NANO MICRO
small

Supporting Information

for *Small,* DOI: 10.1002/smll.201402236

A Pillararene-Based Ternary Drug-Delivery System with Photocontrolled Anticancer Drug Release

*Guocan Yu, Wei Yu, Zhengwei Mao, Changyou Gao, and Feihe Huang**

Supporting Information

A Pillararene-Based Ternary Drug Delivery System with Photo-Controlled Anticancer Drug Release

Guocan Yu,[†] Wei Yu,[‡] Zhengwei Mao,[‡] Changyou Gao,[‡] and Feihe Huang[*,†]*

1. Materials and methods

1-(Hydroxymethyl)pyrene, block copolymer methoxy-poly(ethyleneglycol)$_{114}$- *block*-poly(L-lysine hydrochloride)$_{200}$ (PEG-*b*-PLKC), chlorambucil and other reagents were commercially available and used as received. Solvents were either employed as purchased or dried according to procedures described in the literature. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance Ⅲ-400 spectrometry. Mass spectra were obtained on a Bruker Esquire 3000 plus mass spectrometer (Bruker-Franzen Analytik GmbH Bremen, Germany) equipped with an ESI interface and an ion trap analyzer. HRMS were obtained on a WATERS GCT Premier mass spectrometer. UV-vis spectra were taken on a Shimadzu UV-2550 UV-vis spectrophotometer. Transmission electron microscopic (TEM) investigations were carried out on a HT-7700 instrument. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). Dynamic Light Scattering (DLS) experiments were measured on a Nano-ZS ZEN3600 instrument. The energy-minimized structures were calculated using the GAUSSIAN 03 software based on the arithmetic method PM3 (Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision D.01; Gaussian, Inc.: Wallingford, CT, 2005).

Preparation of ternary polyion complex (PIC) micelles. WP6 (50.0 mg) and **Py-Cbl** (2.59 mg) were added to PBS (2 mL, pH = 7.4) and sonicated for 2 h. Insoluble **Py-Cbl** was removed by filtration and the solution was added dropwise to a solution of PEG-*b*-PLKC (66.2 mg, 1 mL PBS) under stirring and sonication. The charge ratio r = 200 [PEG-*b*-PLKC]$^{200+}$/12[**WP6**]$^{12-}$ between PEG-*b*-PLKC and **WP6** was 1:1 to ensure complete complexation. Here [PEG-*b*-PLKC]$^{200+}$ and [**WP6**]$^{12-}$ represent the concentrations of PEG-*b*-PLKC and **WP6**, respectively.

Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) Studies. The self-assembly nanostructures of **Py-Cbl**, **PyOH**, and the ternary PIC micelles were revealed using TEM. A solution of **Py-Cbl** (**PyOH**) (1.00×10^{-5} M) or the ternary PIC micelles (the concentration of the polymer was 0.75 mg mL^{-1}) was prepared first in water. TEM samples were prepared by drop-coating the solution onto a carbon-coated copper grid. TEM experiments were performed on an HT-7700 instrument. The corresponding solution was left to stand overnight and the insoluble precipitate was eliminated by using a microporous membrane before DLS tests. Dynamic light scattering (DLS) measurements were carried out using a 200 mW polarized laser source Nd:YAG (λ = 532 nm). The polarized scattered light was collected at 90° in a self-beating mode with a Hamamatsu R942/02 photomultiplier. The signals were sent to a Malvern 4700 submicrometer particle analyzer system.

Determination of the photodegradation process. A suspension of **Py-Cbl** or **WP6⊃Py-Cbl** (1.00×10^{-5} M) was prepared in water. After UV irradiation (365 nm) for different times, fluorescence spectroscopy was utilized to monitor the changes in the fluoresence of the solution. Here, we define the released percentage as follows: release% = $(F_t - F_0)/(F_{final} - F_0) \times 100\%$, where F_t means the fluorescence intensity at 400 nm after UV irradiation for different times, F_{final} means the fluorescence intensity at 400 nm after UV irradiation for enough time (10 min), and F_0 means the initial fluorescence intensity at 400 nm before UV irradiation.

