Supplementary Material (ESI) for Organic & Biomolecular Chemistry


# Supporting Information

for

# Fluorescent Organic Nanoparticles Formation in Lysosomes for Cancer Cell Recognition

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## 1. Materials.

General chemicals were of the best grade available, supplied by Acros Organic Co., Merck Ltd, or Aldrich Chemical Co. and were used without further purification. Cell culture medium and organelle markers were from Invitrogen. All the solvents employed were of spectrometric grade.

## 2. Apparatus.

a. Absorption spectra were taken on a *Thermo Genesys 6* UV-visible spectrophotometer.
b. Fluorescence spectra were recorded on a *HORIBA JOBIN-YVON Fluoromas-4* spectrofluorometer with a 1-nm band-pass in a 1-cm cell length at room temperature
c. Transmission electron microscopy (TEM) was performed on a Zeiss EM 902A operated at 80 kV. A solution of the PTZ derivatives (0.2 wt%) in deionized water was deposited onto a carbon-coated copper grid.
d. The *Leica TCS SP5* confocal microscopy was used to capture cellular images and fluorescence spectra.

## 3. Determination of Quantum Yields.

The quantum yields of PTZ derivatives were determined according to the literature.[1]

$\Phi_u = \Phi_s \times (A_{fu} \times A_s \times \lambda_{exs} \times \eta_u) / (A_{fs} \times A_u \times \lambda_{exu} \times \eta_s)$

Where $\Phi_u$ is quantum yield of unknown; $A_f$ is integrated area under the corrected emission spectra; **A** is absorbance area at the excitation wavelength; $\lambda_{ex}$ is the excitation wavelength; **η** is the refractive index of the solution; the subscripts **u** and **s** refer to the unknown and the standard, respectively. Here, we using Rhodamine Green as a standard and for the same $\lambda_{ex}$, we chose BMVC as the standard, which has the quantum yield of 0.25 in glycerol and 0.02 in DMSO.[2]

## 4. Cell Culture Condition and compound Incubation.

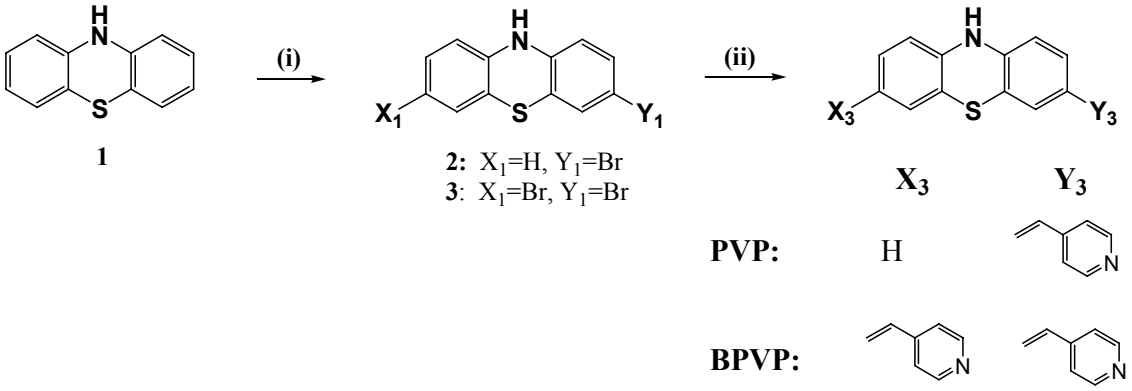
The human Osteosarcoma MG63 and MRC-5 normal fibroblast cells were grown in DMEM with non-essential amino acid supplemented with 10% fetal calf serum (FCS). The human lung adenocarcinoma cell lines CL1-0, A549 and a transformed murine fibroblast L929 cancer line were grown in RPMI medium containing 10% fetal bovine serum (FBS). Cell cultures were maintained at 37℃ in a humidified atmosphere of 95% air and 5% $CO_2$. The cells seeded in culture plates or dishes were incubated with different concentrations of PTZ derivatives, whose DMSO stock solutions were diluted in serum-free medium before use.

