

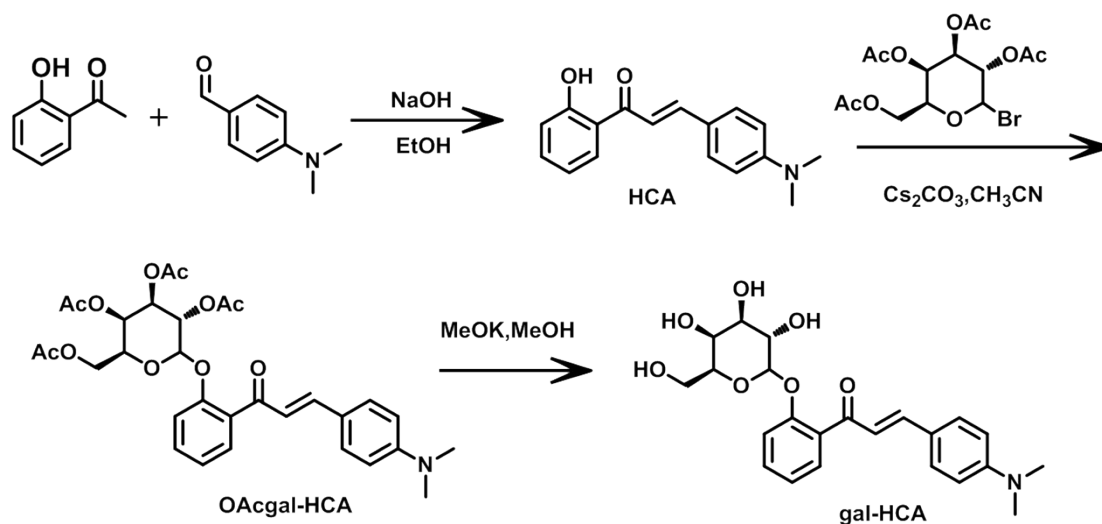
## Supporting Information

### A Chalcone-based ES IPT and AIE Fluorophore for $\beta$ -galactosidase imaging in living cells

Yiran Hu, Haiyan Luo, Luyao Zhao, Xudong Guo, Shuangqing Wang, Rui Hu\* and Guoqiang Yang\*

*Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Photochemistry, Institute of Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100190, China.*

#### 1. Material Synthesis and Characterization



**Scheme S1.** Synthetic route of the probe **gal-HCA**

**Synthesis of OAc-gal-HCA:** A solution of HCA (270 mg, 1 mmol, 1 equiv.) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was treated with Cs<sub>2</sub>CO<sub>3</sub> (650 mg, 2 mmol, 2 equiv.) and stirred at room temperature for 15 min under N<sub>2</sub>. 2,3,4,6-Tetra-O-acetyl- $\alpha$ -D-galactopyranosyl bromide (400 mg, 1 mmol, 1 equiv.) soluted in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was then added dropwise and the reaction was stirred for 8 h. The reaction was diluted with brine (20 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  25 mL). The

combined organic layers were dried over CaCl<sub>2</sub>, filtered, concentrated, and purified by silica gel column chromatography to obtain yellow solid **OAc-gal-HCA** (376 mg, 63%). <sup>1</sup>H-NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 7.53-7.47 (m, 3H), 7.35 (d, *J* = 7.29 Hz, 1H), 7.29-7.13 (m, 3H), 6.89 (d, *J* = 15.89 Hz, 1H), 6.71 (d, *J* = 8.90 Hz, 2H), 5.50 (d, *J* = 7.60 Hz, 1H), 5.32 (s, 1H), 5.24 (m, 1H), 5.14 (m, 1H), 4.44 (t, *J* = 5.66 Hz, 1H), 4.12 (d, *J* = 5.99 Hz, 2H), 2.99 (s, 6H), 2.10 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.90 (s, 3H), 1.86 (s, 3H). <sup>13</sup>C-NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 193.54, 170.30, 170.21, 170.03, 169.21, 154.43, 151.95, 146.52, 132.52, 131.30, 130.56, 129.36, 123.70, 122.43, 117.62, 111.92, 100.88, 77.36, 77.24, 77.04, 76.72, 71.18, 70.92, 68.19, 66.94, 61.38, 40.18, 20.66, 20.63, 20.60, 20.50. ESI-MS: (m/z) [M]<sup>+</sup> Calcd for C<sub>31</sub>H<sub>35</sub>NO<sub>11</sub> 597.221, found: 597.220

**Synthesis of gal-HCA:** **OAc-gal-HCA** (300 mg, 0.5 mmol, 1 equiv.) and sodium methoxide (56 mg, 1.1 mmol) was dissolved in methanol (5 ml) and stirred at room temperature for 5h. Then, the pH of mixture was adjusted to neutral. After purification by silica gel column (DCM/methanol, v:v = 20:1), **gal-HCA** was obtained as yellow solid (174 mg 0.41 mmol 82%). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>) δ 7.63(d, *J* = 8.12 Hz, 2H), 7.60-7.47 (m, 3H), 7.31(d, *J* = 8.62 Hz, 1H), 7.10(t, *J* = 6.63 Hz, 1H), 6.73(d, *J* = 8.62 Hz, 2H), 5.11(d, *J* = 7.62 Hz, 1H), 5.07(d, *J* = 5.14 Hz, 1H), 4.91(d, *J* = 4.97 Hz, 1H), 4.67(t, *J* = 4.97 Hz, 1H), 4.63(d, *J* = 3.45 Hz, 1H), 3.74(s, 1H), 3.64-3.45(m, 5H), 2.99(s, 3H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 191.17, 156.31, 152.29, 143.81, 133.09, 131.09, 130.69, 130.31, 122.74, 122.33, 122.05, 116.29, 112.39, 101.83, 76.30, 74.05, 71.10, 68.59, 60.90, 40.03. ESI-MS: m/z [M+Na]<sup>+</sup> Calcd for C<sub>23</sub>H<sub>27</sub>NNaO<sub>7</sub><sup>+</sup> 452.168, found 452.1861

## 2. Tables

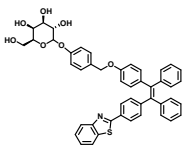
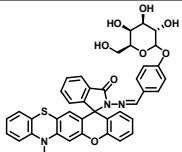
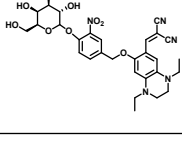
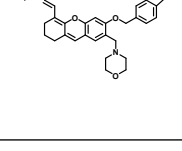
**Table S1** Photophysical properties of **HCA** and **gal-HCA** in different solvent.