For the ^{1}H NMR investigation, a solution of **Py-Cbl** (2.00 mM) was prepared in a mixture of D_2O and acetone-d_6 (2:1, *v*/*v*). After irradiation (365 nm) for different times, ^{1}H NMR spectra were recorded on a Bruker Avance III-400 spectrometer to monitor the photodegradation process of **Py-Cbl**.

Cell Culture. A549 cells, a type of human lung cancer cells, were cultured in Dulbecco's

modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells grew as a monolayer and were detached upon confluence using trypsin (0.5% *w*/*v* in PBS). The cells were harvested from the cell culture medium by incubating in the trypsin solution for 5 min. The cells were centrifuged, and the supernatant was discarded. A 3.00 mL portion of serum-supplemented DMEM was added to neutralize any residual trypsin. The cells were resuspended in serum-supplemented DMEM at a concentration of 1.00×10^4 cells/mL. Cells were cultured at 37 °C and 5% CO_2.

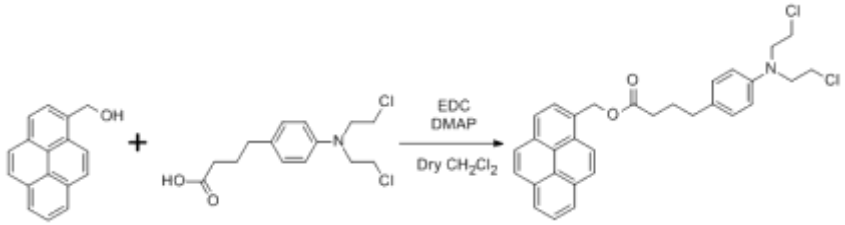
Cytotoxicity Evaluation (before irradiation). The cytotoxicities of **Py-Cbl**, **WP6⊃Py-Cbl**, chlorambucil, and the ternary PIC micelles against A549 cells were determined by 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in 96-well cell culture plates. A549 cells were seeded at a density of 1.00×10^4 cells/well, and incubated for 24 h for attachment. Cells were then incubated with fresh serum-supplemented DMEM without/with **Py-Cbl**, **WP6⊃Py-Cbl**, chlorambucil, and the ternary PIC micelles at various concentrations for 24 h. Then 20 μL of MTT solution (5.00 mg/mL) were added to each well. After 4 h of incubation at 37 °C, the MTT solution was removed, and the resultant insoluble formazan crystals were dissolved in 100 μL of dimethylsulfoxide (DMSO). The absorbance of the formazan product was measured at 570 nm using a spectrophotometer (Bio-Rad Model 680). Untreated cells in media were used as a control. All experiments were carried out with three replicates.

Cytotoxicity Evaluation (after irradiation). A549 cells were seeded at a density of 1.00×10^4 cells/well, and incubated for 24 h for attachment. The cells were incubated with fresh serum-supplemented DMEM without/with **Py-Cbl**, **WP6⊃Py-Cbl**, and the ternary PIC micelles at various concentrations for 12 h. Then the cells were irradiated (keeping the cell-culture plate 5 cm apart from the light source) using an 8 W medium pressure Hg lamp through a UV filter. After irradiation, the medium was removed and new medium was added, and the cells were again incubated for 12 h. Then cytotoxicity was measured using the MTT assay as described above for the case before irradiation. In this part, untreated cells (incubated with fresh serum-supplemented DMEM) exposed to UV light were used as a control. Therefore, the influence of the UV light on the cell viability could be eliminated.

Cell imaging studies. A549 cells were seeded at a density of 5×10^3 cells per well and allowed to attach for 24 h at 37 °C and 5 % CO_2. The ternary PIC micelles were added (5.00 ×

10^{-5} M). After 24 h, the medium was removed and washed with PBS. Thereafter, cells were fixed in paraformaldehyde for 15 min and washed twice with PBS and then visualized under a fluorescence microscope (Olympus IX81).

2. Synthesis of ***Py-Cbl***



Scheme S1. Synthetic route to **Py-Cbl**.

