Real-time Monitoring Etoposide Prodrug Activated by Hydrogen Peroxide with Improved Safety

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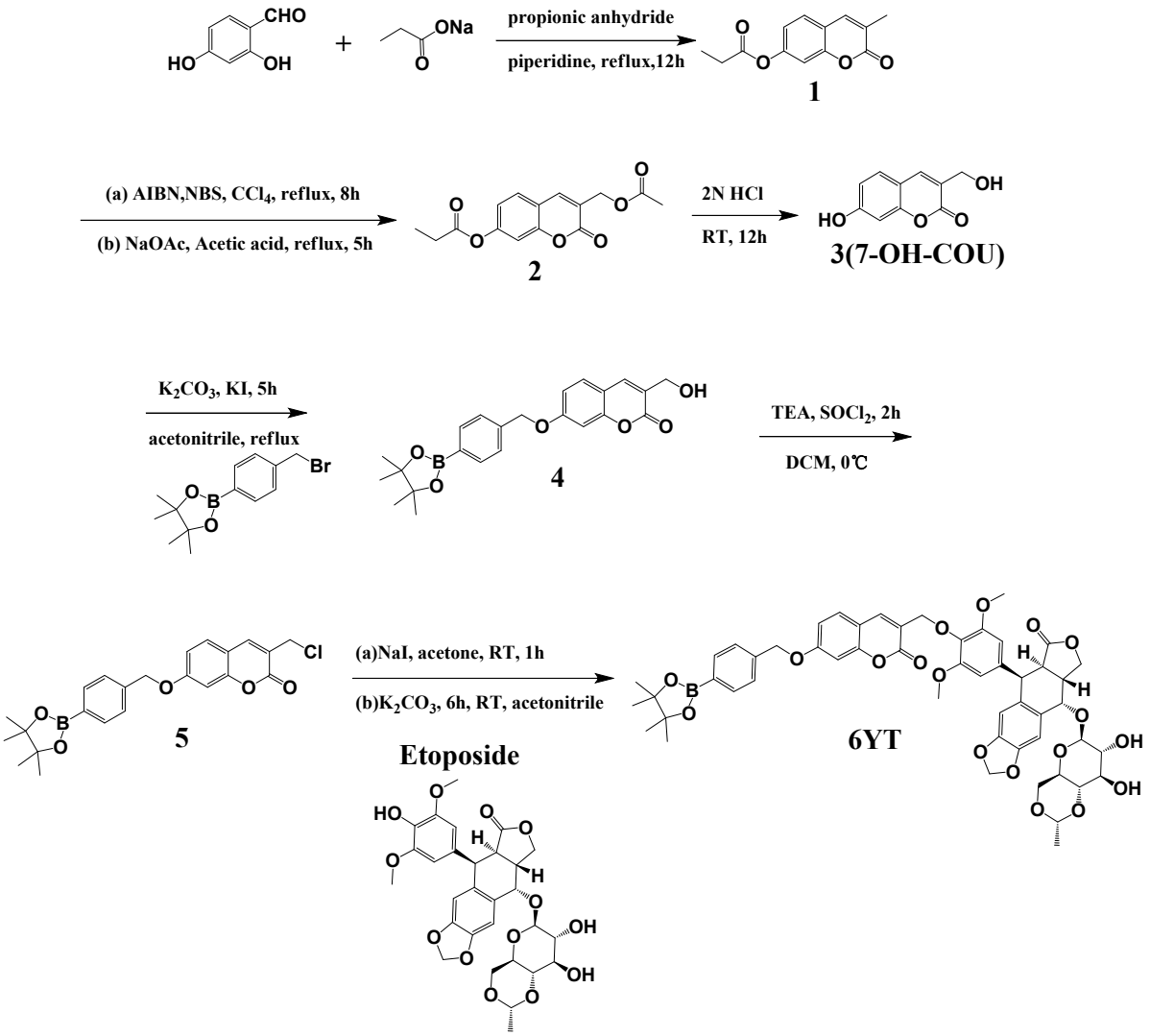
Supporting Information