## 5. Confocal Cellular Localizations.

Prior to perform the localization of cellular PTZ, cells were seeded into coverslips and incubated for 24 hr. On the next day, cells were incubated with 5μM of PTZ for 4 hr, and then with MitoTracker® Red CMXRos or Lyso Tracker® Red DND-99 for 30 min, then the λ scanning and fluorescence images were taken under *Leica TCS SP5* confocal fluorescence microscopy. The excitation source was a 405 nm diode laser for PTZ compounds and 561nm DPSS green laser for markers. Fluorescence photographs were taken through a related 420-550 nm and 580-700nm ranges. λ scanning was perform with 405nm diode laser and collected under the wavelength 420-650nm.

## 6. General Procedure for the Synthesis of Phenothiazine Derivatives.

Synthesis of these phenothiazines derivatives are shown in Scheme 1. 10*H*-phenothiazine containing solution was bromination with NBS/THF in additional funnel and then, followed by Heck coupling reaction[3] with 4-vinyl pyridine under catalyst $Pd(OAc)_2$.




**Scheme S** : Synthesis of PVP and BPVP. Reaction reagents and conditions: (i) NBS/THF, rt. (ii) $Pd(OAc)_2$/(*o*-tol)$_3$P, 4-vinylpyridine and $Et_3N$/MeCN as solvent pair; under $N_2$. 48hr

### 6.1. Synthesis of 3-Bromo-10*H*-phenothiazine (2).

A double-necked round bottomed flask was charged with phenothiazine (PTZ) 10 mmole in THF solution (20 mL). Then NBS (10 mmole) was dissolved in 20 mL THF and was added dropwise over 1h with an addition-funnel. The reaction was stirred at ice bath until the complete consumption by TLC monitoring. The solvent was evaporated in vacuum and the residue purified via column chromatography (silica, ethyl acetate / hexane. 1:8, v/v, Rf =0.38) to remove the dibromo-substituted side product. The final light green products were crystallized from acetone / EA. (yield: 55%). Data for **2** : mp: 175.2~176.0 ℃. $^1$H NMR ( 400Hz , DMSO-d6) : δ= 8.706(s , 1H) , 7.114( m , 2H) , 6.984 (t, *J*=8Hz, 1H), 6.896(d, *J*=8Hz, 1H) , 6.750(t, *J*=8Hz, 1H), 6.650(d, *J*=8Hz, 1H), 6.590(d, *J*=8Hz, 1H) ppm.

### 6.2. 3,7-Dibromo-10*H*-phenothiazine (3).

A double-necked round bottomed flask was charged with phenothiazine (PTZ) 10 mmole in THF solution (20 mL). Then NBS (22 mmole) was dissolved in 45 mL THF and was added dropwise over 1h with an addition-funnel. The reaction was stirred at ice bath until the complete consumption by TLC monitoring. The solvent was evaporated in vacuum and the residue purified via column chromatography (silica, ethyl acetate / hexane. 1/8, v/v, Rf =0.34). The final light green products were crystallized from acetone / EA. (yield: 70%). Data for **3** mp: 195.6~196.8 ℃. $^1$H NMR ( 400Hz , DMSO-d6) :δ= 8.848 (s, 1H), 7.122 ( m, 4H ) 6.590 (d , *J* = 8 Hz , 2H ) ppm.

### 6.3. 3-((*E*)-2-(Pyridin-4-yl)vinyl)-10*H*-phenothiazine (PVP).

The compound **2** (5mmole) was added into a high pressure bottle containing the mixture of palladium (II) acetate (8mg, strem) and tri-*o*-tolyl phosphine (80mg, Aldrich), then to which was added the solvent pair (triethylamine 5mL / acetonitrile 15mL) and 4-vinylpyridine (10mmole, Merck). The bottle was sealed after bubbling 10 min with nitrogen. After keeping the system under ~105℃ for three days, the system was cooled to room temperature and then extracted with $CH_2Cl_2$ / $H_2O$ twice. The solvent was dried by MgSO4 and evaporated in vacuum. The residue was chromatographed on silica gel by Hexane / Acetone (1/1). The orange solid compound was obtained by recrystallizing with acetone / EA (yield: 72%). Data for **PVP** :

mp: 246.4~248.2 ℃.

Anal. Calcd. (%). Observation: C, 71.24; H, 5.03; N, 8.75. Calc. **PVP•$H_2O$**: $C_{19}H_{16}N_2OS$: C, 71.22; H, 5.03; 8.74. Calc. **PVP**: $C_{19}H_{14}N_2S$ : C, 75.47; H, 4.67; N, 9.26.

HRMS ($EI^+$) m/z: 302.09 [$M^+$].