To a solution of 1-pyrenemethanol (1.16 g, 5.00 mmol) and chlorambucil (0.757 g, 2.50 mmol) in dry CH_2Cl_2 (100 mL), 4-dimethylaminopyridine (DMAP, catalytic amount) and 1-(3'-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 1.79 g, 10.0 mmol) were added under nitrogen atmosphere. The mixture was stirred overnight at room temperature. The solution was evaporated under vacuo and the residue was purified by flash column chromatography on silica gel (dichloromethane/petroleum ether = 2:1, *v*/*v*) to afford **Py-Cbl** as an oil (0.797 g, 63%). The proton NMR spectrum of **Py-Cbl** is shown in Figure S1. ^{1}H NMR (400 MHz, chloroform-*d*, room temperature) δ (ppm): 8.30 (d, J = 8 Hz, 1H), 8.24–8.16 (m, 4H), 8.11–8.02 (m, 8H), 6.89 (d, J = 8 Hz, 2H), 6.47 (d, J = 8 Hz, 2H), 5.85 (s, 2H), 3.63 (t, J = 4 Hz, 4H), 3.56 (t, J = 4 Hz, 4H), 2.48 (t, J = 8 Hz, 2H), 2.39 (t, J = 8 Hz, 2H), 1.94–1.87 (m, 2H). The ^{13}C NMR spectrum of **Py-Cbl** is shown in Figure S2. ^{13}C NMR (100 MHz, chloroform-*d*, room temperature) δ (ppm): 173.52, 155.89, 144.25, 131.81, 131.78, 131.24, 130.69, 130.46, 129.64, 128.23, 127.89, 127.85, 127.41, 126.13, 125.55, 125.47, 124.94, 124.65, 123.00, 112.06, 64.60, 53.57, 40.49, 33.87, 33.71, 26.82. LRESIMS is shown in Figure S3: *m*/*z* 539.9 $[M + Na]^+$ (100%). HRESIMS: *m*/*z* calcd for $[M + H]^+$ $C_{31}H_{30}Cl_2NO_2$, 519.4735, found 519.4744, error 1.7 ppm.

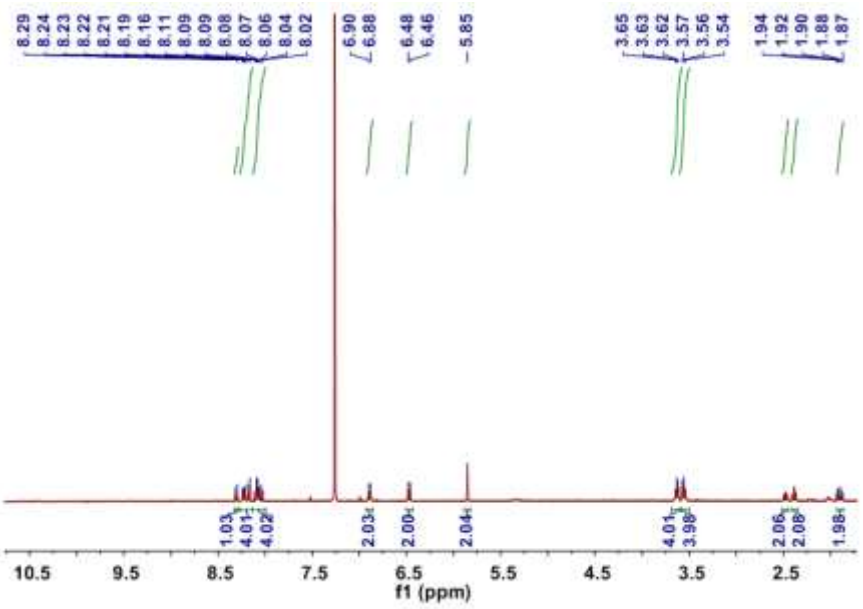


Figure S1. ^{1}H NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **Py-Cbl**.