Contents

1. Synthetic procedure of 6YT



Scheme S1. Synthesis of **6YT**

2. The spectra of ^{1}H NMR, ^{13}C NMR

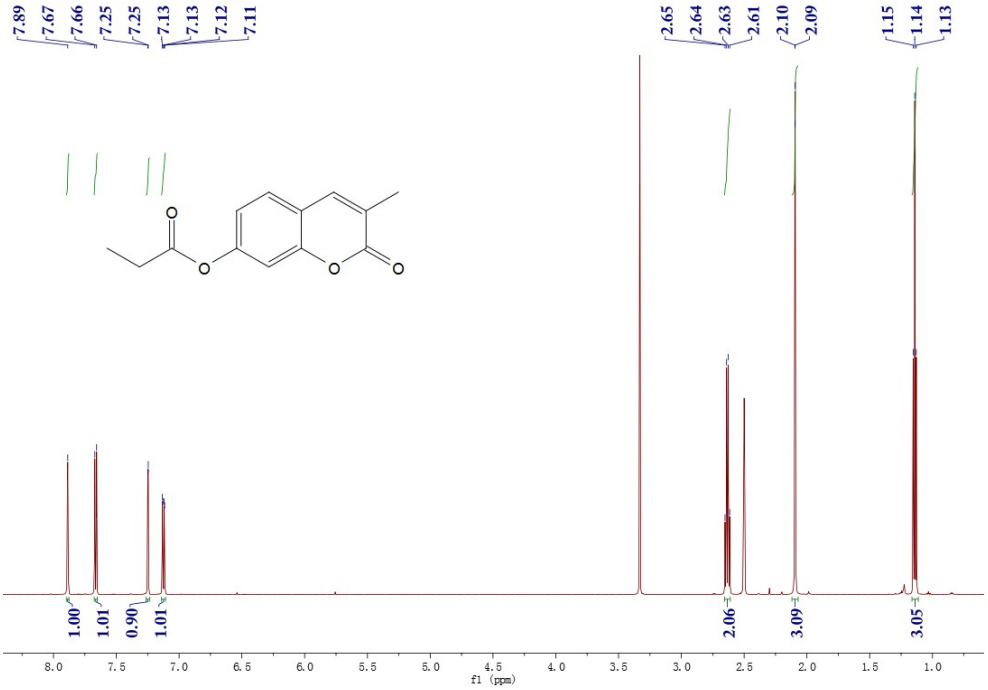


Figure S1.^{1}H NMR spectrum (600 MHz) of compound **1** in DMSO-d_6.

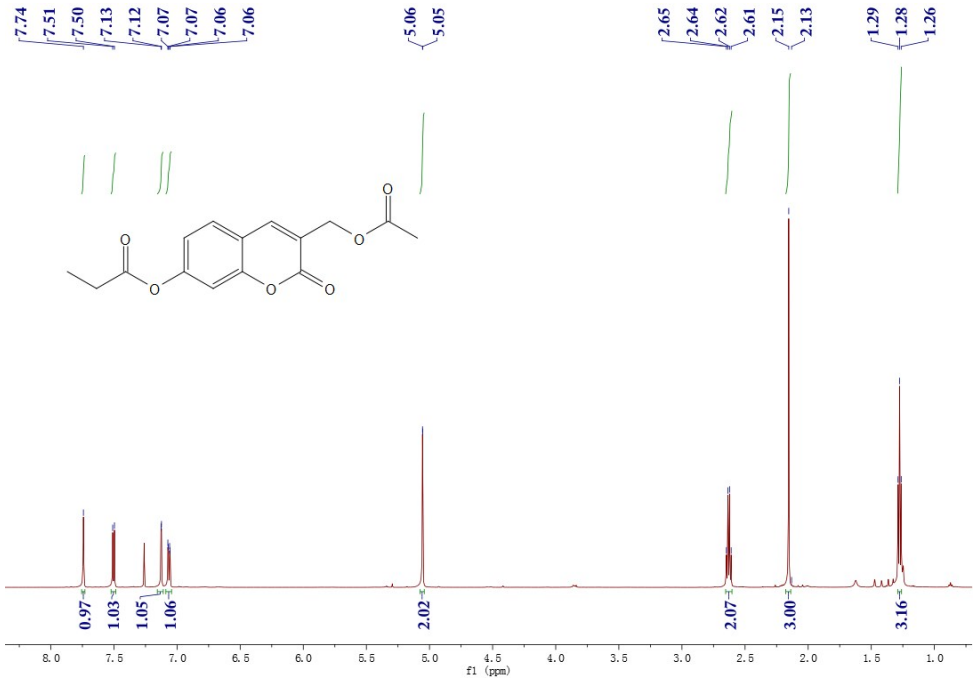


Figure S2.^{1}H NMR spectrum (600 MHz) of compound **2** in $CDCl_3$.

