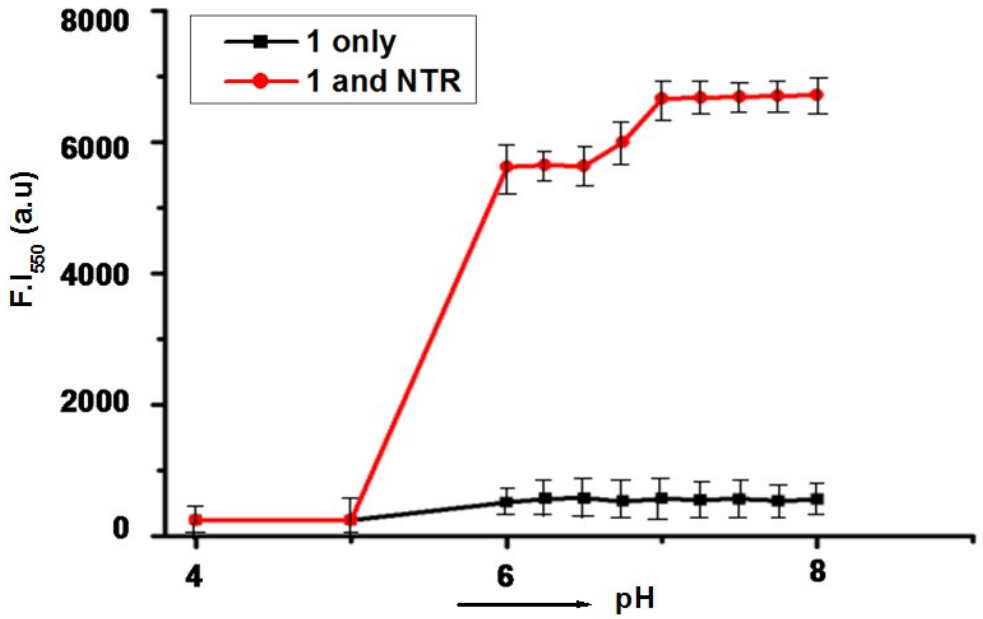


Fig. S5 Fluorescence response of **1** (20 μM) in the presence of NTR (15 ng/mL) and in absence of NTR in variable pH ranges (4-8). The excitation wavelength (λ_{ex}) was 510 nm and both the slit width set at 5 nm/5 nm. The error bars were obtained from average data of three successive experiments.

	Hacat	L6	Hepg2	A549	SKOV
EC50/μM	3.76	6.15	6.42	1.99	2.67

Table. S1 Cytotoxicity of **1** on different cells in presence NTR.