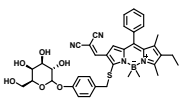
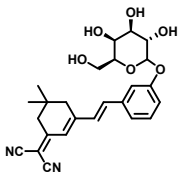
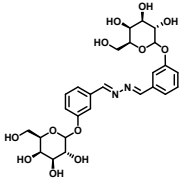
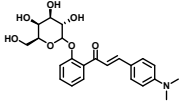
Solvent	HCA					gal-HCA				
	$\lambda_{\text{abs}}$ /nm	$\lambda_{\text{emi}}$ /nm	Stokes Shift /nm	$\epsilon$ /cm <sup>-1</sup> M <sup>-1</sup>	QY	$\lambda_{\text{abs}}$ /nm	$\lambda_{\text{emi}}$ /nm	Stokes Shift /nm	$\epsilon$ /cm <sup>-1</sup> M <sup>-1</sup>	QY
DMSO	450	541	91	32500	0.008	412	540	128	20600	0.388
CH <sub>3</sub> CN	435	560	125	34200	<0.001	403	532	129	23100	0.131
EtOH	433	559	126	33800	<0.001	410	544	134	19600	0.030
THF	435	-	-	34300	-	402	499	97	17100	0.190
EA	428	-	-	31800	-	397	500	103	20800	0.178
DCM	437	-	-	34800	-	409	519	110	22800	0.264
toluene	428	-	-	31900	-	406	479	73	18700	0.047

$\epsilon$ : molar extinction coefficient;

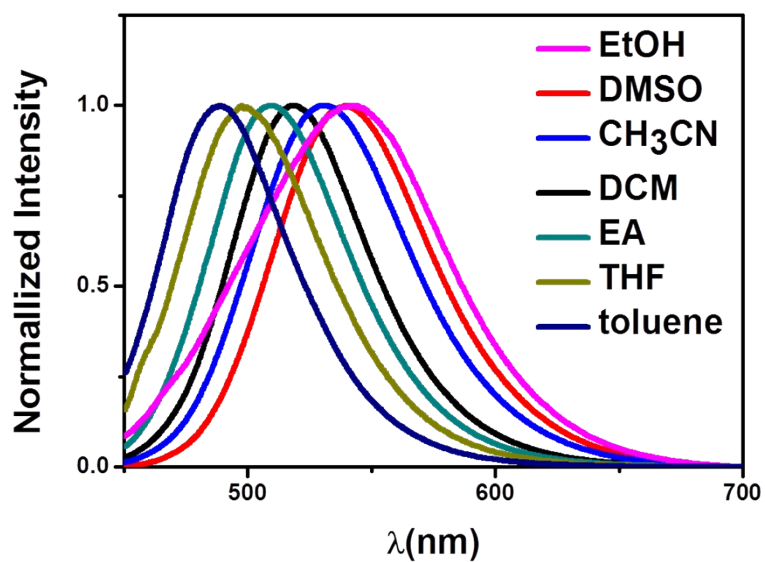
QY: quantum yield, calculated according to a reference of Rhodamine B in MeOH ( $\Phi_F = 0.69$ ).

**Table S2** Comparison of photophysical and response ability to  $\beta$ -gal of **gal-HCA** and reported fluorescence probes.

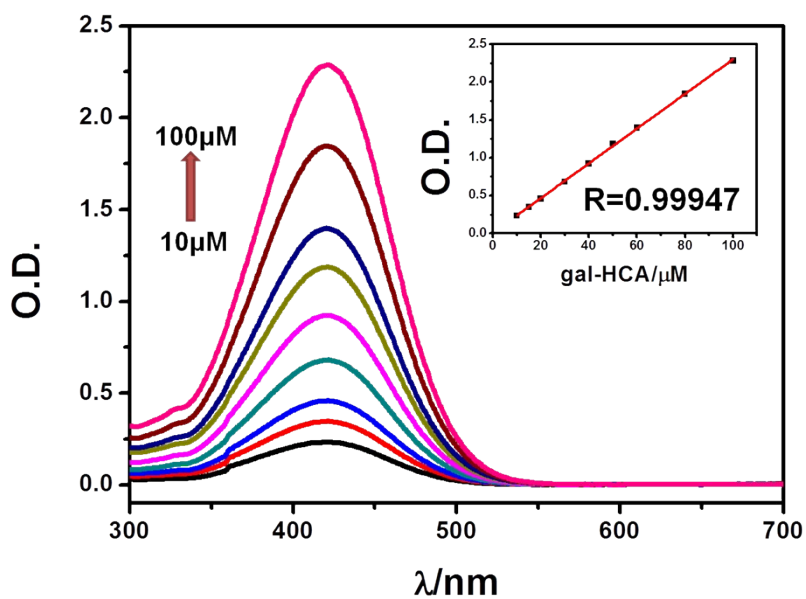
Doi	Structure of the probe	Solvent environment	Excitation wavelength/ Emission wavelength/ Stokes shift	LOD/ U/L	Km/ $\mu$ M	Imaging in senescent cells	Imaging in ovarian cancer cells
10.1039/d3qo00605k		PBS:DMSO (v:v 7:3)	360nm/ 560nm/ 200nm	30	27	no	no
10.1016/j.aca.2023.341482		PBS:CH <sub>3</sub> CN (v:v 7:3)	596nm/ 738nm/ 142nm	2.9	2.93	no	yes
10.1016/j.dyepig.2022.111004		PBS:DMSO (v:v 95:5)	490nm/ 640nm/ 150nm	19.7	-	no	yes
10.1021/acs.analchem.0c02670		PBS:DMSO (v:v 7:3)	651nm/ 727nm/ 76nm	14	34.6	yes	no

10.1021/ acs.anal chem.9b 05121		PBS:DMSO (v:v 8:2)	690nm/ 725nm/ 45nm	22	-	no	yes
10.1016/ j.talanta. 2020.12 1307		PBS:DMSO (v:v 6:4)	550nm/ 675nm/ 125nm	3.2	13.87	no	yes
10.1039/ c5tb019 38a		PBS	387nm/ 545nm/ 158nm	14	-	yes	no
This work		PBS	460nm/ 615nm/ 155nm	12.5	14.92	yes	yes