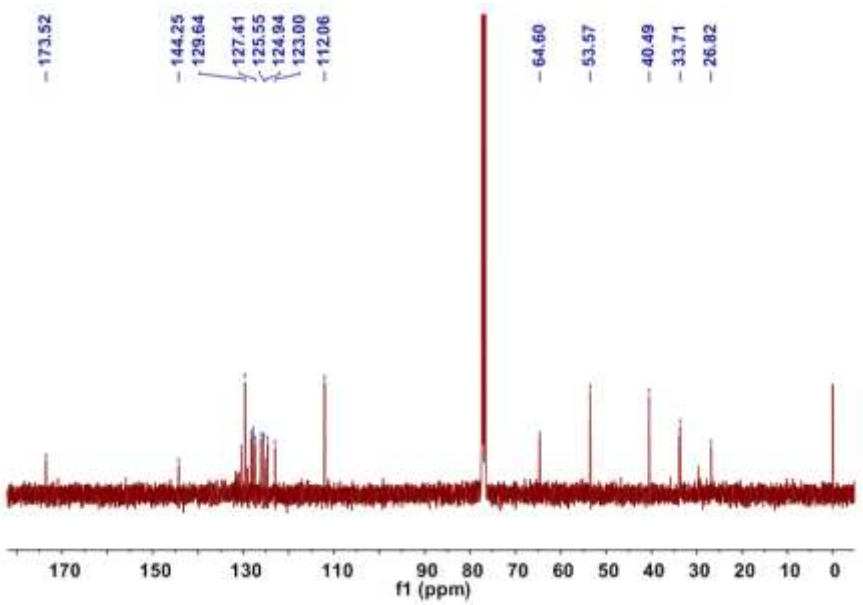


Figure S2. ^{13}C NMR spectrum (100 MHz, chloroform-*d*, room temperature) of **Py-Cbl**.

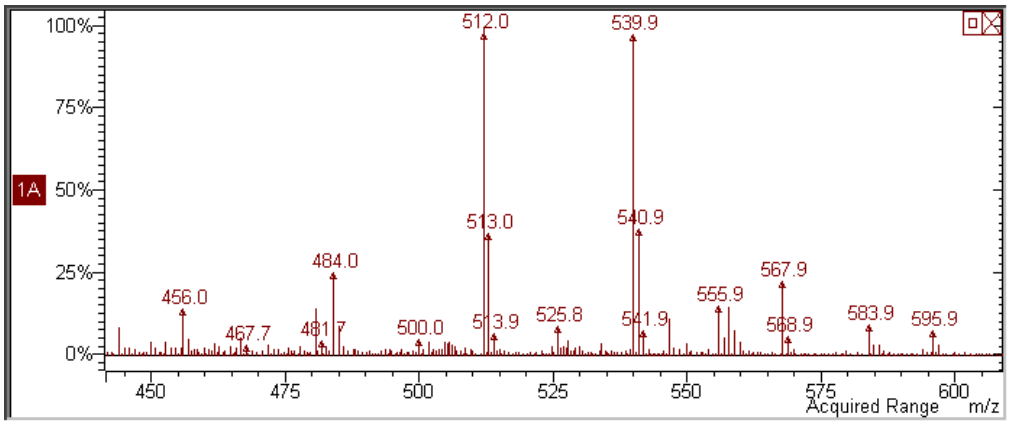


Figure S3. Electrospray ionization mass spectrum of **Py-Cbl**. Assignment of the main peak: *m*/*z* 539.9 $[M + Na]^{+}$ (100%).

*3. DLS data of **Py-Cbl** before and after UV irradiation*

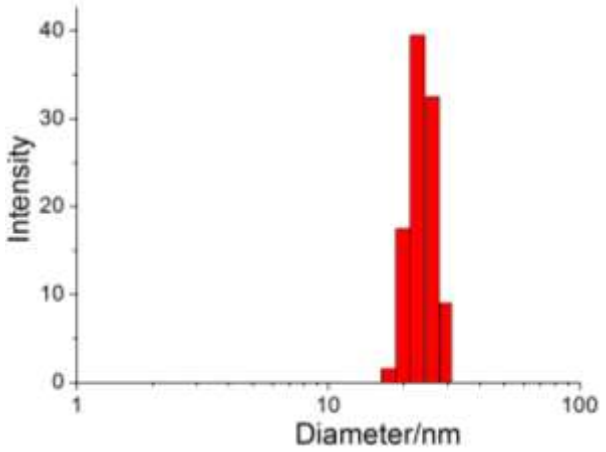


Figure S4. DLS result of the nanoparticles formed by **Py-Cbl**.