$^1$H NMR (400Hz, DMSO-d6): δ= 8.807 (s, 1H), 8.480 (d, *J*= 5.4 Hz, 2H), 7.46 (d, *J*=5.4 Hz, 2H ), 7.33(d, *J*=16.2 Hz ,1H ), 7.24 (m , 2H ) , 7.01(d , *J*=16Hz ,1H ), 6.997 (t, *J*=8Hz, 1H), 6.935(d, *J*=8Hz, 1H) , 6.749(t, *J*=8Hz, 1H), 6.66(m, 2H).

$^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ =149.9 144.6, 142.2, 141.1, 132.0, 130.0, 127.6, 127.1, 126.2, 124.5, 123.0, 122.1, 120.5, 116.8, 116.0, 114.6, 114.4.

**6.4. 3,7-bis((*E*)-2-(Pyridin-4-yl)vinyl)-10*H*-phenothiazine (BPVP).**

The compound **3** (5mmole) was added into a high pressure bottle containing the mixture of palladium (II) acetate (16mg, strem) and tri-*o*-tolyl phosphine (160mg, Aldrich), then to which was added the solvent pair (triethylamine 5mL / acetonitrile 15mL) and 4-vinylpyridine (20mmole, Merck). The bottle was sealed after bubbling 10 min with nitrogen. After keeping the system under ~105℃ for three days, the system was cooled to room temperature and then extracted with $CH_2Cl_2$ / $H_2O$ twice. The solvent was dried by MgSO4 and evaporated in vacuum. The residue was chromatographed on silica gel by Hexane / Acetone (1/2). The dark-red solid compound was obtained with recrystallizing under acetone / EA (yield: 68%). Data for **BPVP**:

mp: 228.4~230.0 ℃.

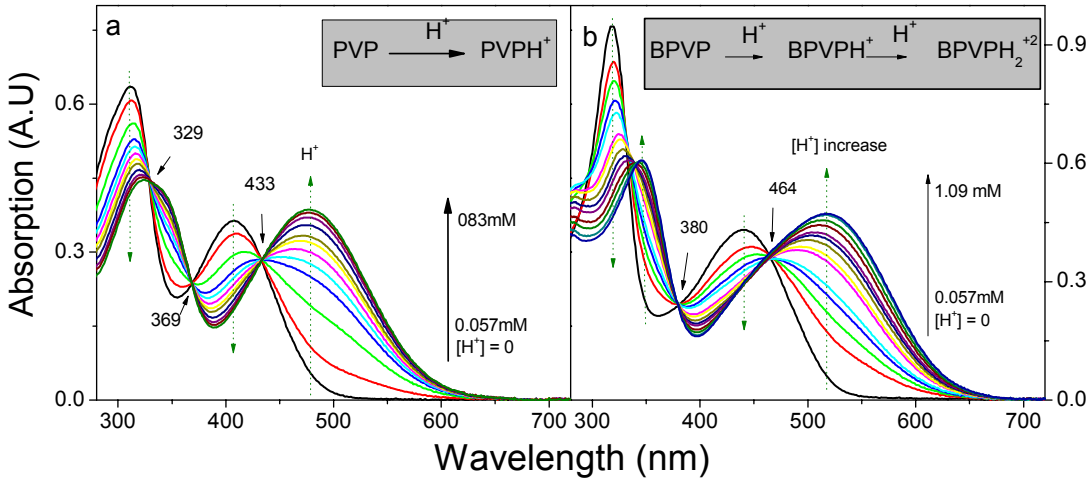
Anal. Calcd. (%).Observation: C, 67.98; H, 5.50; N, 9.11. Calc. **BPVP•3$H_2O$:** $C_{26}H_{21}N_3OS$: C, 67.95; H, 5.48; N, 9.14. Calc. **BPVP:** $C_{26}H_{19}N_3S$: C, 77.01 ; H, 4.72 ; N, 10.36.

HRMS ($EI^+$) m/z: 405.13 [$M^+$].