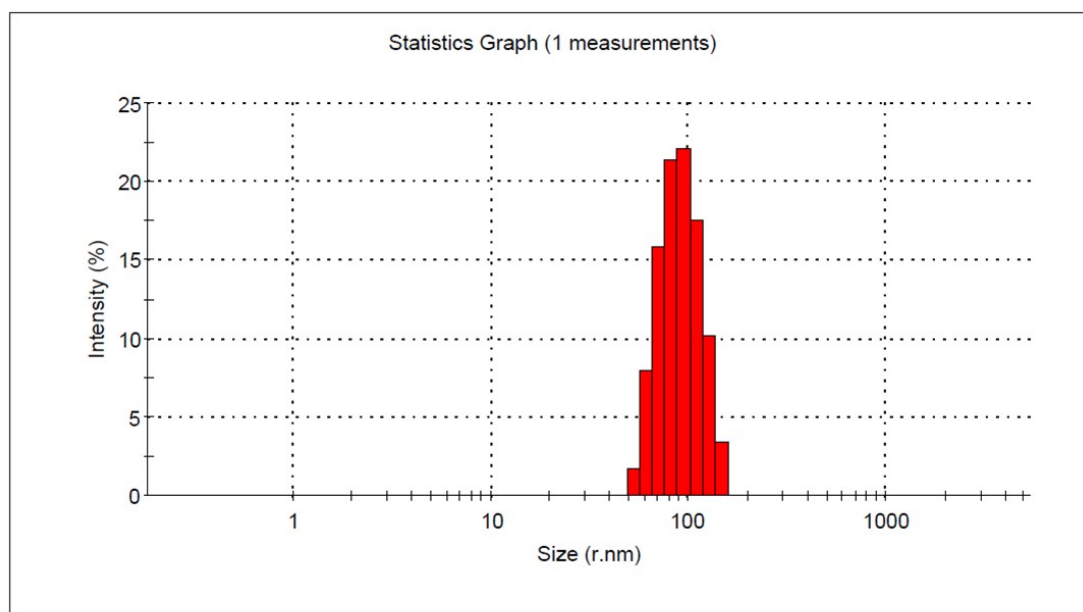
### 3. Figures



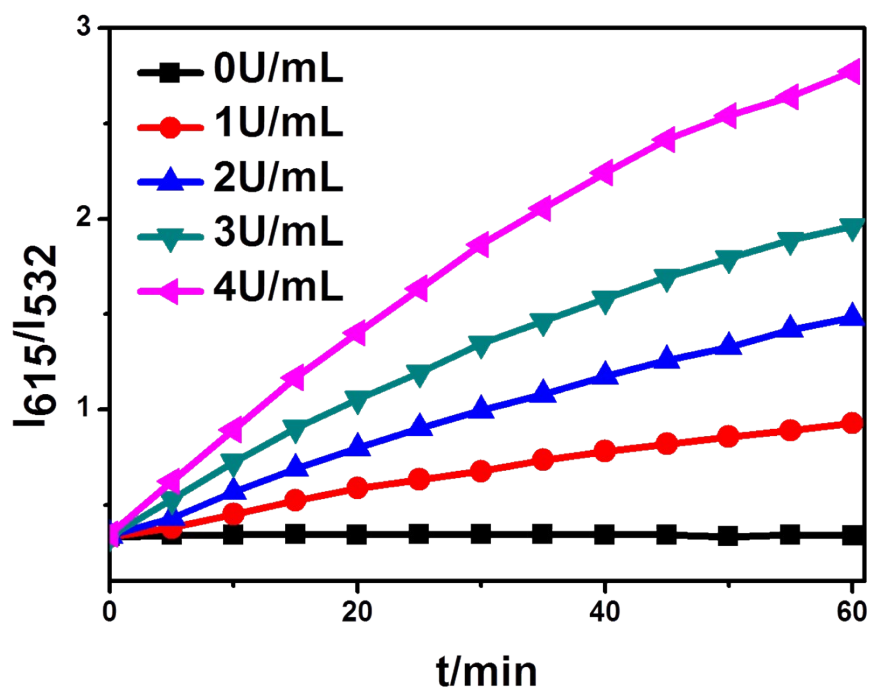
**Figure S1.** The Normalized fluorescence spectrum of gal-HCA (10  $\mu$ M) in different organic solvent.



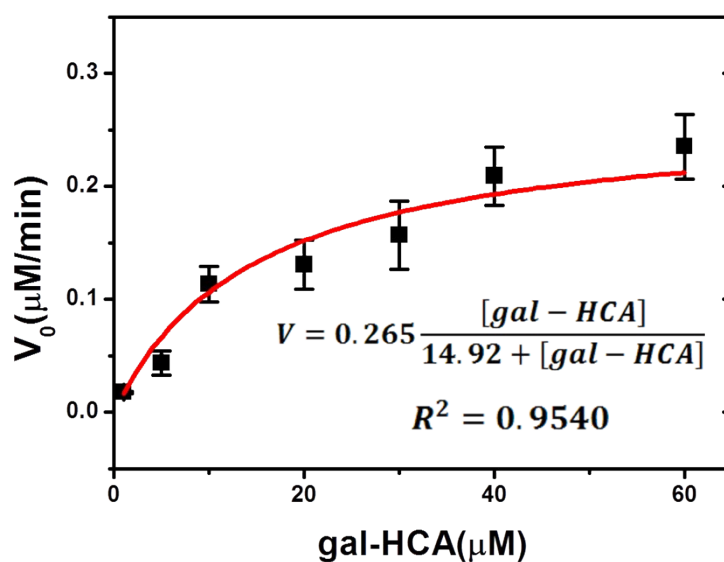
**Figure S2.** The absorption spectra of **gal-HCA** (0~100  $\mu$ M) in PBS buffer (pH = 7.4). Inset: linear fitting curve of the absorption at 422nm to concentration of **gal-HCA**.



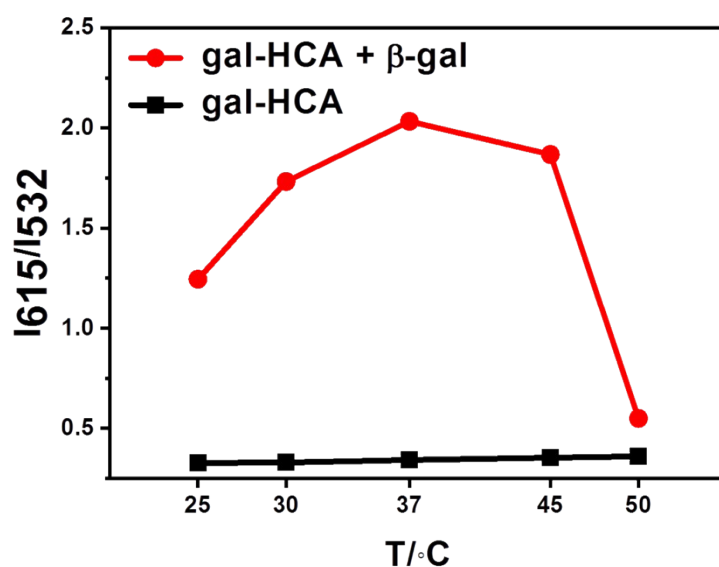
**Figure S3.** Dynamic light scattering of **HCA** (40  $\mu$ M) in PBS buffer containing 4% THF, pH = 7.4.