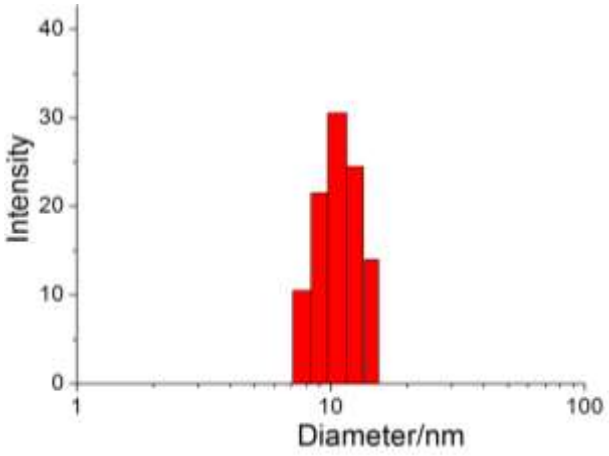


Figure S5. DLS result of the nanoparticles formed by **Py-Cbl** after UV irradiation for 10 min (8 W).

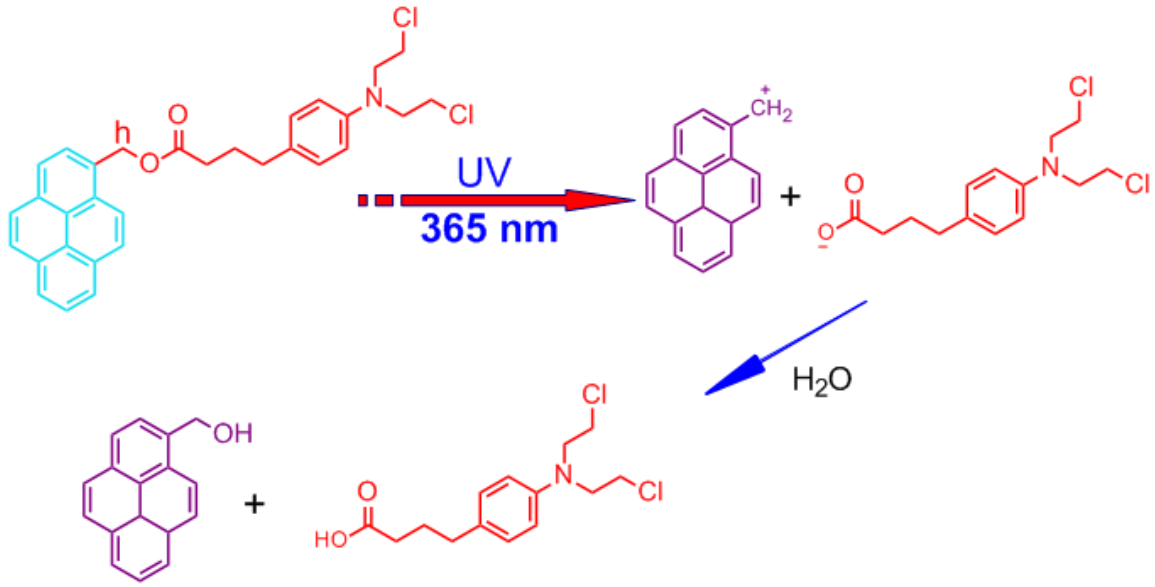


Figure S6. Photodegradation process of **Py-Cbl** upon UV irradiation.[S1]

*4. Enhancement of the solubility of **Py-Cbl** upon formation of host–guest complex **WP6⊃Py-Cbl***

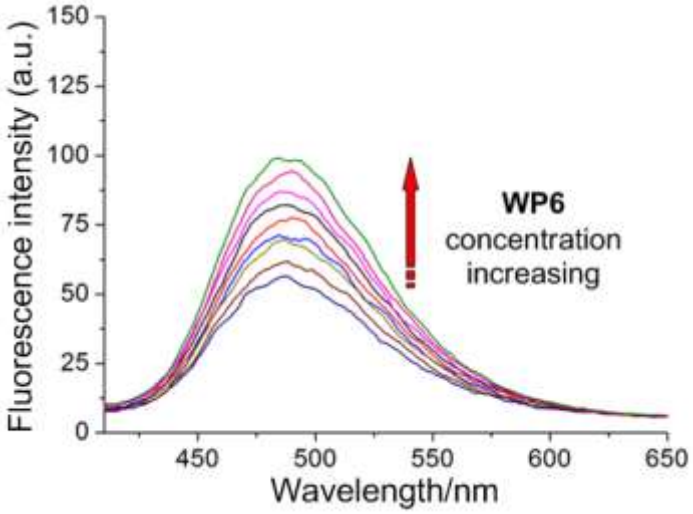


Figure S7. Fluorescence emission spectra of **Py-Cbl** (2.00×10^{-5} M, λ_{ex} = 340 nm) in water at room temperature with different concentrations of **WP6** (from 0 to 5.00 equiv).