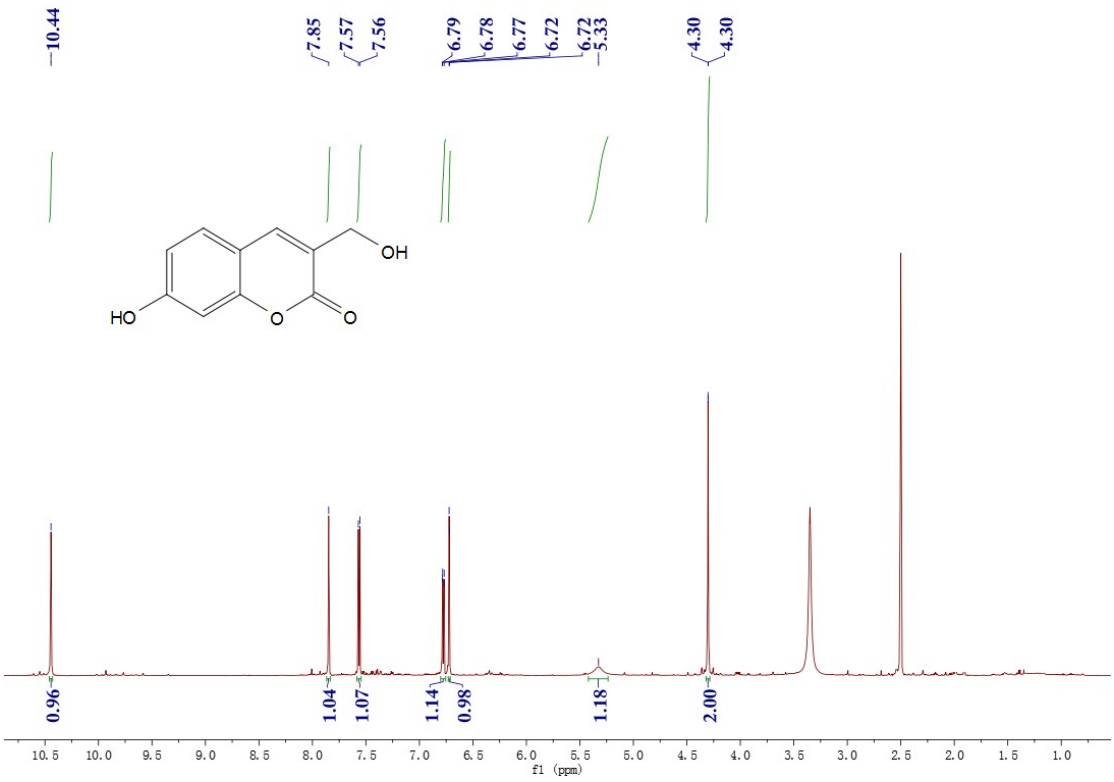


Figure S3.[1]H NMR spectrum (600 MHz) of compound **3** in DMSO-d_6.

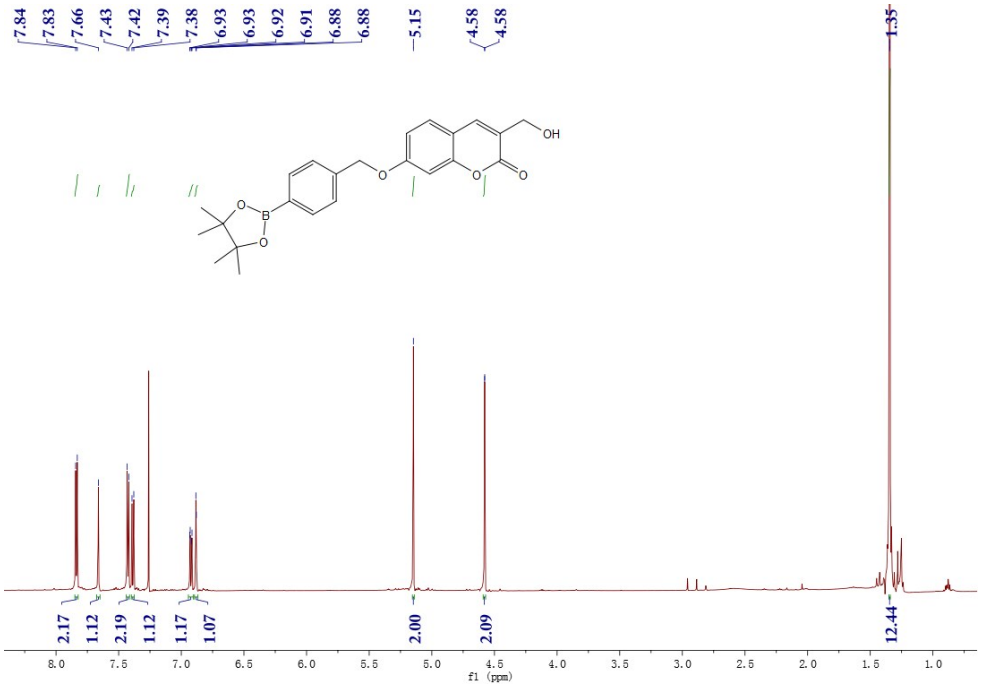


Figure S4.[1]H NMR spectrum (600 MHz) of compound **4** in $CDCl_3$.