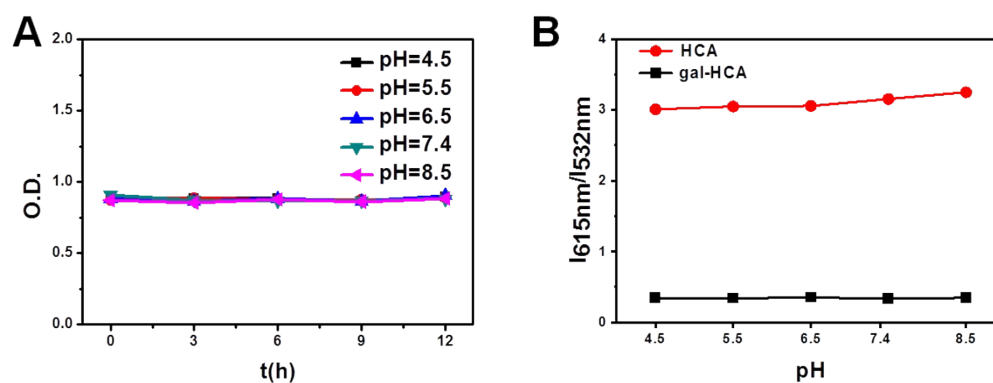
**Figure S4.** Time dependent fluorescence ( $I_{615\text{nm}}/I_{532\text{nm}}$ ) responses of **gal-HCA** towards 0~4 U/ml  $\beta$ -gal in PBS (pH=7.4) in 1 h,  $\lambda_{\text{ex}} = 450$  nm.



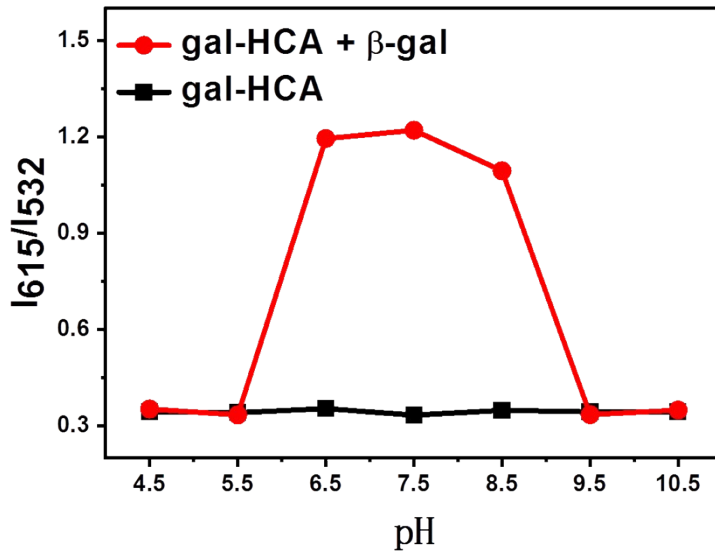
**Figure S5.** Nonlinear Michaelis-Menten curve fitting of **gal-HCA** towards 1U/mL  $\beta$ -gal in PBS. Error bar: standard deviation of 3 parallel experiments.



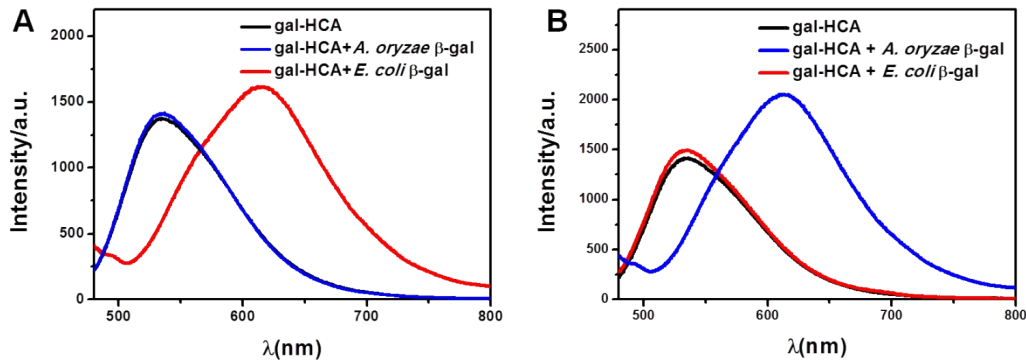
**Figure S6.** Fluorescence ( $I_{615nm}/I_{532nm}$ ) responses of **gal-HCA** (40  $\mu$ M) towards  $\beta$ -gal (3 U/ml, in pH = 7.4 PBS buffer for 1 h) at different temperatures,  $\lambda_{ex}$  = 450 nm.



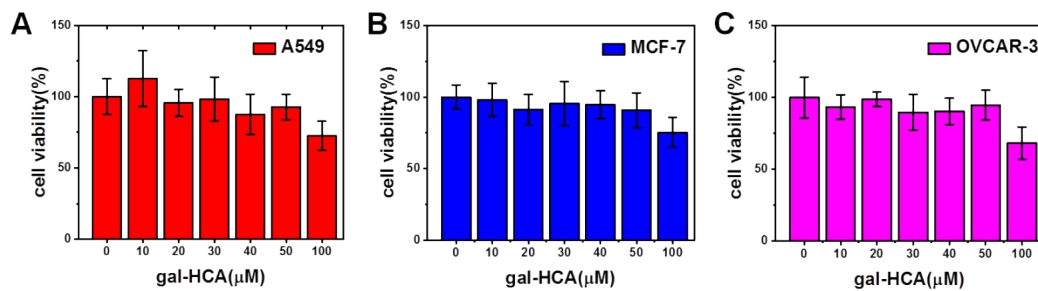
**Figure S7 (A)** Absorbance of **gal-HCA** (40  $\mu$ M) at 422 nm in PBS buffer (pH = 4.5~8.5) in 12h. **(B)** Fluorescence ratio ( $I_{615nm}/I_{532nm}$ ) of **HCA** (40  $\mu$ M) and **gal-HCA** (40  $\mu$ M) in PBS buffer (pH = 4.5~8.5).



**Figure S8.** Fluorescence ( $I_{615nm}/I_{532nm}$ ) responses of gal-HCA(40  $\mu$ M) towards *E. coli*  $\beta$ -gal (2 U/ml) at different pH conditions for 1 h,  $\lambda_{ex}$  = 450 nm.

