Supporting Information

Pyridine Embedded Phenothiazinium Dyes as Lysosome-targeted Photosensitizers for Highly Efficient Photodynamic Antitumor Therapy

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a. Synthesis of key intermediate 2



Figure S1. Synthetic scheme of pyridophenothiazinium dyes.



Figure S2. ¹H NMR of compound 10.



Figure S3. ¹³C NMR of compound 10.



Figure S4. ¹H NMR of compound 11.



Figure S5. ¹³C NMR of compound 11.



Figure S6. ¹H NMR of compound 4.



Figure S7. ¹³C NMR of compound 4.



Figure S8. ¹H NMR of compound 5.



Figure S9. ¹³C NMR of compound 5.



Figure S10. ¹H NMR of compound 6.



Figure S11. ¹³C NMR of compound 6.



(b)

Accurate Mass Measurement



Molecular formula $C_{20}H_{21}SN_4Cl$ [M- Cl]⁺ (theoretical) = 349.1481

Figure S12. ESI mass spectrum (a) and HRMS spectrum (b) of compound 4.





(a)

Accurate Mass Measurement



Figure S13. ESI mass spectrum (a) and HRMS spectrum (b) of compound 5.

Bruker 9.4T FTICR MS Analysis Report

Faculty of Science, The Chinese University of Hong Kong

Analysis Info

| Sample Name : | xql-131 | Reference No. : | xhkl012 |
|------------------|------------------------------|-----------------------|-------------------|
| Applicant Name : | Xiao Qicai Analysis Date : | | 1/4/2016 11:23:21 |
| Analysis Path : | xhkl012_000001.d | | |
| Instrument : | solariX | Polarity | Positive |
| Method | 4_17_mass_range_pos_7T | Acquired Scans | 31 |
| Comment : | 4.5kV, 150ul/hr, 1.0 bar neb | ulizer gas, TOF = 0.6 | |

Accurate Mass Measurement

| C27H35ClN4S |
|-------------|
| 447.257695 |
| 447.257695 |
| 447.25728 |
| 0.6 |
| |



Figure S14. HRMS spectrum of compound 6.



Figure S15. a) Heteronuclear Single Quantum Correlation (HSQC) of compound 5.
b) 1D ¹H NMR analysis of compound 5.



Figure S16. A) Heteronuclear Multiple Bond Correlation (HMBC) of compound 5.B) Significant correlations in the quinoline moiety of compound 5.



Figure S17. HPLC spectrum of compound 4 ($t_R = 16.06 \text{ min}$).



Figure S18. HPLC spectrum of compound 5 ($t_R = 22.26 \text{ min}$).



Figure S19. HPLC spectrum of compound 6 ($t_R = 29.27$ min).



Figure S20. UV-Vis spectrum of compounds **4-6**, the insert plot the Q band absorption versus the concentration.



Figure S21. Cellular uptake of compound 4 (0.5 μ M) at different time slots in HT29 cells. Data are expressed as mean \pm SD of three independent experiments.



Figure S22. Colocalization images of compound 5 (0.5 μ M; in red, column 2) with Lyso-Tracker and Mito-Tracker. The corresponding superimposed images and profiles are shown in column 3 and column 4.



Figure S23. Colocalization images of compound 4 (0.5 μ M; in red, column 2) with Lyso-Tracker and Mito-Tracker. The corresponding superimposed images and profiles are shown in column 3 and column 4.



Figure S24. Colocalization images of compound 6 (0.5 μ M; in red, column 2) with Lyso-Tracker and Mito-Tracker. The corresponding superimposed images and profiles are shown in column 3 and column 4.



Figure S25. Mean fluorescence intensity of HT29 tumor-bearing nude mice after *i.v* injection of compound **5** (2.0 mg kg⁻¹) at different time slots.

| Comps | t _R (min) | Purity ^a | HRMS calcd | HRMS⁵ |
|-------|----------------------|---------------------|------------|----------|
| 4 | 16.06 | 99% | 349.1481 | 349.1483 |
| 5 | 22.26 | 99% | 391.1951 | 391.1953 |
| 6 | 29.27 | 99% | 447.2577 | 447.2573 |

Table S1. HPLC and HRMS data of compounds 4-6.

^a Analytical HPLC method: GL Sciences Inertsil ODS-4 column (150 mm × 4.6 mm, 5 μ m); solvent system: A, MeCN; B, 0.1% TFA in water; A from 30% -100% over 30 min (for compounds **4** and **5**) or 50 min (for compound **6**); flow rate, 1.0 mL/min; wavelength 254 nm; injection volume 10 μ L. ^b Purified products were further characterized by high resolution mass spectrometry.