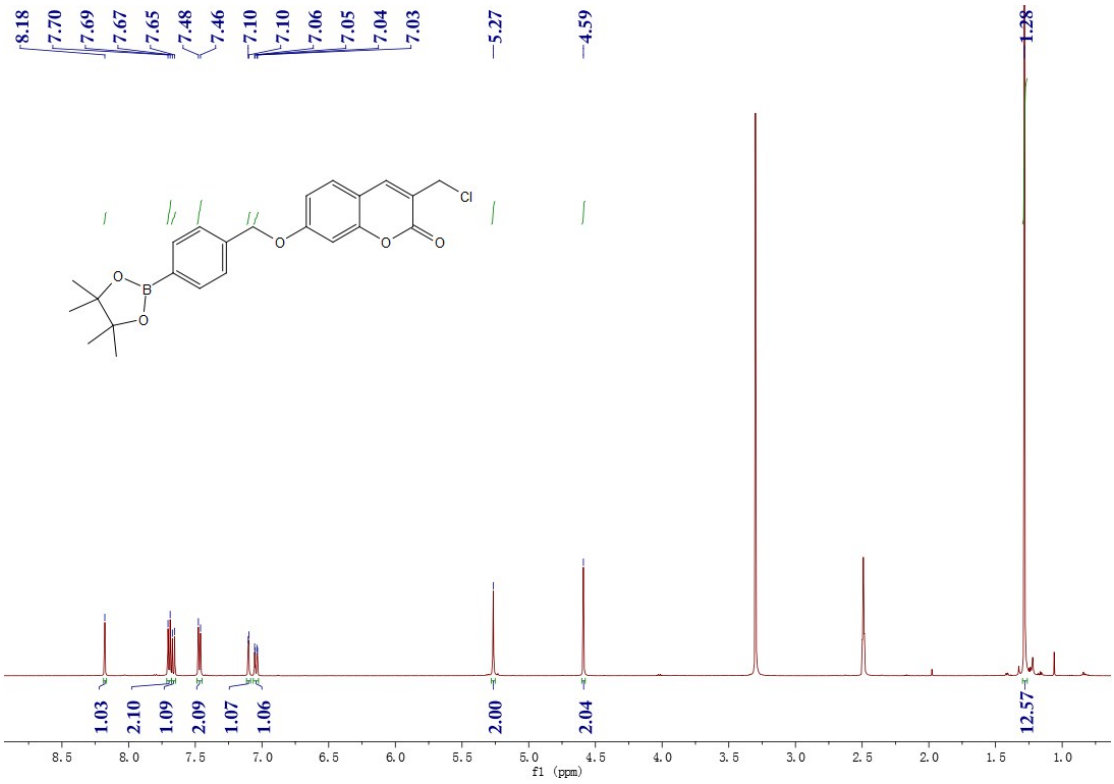


Figure S5.[1]H NMR spectrum (500 MHz) of compound **5** in DMSO-d_6.

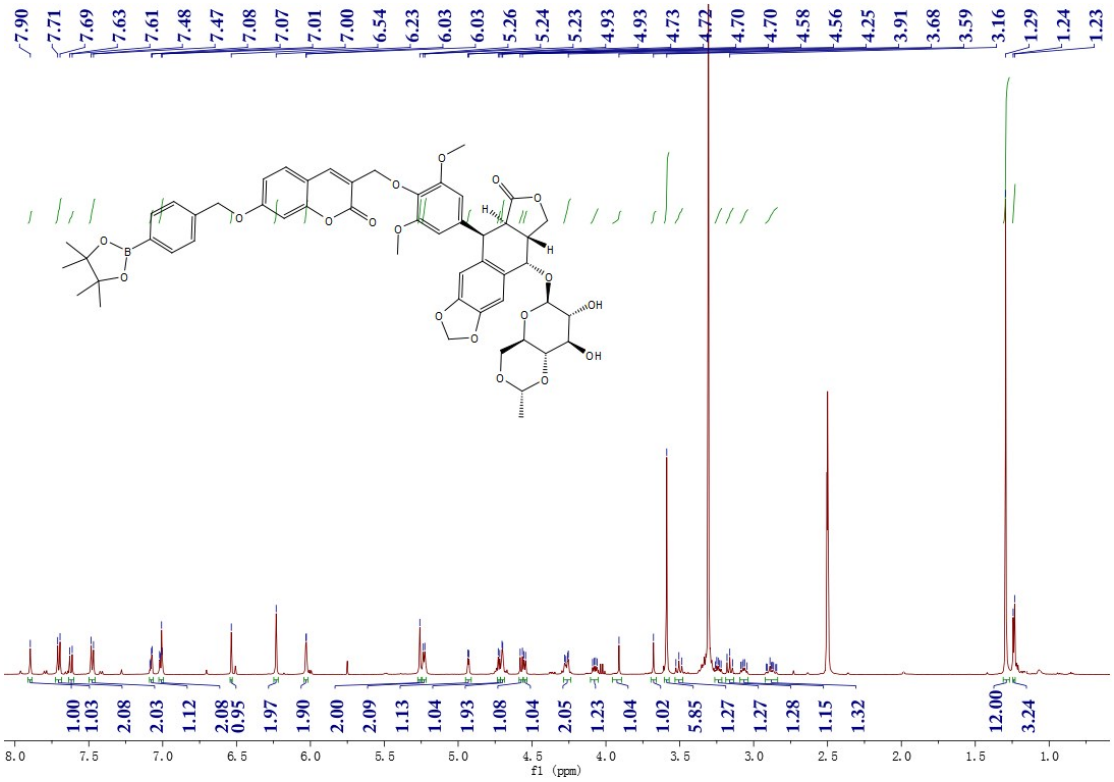


Figure S6.[1]H NMR spectrum (500 MHz) of **6YT** in DMSO-d_6.