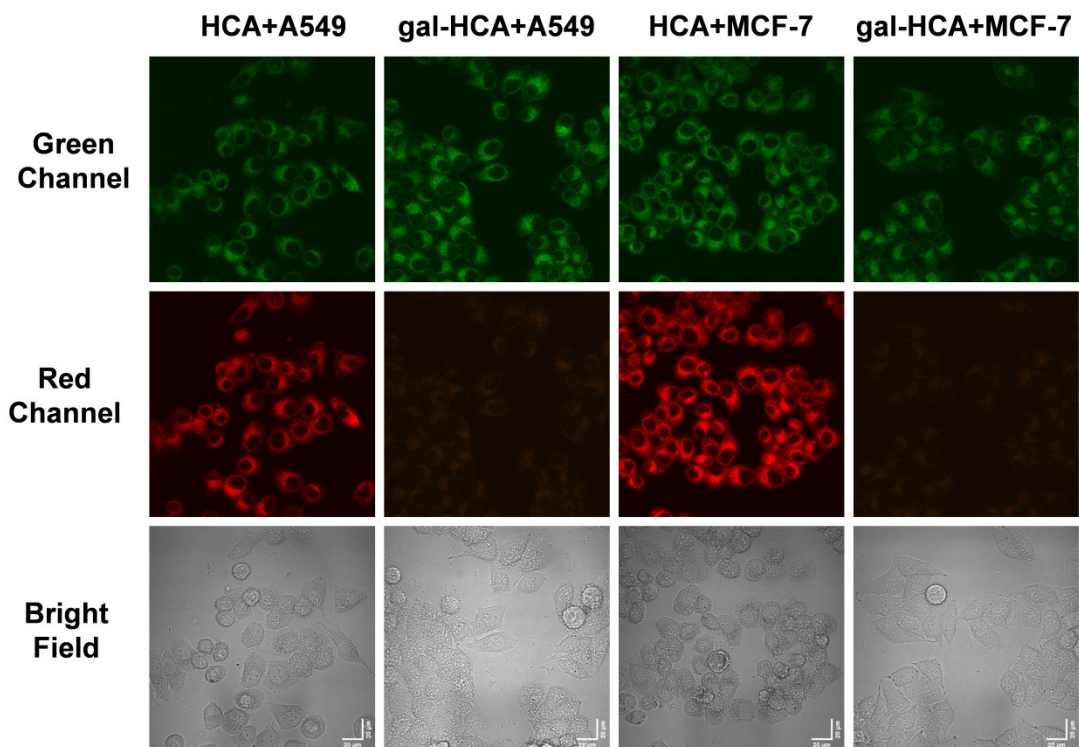


**Figure S9.** Fluorescence Spectra of gal-HCA (40  $\mu$ M) with *E. coli*  $\beta$ -gal or *A. oryzae*  $\beta$ -gal for 60 min at different pH conditions. (A) pH = 7.4; (B) pH = 4.5,  $\lambda_{ex}$  = 450 nm.

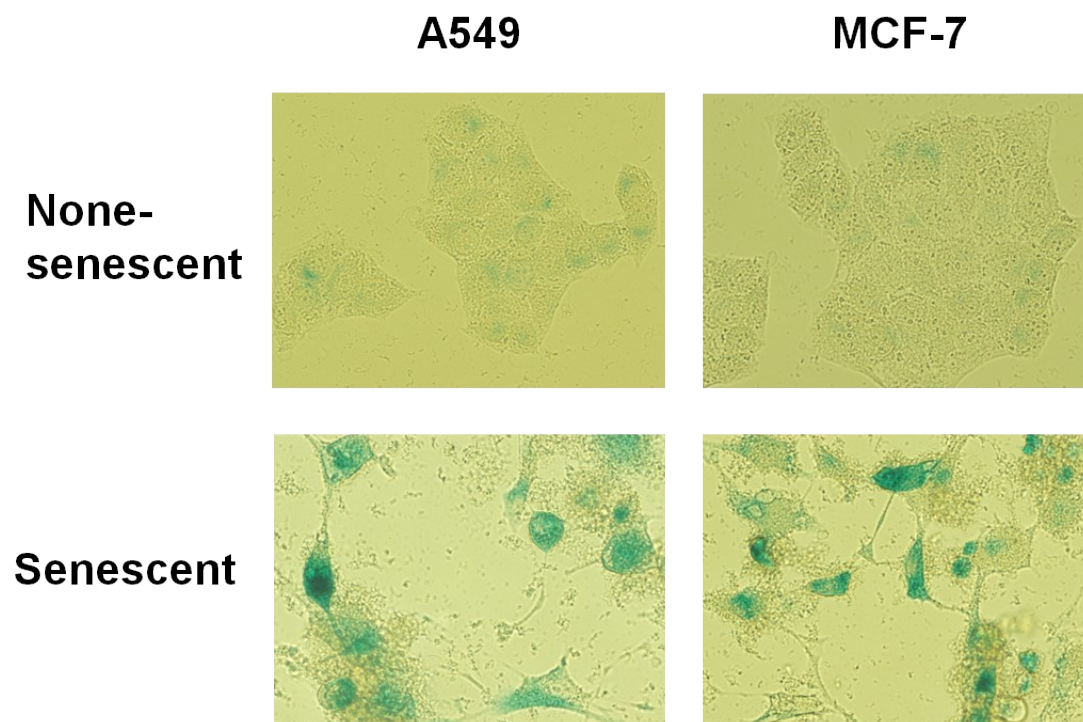


**Figure S10.** Gal-HCA–dose dependent cell viability of (A) A549 cells, (B) MCF-7 cells and (C) OVCAR-3 cells. Error bar: standard deviation of 6 parallel experiment.