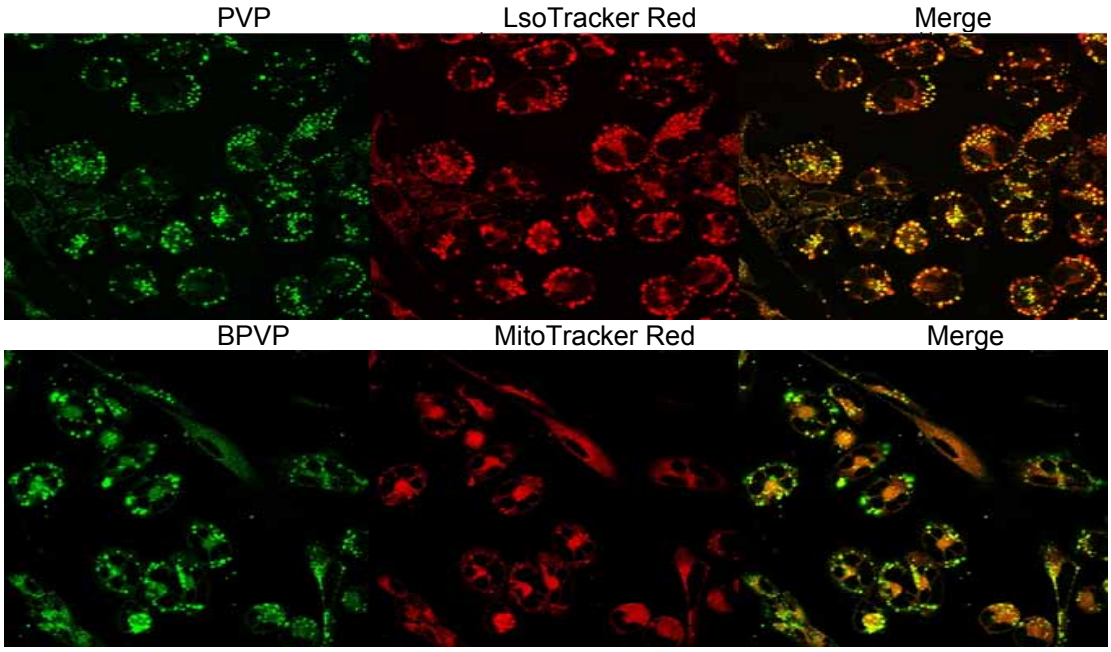
$^1$H NMR (400Hz, DMSO-d6): δ= 9.03 (s, 1H ), 8.48 (d, *J*= 5.4Hz , 4H), 7.45 (d, *J*=5.8Hz, 4H), 7.37 (d, *J*=16.2 Hz, 2H), 7.24 (m , 4H), 7.03(d , *J*=16.2 Hz , 2H), 6.65 (d , *J*=8.0 Hz, 2H).

$^{13}$C NMR (100 MHz, DMSO-d6) $\delta$ =150.2 145.7, 141.8, 132.9, 130.9, 127.9, 125.2, 123.8, 121.3, 117.1, 115.3.

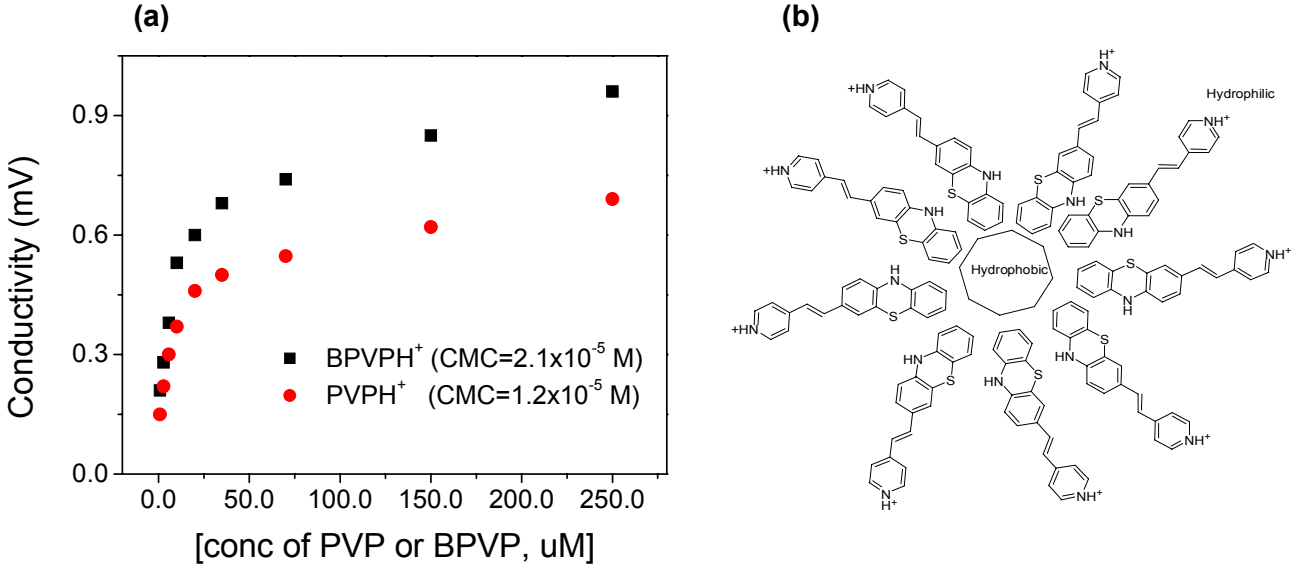
**7. Figure S1**. Absorption spectra of 25 $\mu$M (a) PVP and (b) BPVP in DMSO as a function of [$H^+$]. It is clear that only one protonation process in PVP (one isosbestic point) while there are two protonated steps are observed in BPVP.