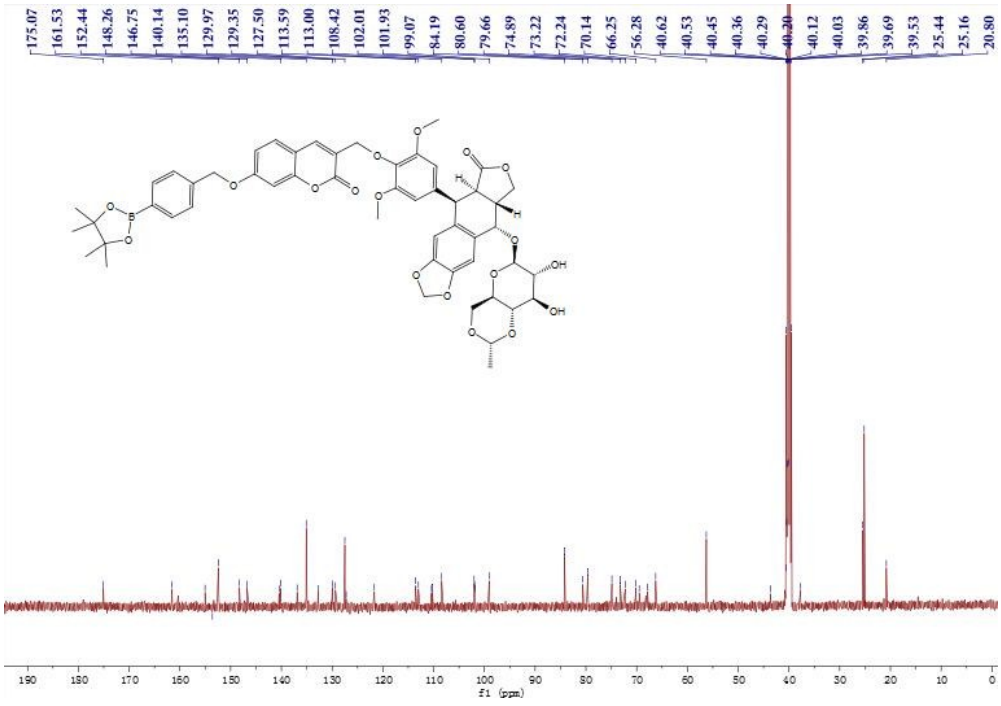
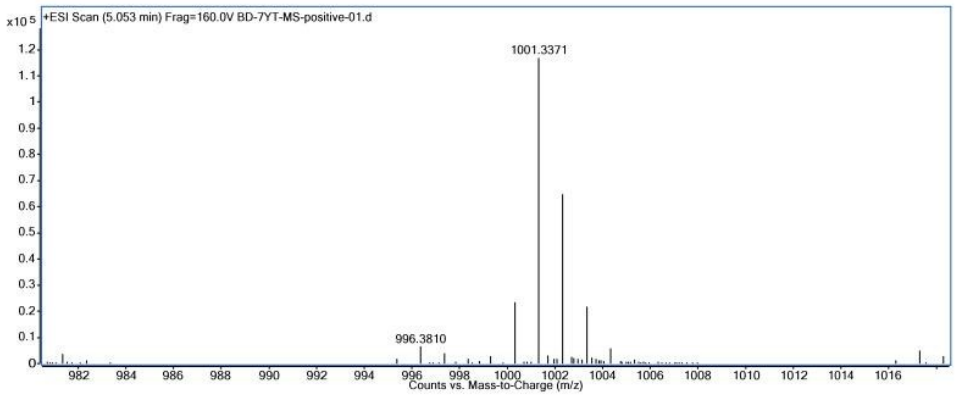


Figure S7.^{13}C NMR spectrum (126 MHz) of **6YT** in DMSO-d_6.

3. HR-ESI-MS spectrum of compound 6YT



Elemental Composition Calculator

Target m/z:	1001.3371	**Result type:**	Positive ions	**Species:**	$[M+Na]^+$
Elements:		C (0-80); H (0-120); O (0-30); Na (0-5) ; B(0-5)			
Ion Formula		**Calculated m/z**		**PPM Error**	
C52H55[11B]NaO18		1001.3374		0.23	

Figure S8.HR-ESI-MS spectrum of compound **6YT**.