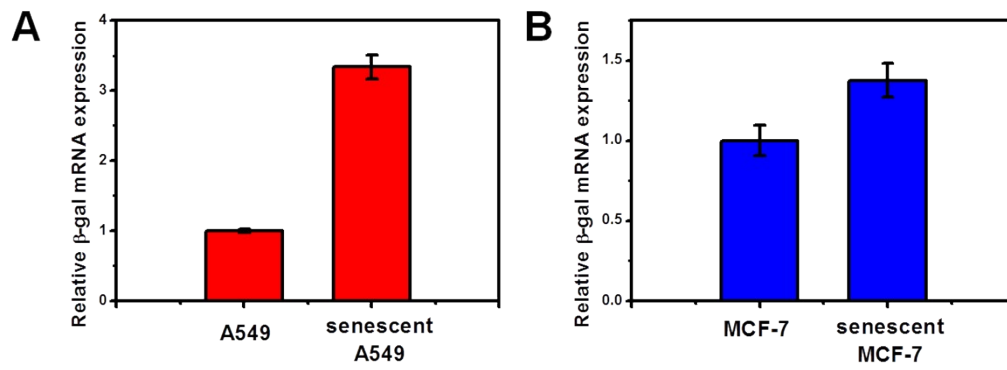




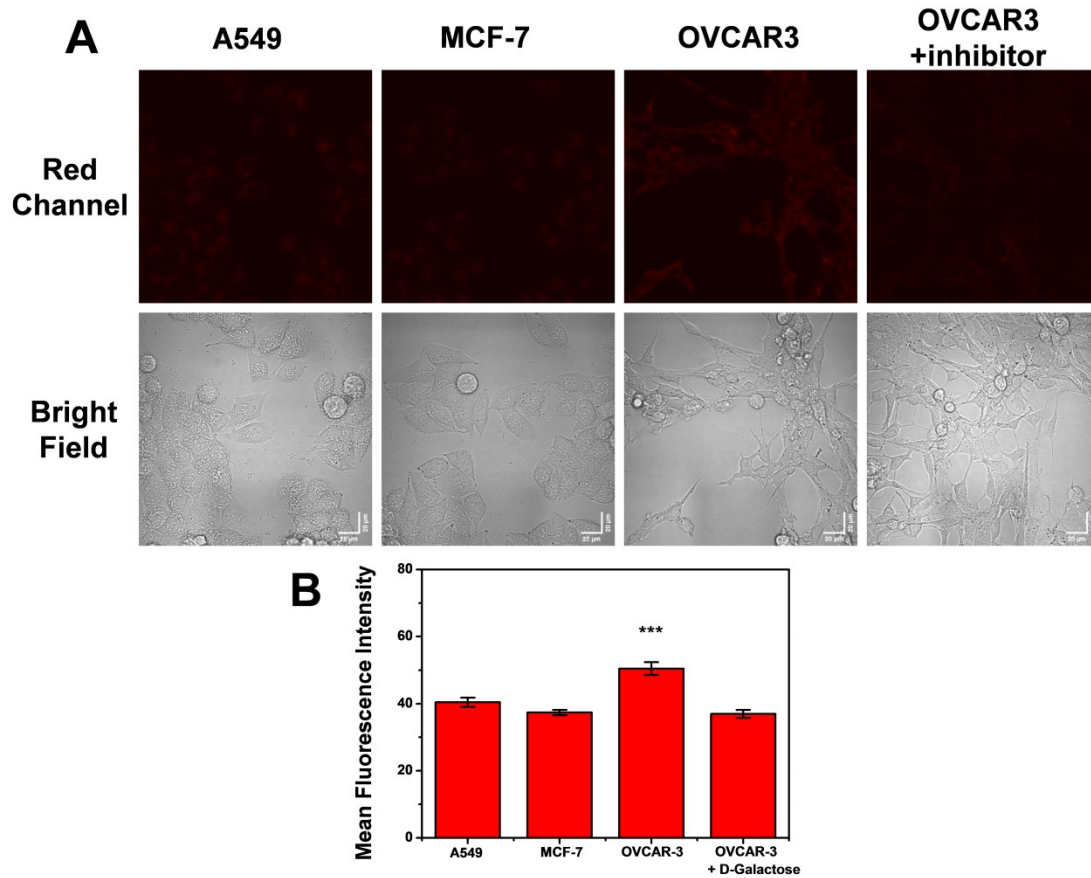
**Figure S11.** Confocal fluorescence images of A549 and MCF-7 cells stained with **HCA** (20  $\mu$ M) and **gal-HCA** (20  $\mu$ M) respectively for 60 min.  $\lambda_{ex}$  = 450 nm. Green channel = 460~560 nm, red channel = 580~680 nm.



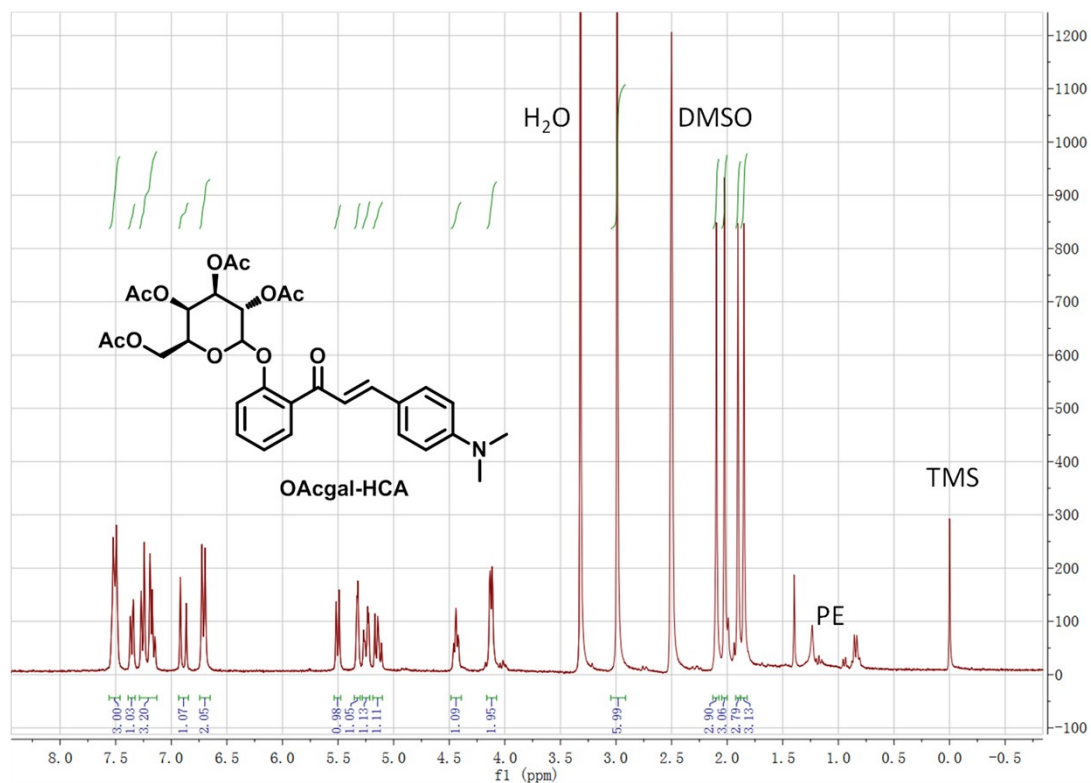
**Figure S12.** CLSM images of X-gal stained none-senescent and senescent A549 and MCF-7 cells, respectively.