a
PVP
H+
PVPH+
329
433
H+
369
083mM
0.057mM
[H+] = 0
b
BPVP
H+
BPVPH+
H+
BPVPH2+2
[H+] increase
380
464
1.09 mM
0.057mM
[H+] = 0
Absorption (A.U)
Wavelength (nm)
0.0
0.3
0.6
0.9
300
400
500
600
700

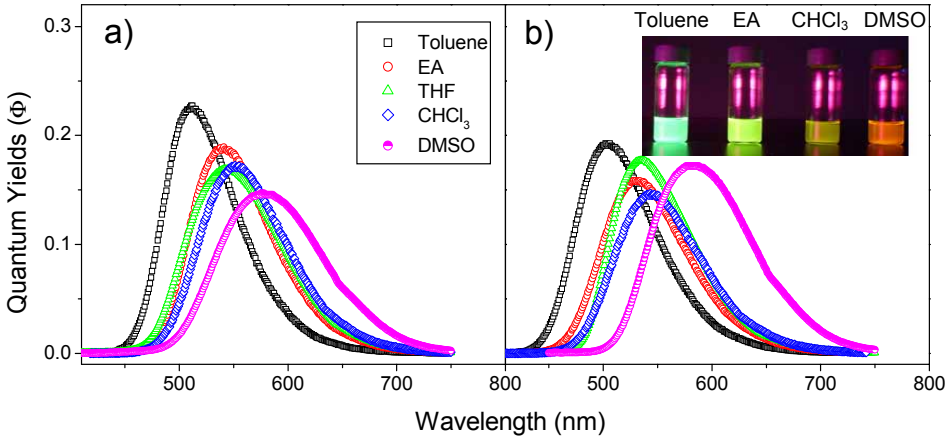
**8. Figure S2.** Immunofluorescence staining: The intracellular localization images of 4 $\mu$M PTZ compounds under MRC-5 normal cell co-cultured CL1-0 lung cancer cell. Cells were incubated first with compound for 4 hours, followed by 1 $\mu$M organelle probes (MitoTracker red CMH2XRos for 30 min at 37 °C, LysoTracker red DND-99 for 30 min at 37 °C). The excitation source was a 405 nm diode laser for PTZ compounds and 561nm DPSS green laser for markers. Fluorescence photographs were taken through a related 420-550 nm and 580-700nm ranges.




**9. Figure S3.** (a) Conductivity measurement and determination of CMC (critical micelle concentration) of PVP and BPVP under acidic aqueous solutions (pH = 3.95 for PVP, 4.21 for BPVP). (b) The aggregation model of surfactant-like molecules.



**10. Figure S4.** The emission spectra of solvent effects for (a) $PVPH^+$ and (b) $BPVPH^+$. These spectra are collected under first end point of protonation titration process in every solvent. Excited wavelength is 400nm for $PVPH^+$ and 440nm for $BPVPH^+$. The insets show the corresponding fluorescence emission photographs.




## 11. Reference

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2. Chang, C.C.; Kuo, S.H.; Kang, C.C.; Chang, T.C. *Journal of Luminescence* **2006**, *119-120*. 84-90.
3. a). Chang, C. C.; Chen, K. J.; Yu, L.J. *J Org Chem*. 1999, *64*(15), 5603-5610; b). Chang, C.C.; Wu, Y, J,; Chang, T.-C. *Journal of the Chinese Chemical Society* **2003**, *50*, 185-188.