4. pH dependence of 6YT in the absence and presence of H_2O_2

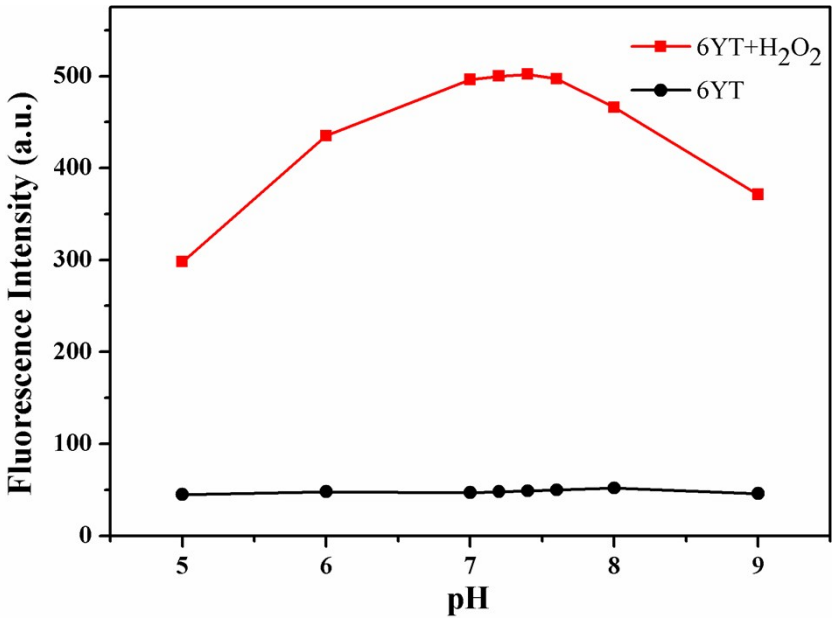


Figure S9.Fluorescence emission intensity (458 nm) of 6YT (10 µM) and 6YT + H_2O_2 (300 µM) to various pH (5.0-9.0) in PBS buffer (10 mM, pH 7.4, 0.1% DMSO) after incubation for 60 min.

5. Monitoring of Etoposide release by RP-HPLC

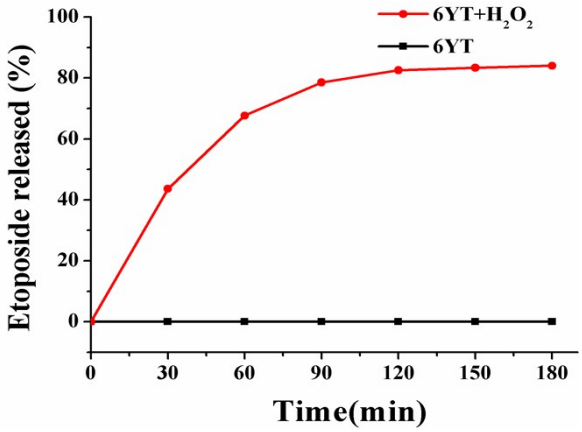


Figure S10.Etoposide release (%) from **6YT** (100µM) in PBS (pH 7.4) in the presence (red squares) or absence (black circles) of H_2O_2 (10mM).