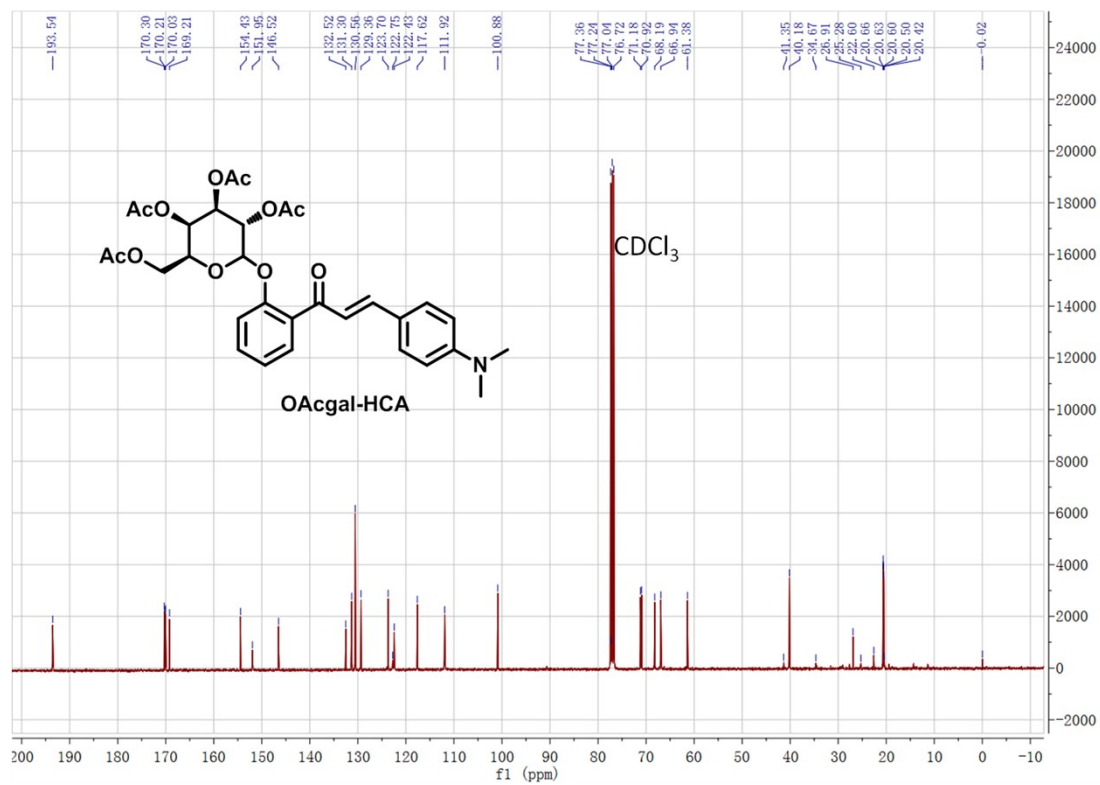
**Figure S13.** The relative expression level of  $\beta$ -gal mRNA by RT-qPCR in none-senescent and senescent A549 and MCF-7 cells,  $n \geq 4$ .



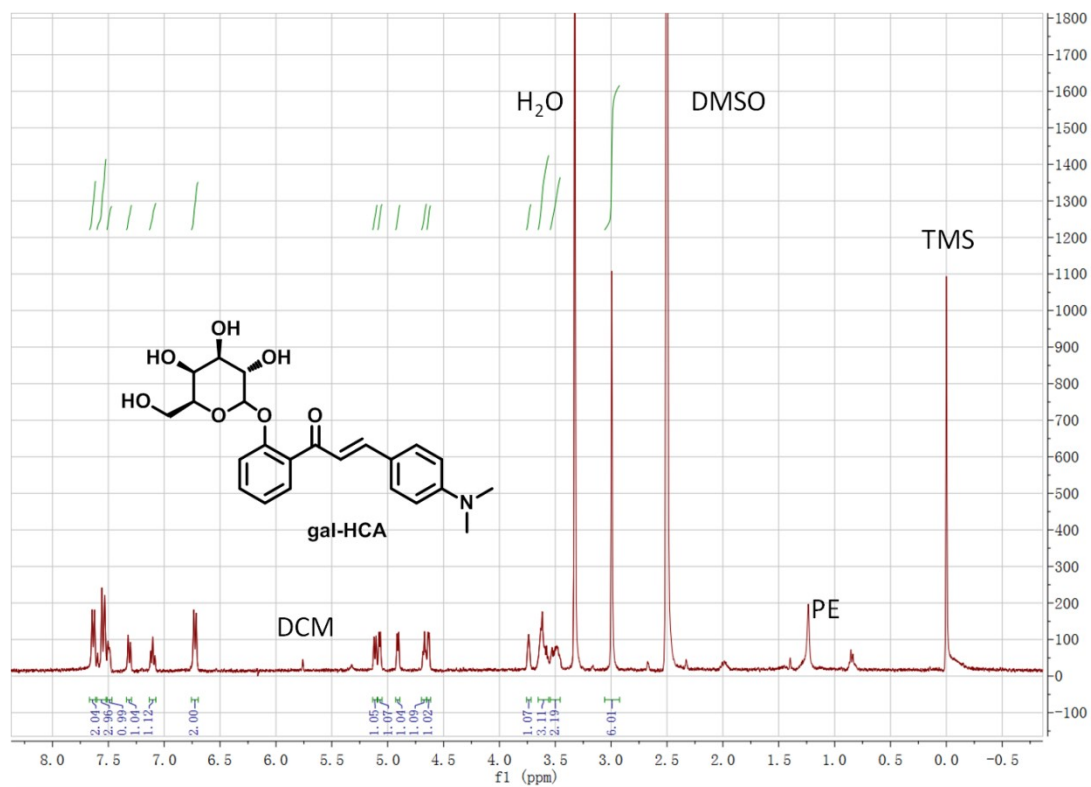
**Figure S14.** Confocal fluorescence images of (A) A549 cells, (B) MCF-7 cells, (C) OVCAR3 cells and (D) OVCAR3 cells pretreated by D-gal incubated with gal-HCA (20  $\mu$ M) for 1 h,  $\lambda_{ex} = 450$  nm, Red Channel = 580~680 nm. (D) Mean fluorescence intensities in the red channels, \*\*\*  $p < 0.01$ . Error bar: standard deviation of mean fluorescence intensities of cells in sight.



