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

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Supporting Information

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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

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Figure S8. HR-ESI-MS spectrum of compound 6YT.
4. pH dependence of 6YT in the absence and presence of H$_2$O$_2$

![Graph showing fluorescence intensity vs pH](image)

**Figure S9.** Fluorescence emission intensity (458 nm) of 6YT (10 µM) and 6YT + H$_2$O$_2$ (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

![Graph showing etoposide release vs time](image)

**Figure S10.** Etoposide release (%) from 6YT (100µM) in PBS (pH 7.4) in the presence (red squares) or absence (black circles) of H$_2$O$_2$ (10mM).
6. Fluorescence image of compound 6YT activated by different concentration of exogenous H$_2$O$_2$

![Image](image.png)

**Figure S11.** (A) Fluorescence images of A549 cell lines treated with increasing concentrations of H$_2$O$_2$ (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 $\text{H}_2\text{O}_2$ 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).