6. Fluorescence image of compound 6YT activated by different concentration of exogenous H_2O_2

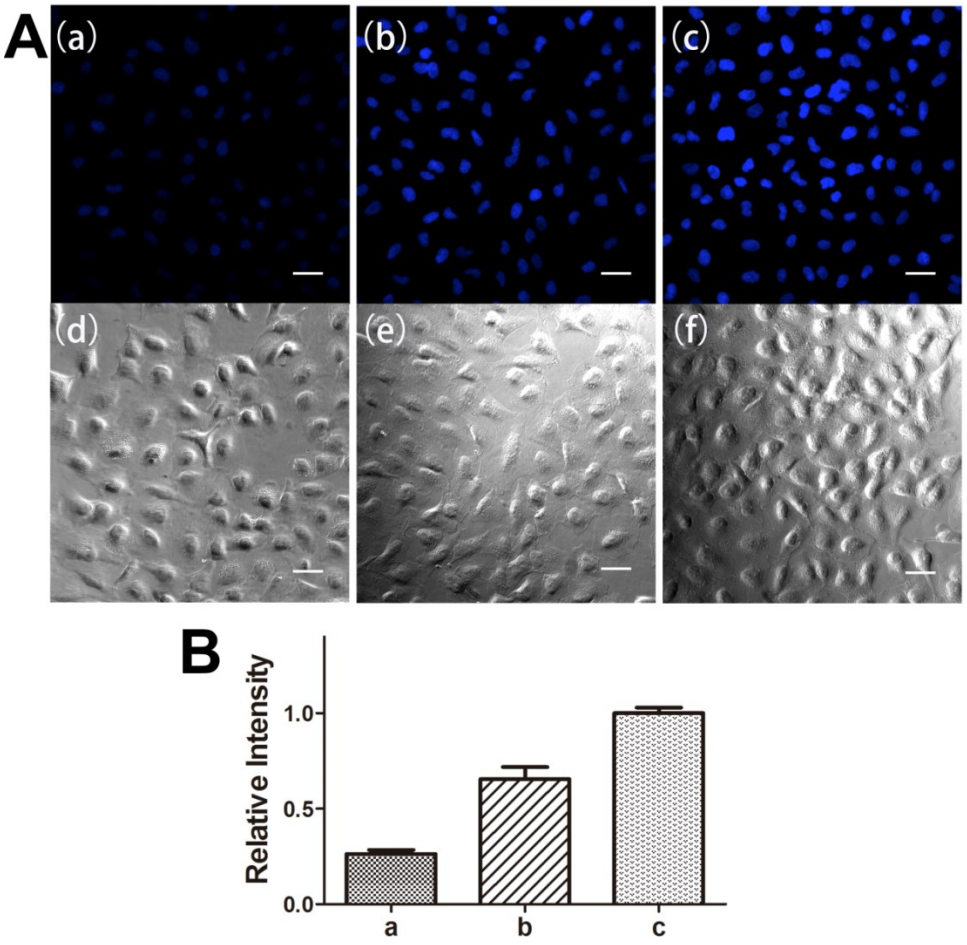


Figure S11. (A) Fluorescence images of A549 cell lines treated with increasing concentrations of H_2O_2 (0, 100 and 200 μM) for 2h after incubated with compound **6YT** (10 μM) for another 2 h(a-c), and the bright field(d-f). Scale bar = 20 μm. (B) The relative fluorescence intensities of a-c were measured at three regions in each dish. Error bars represent standard deviation (n = 3).

7. Fluorescence image of compound 6YT activated by endogenous H_2O_2 in different cell lines.

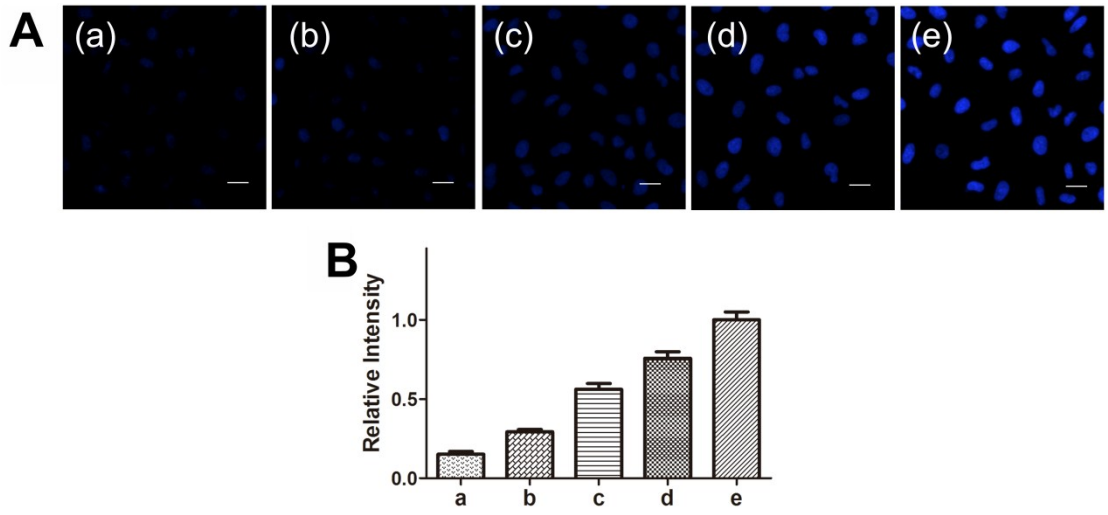


Figure S12. (A) Fluorescence images of A549 cell lines incubated with compound **6YT** (10 μM) for (a)0h, (b)2 h, (c)4h, (d)8h and (e)12h. Scale bar = 20 μm. (B) The relative fluorescence intensity of a-e were measured at three regions in each dish. Error bars represent standard deviation (n = 3).

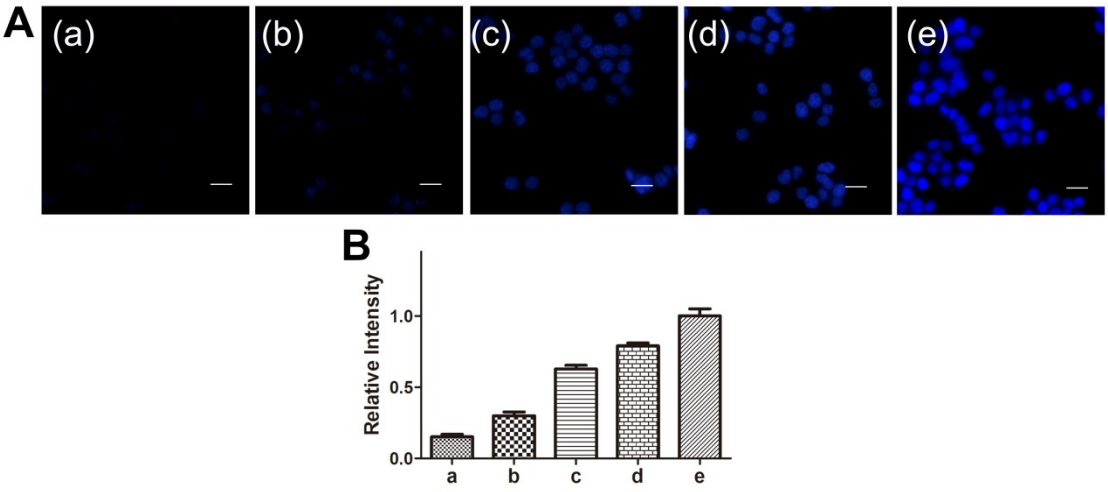


Figure S13. (A) Fluorescence images of HCT-116 cell lines incubated with

compound **6YT** (10 μM) for (a)0h, (b)2 h, (c)4h, (d)8h and (e)12h. Scale bar = 20 μm. (B) The relative fluorescence intensity of a-e were measured at three regions in each dish. Error bars represent standard deviation (n = 3).