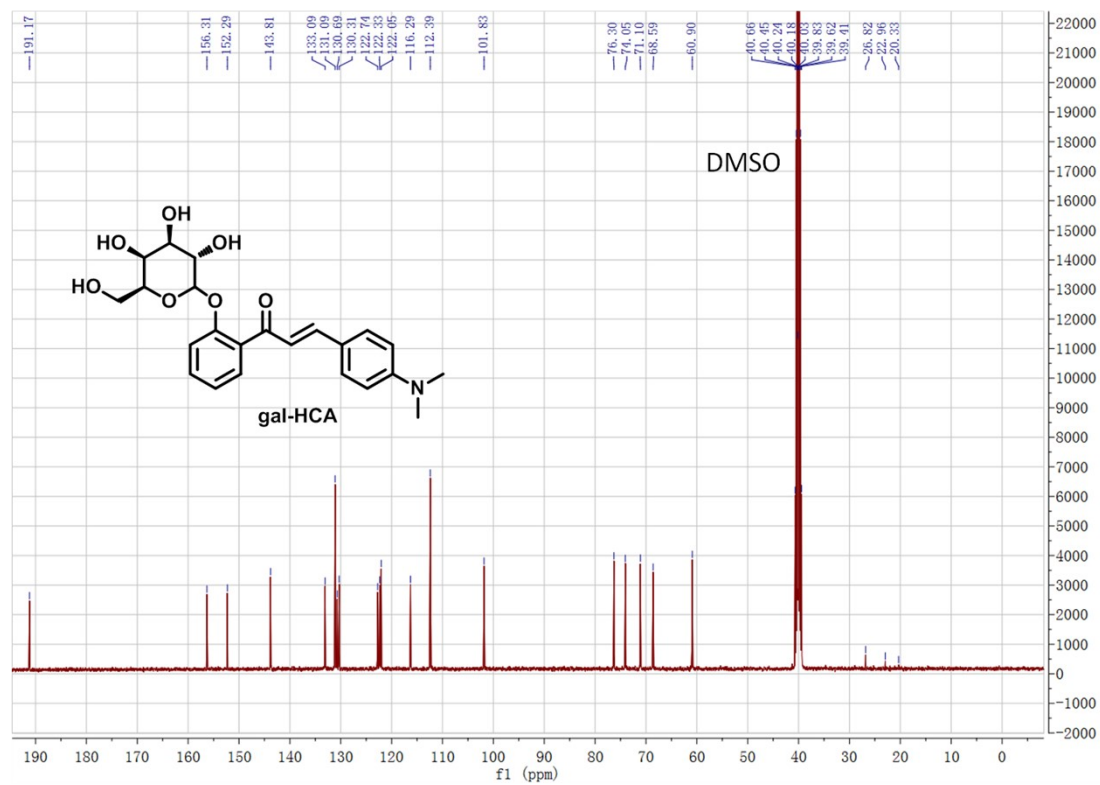
**Figure S15.**  $^1\text{H-NMR}$  spectrum of **OAcgal-HCA** (300 MHz,  $\text{DMSO-}d_6$ ).



**Figure S16.**  $^{13}\text{C-NMR}$  spectrum of **OAcgal-HCA** (100 MHz,  $\text{CDCl}_3$ ).



**Figure S17.**  $^1\text{H-NMR}$  spectrum of gal-HCA (300 MHz,  $\text{DMSO-d}_6$ ).



**Figure S18.**  $^{13}\text{C-NMR}$  spectrum of gal-HCA (100 MHz,  $\text{DMSO-d}_6$ ).

**Acquisition Parameter**

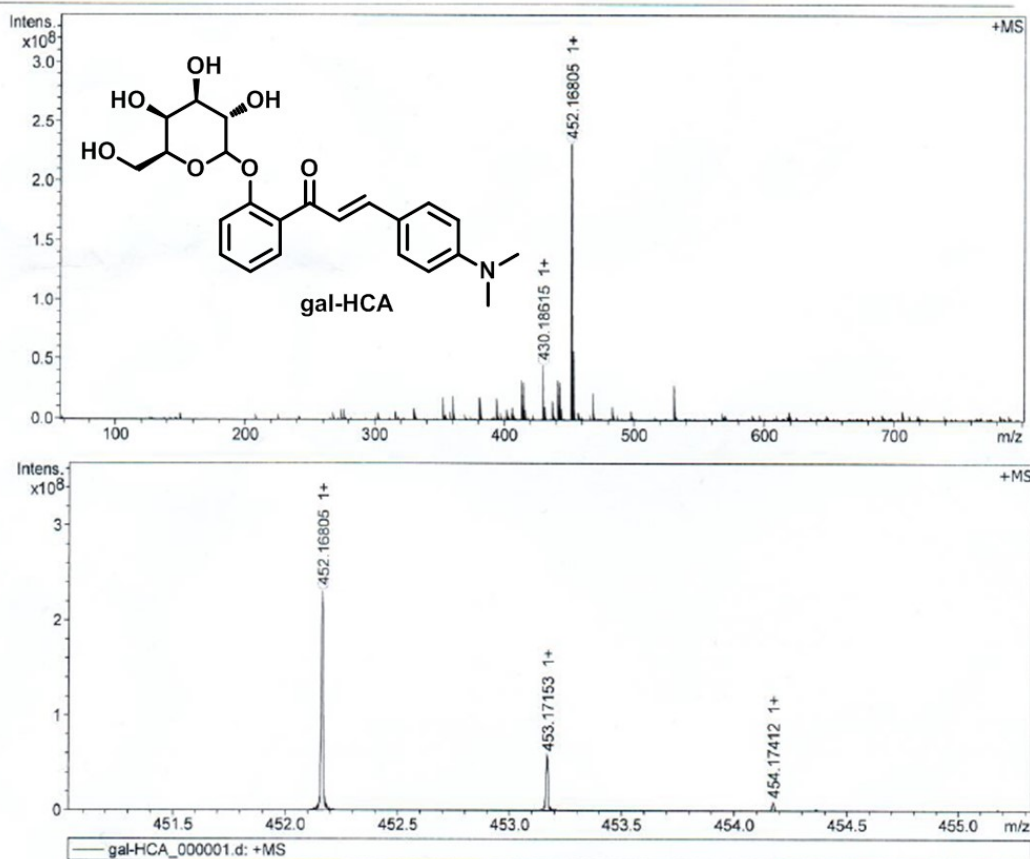
Acquisition Mode Single MS

Acquired Scans 10

Polarity Positive

Broadband Low Mass 57.7 m/z

Broadband High Mass 800.0 m/z

**Figure S19.** HR-ESI mass spectrum of gal-HCA.