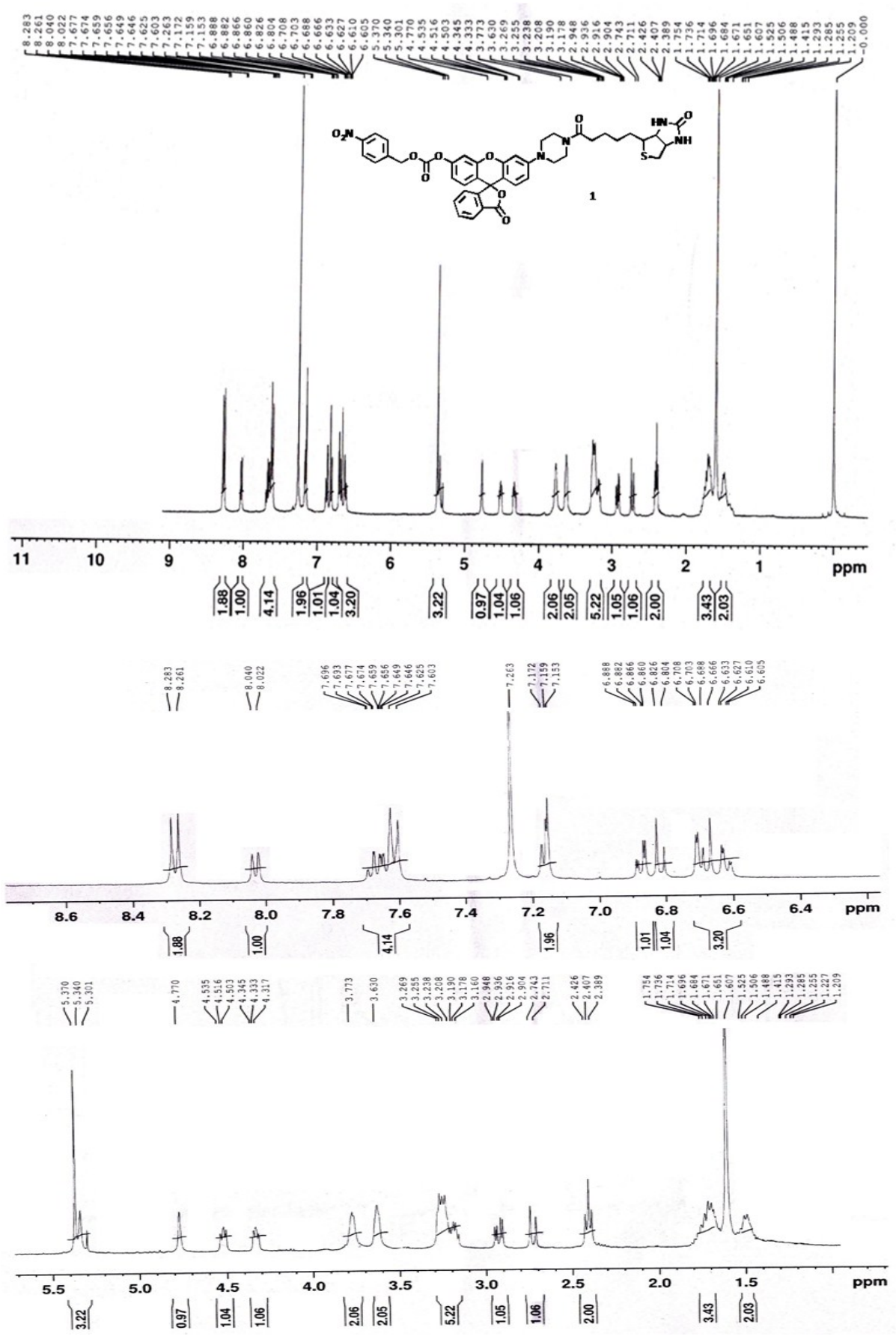


Fig. S6 ^{1}H-NMR of **1** in DMSO-d_6

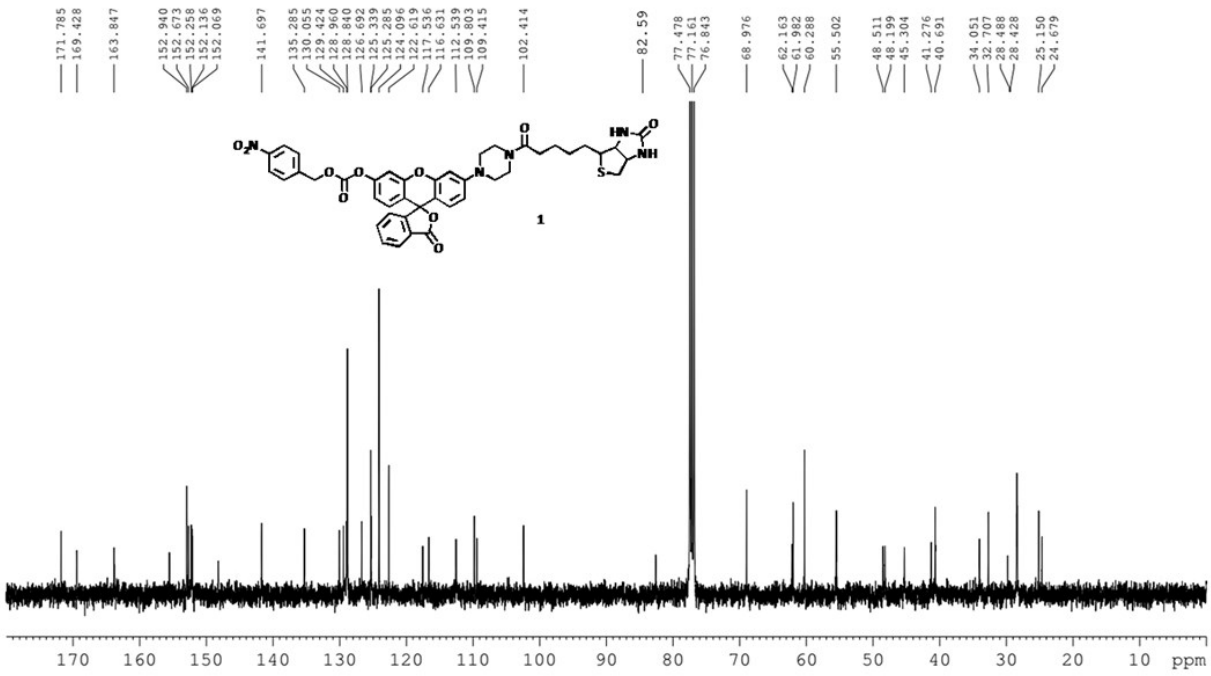


Fig.S7 ^{13}C-NMR of **1** in DMSO-d_6

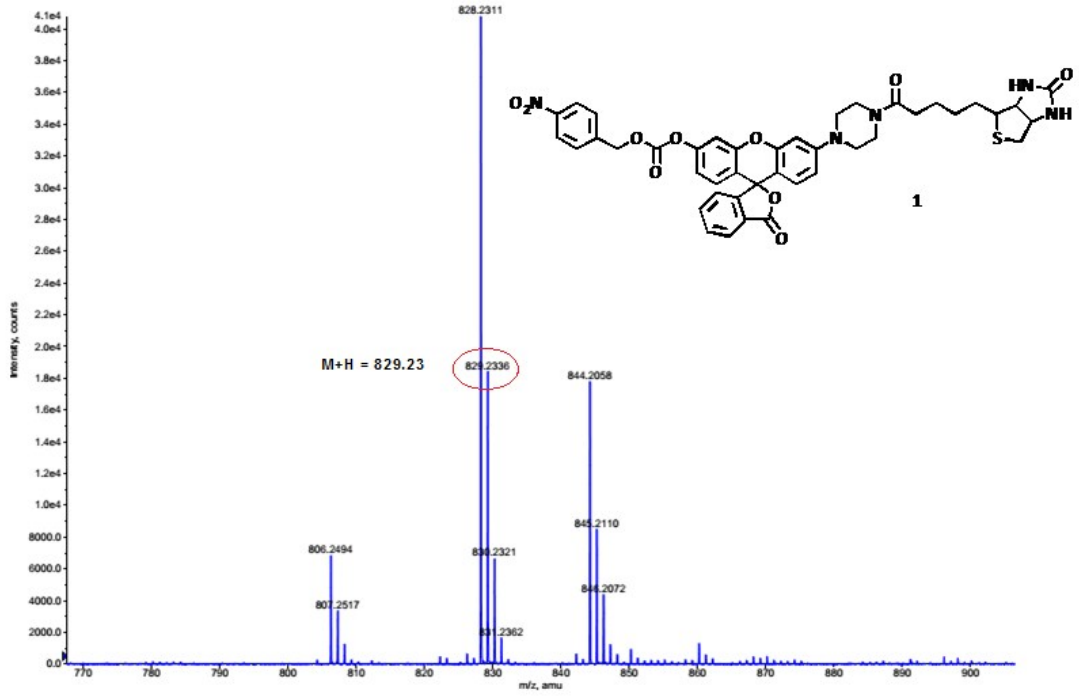
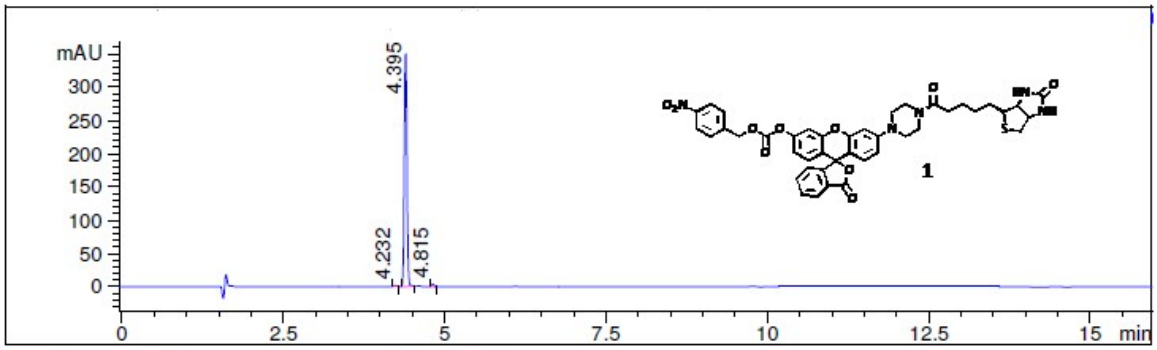


Fig.S8 HRMS of **1**

HPLC REPORT

olumn : X-BRIDGE C18 (4.6X150mm) 5.0µm
Mobile Phase : A:, 0.01M Ammonium acetate in Water , B: Acetonitrile
T/%B : 0/5 , 1.2/5,3/55,5/70, 7/95, 10/95, 12/100, 14/5, 16/5
Flow Rate : 1.2 mL/min
Temp : 35°C
Diluent : ACN+H2O



Peak #	RT [min]	Area	Area %
1	4.232	7.011	0.671
2	4.395	1.025e3	98.106
3	4.815	12.766	1.222

Fig. S9 HPLC of **1**