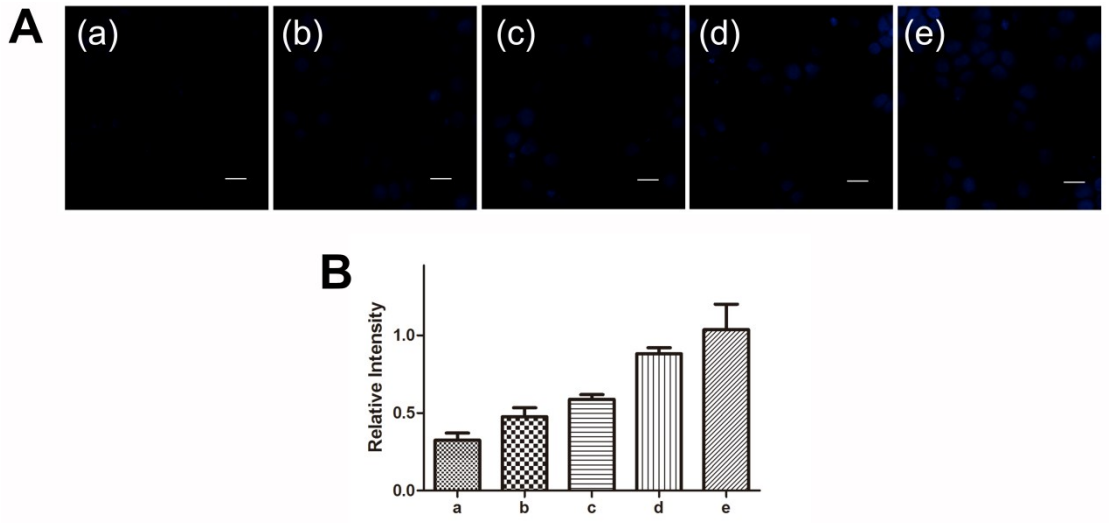


Figure S14. (A) Fluorescence images of MCF-10A cell lines incubated with compound **6YT** (10 μM) for (a)0h, (b)2 h, (c)4h, (d)8h and (e)12h. Scale bar = 20 μm. (B) The relative fluorescence intensity of a-e were measured at three regions in each dish. Error bars represent standard deviation (n = 3).

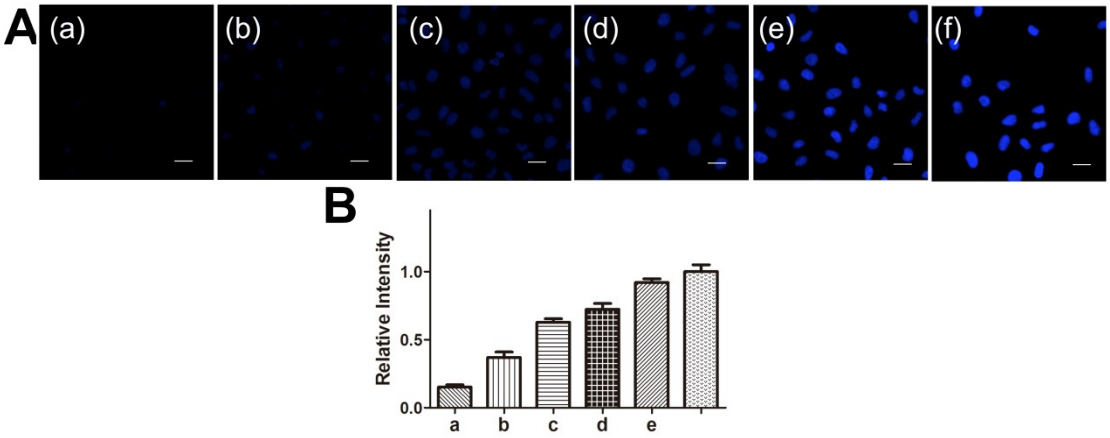


Figure S15. (A) Fluorescence images of A549 cell lines incubated with

increasing concentration of compound **6YT** (a)0μM, (b)1.25μM, (c)2.5μM, (d)5μM, (e)10μM and (f)20μM for 12h. Scale bar = 20 μm. (B) The relative fluorescence intensity of a-f were measured at three regions in each dish. Error bars represent standard deviation (n = 3).