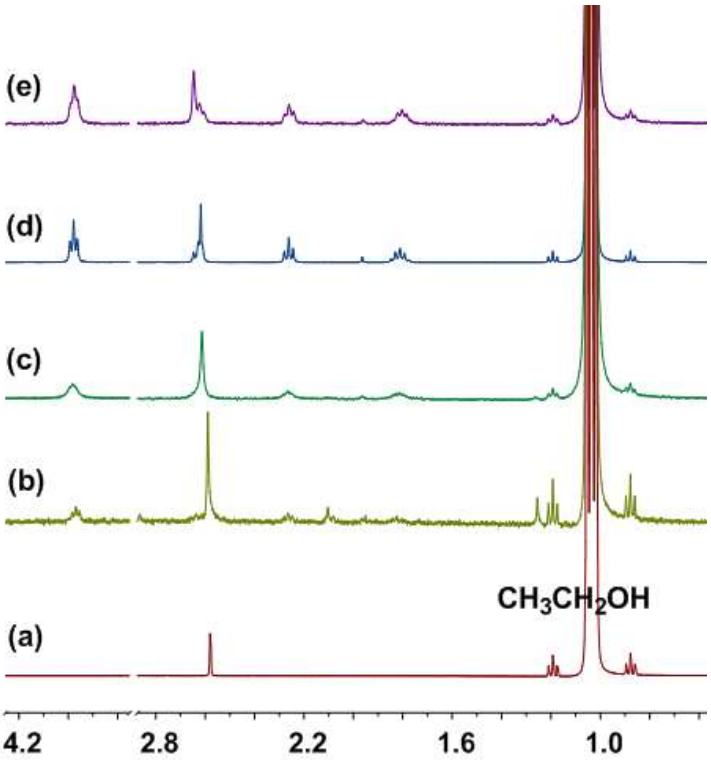


Figure S8. Partial ^{1}H NMR spectra (500 MHz, D_2O, room temperature): (a) **Py-Cbl** (0.500 mM); (b) **Py-Cbl** (0.500 mM) and **WP6** (1.00 mM); (c) **Py-Cbl** (0.500 mM) and **WP6** (2.00 mM); (d) **Py-Cbl** (0.500 mM) and **WP6** (3.00 mM); (e) **Py-Cbl** (0.500 mM) and **WP6** (5.00 mM). Ethanol was employed as an internal standard, and the concentration of ethanol was kept at 10.0 mM.

40–70% of new drug candidates can not be formulated on their own because of their poor solubilities in water. Therefore, sophisticated methods that can effectively improve the solubilities of poorly soluble drug candidates have attracted a great deal of attention. Recently, various methods have been developed to improve the water-solubilities of poorly soluble anticancer drugs. Covalent functionalization improves the solubility of the drugs, but it is necessary to alter their chemical structures, which may affect their therapeutic efficacy. Compared with covalent modification, supramolecular functionalization approaches to enhance the solubility of the anticancer drugs are especially important for their bioapplications, because the unique properties of drugs can be effectively preserved. Solubility enhancement studies were conducted by using the phase solubility method.[S2] Briefly, excess produg was added to a solution containing a known concentration of **WP6** under sonication for 2 h. After removal of the insoluble **Py-Cbl** by centrifugation, the concentration of soluble **Py-Cbl** in the supernatant was determined by ^{1}H NMR integration of **Py-Cbl** resonances versus ethanol, which was employed as an internal standard. As shown in Figures S8 and S9, the solubility of **Py-Cbl** increased gradually upon addition of **WP6**, demonstrating that **WP6** could be utilized as an excellent supramolecular container to greatly enhance the solubility of the poorly soluble anticancer prodrug.

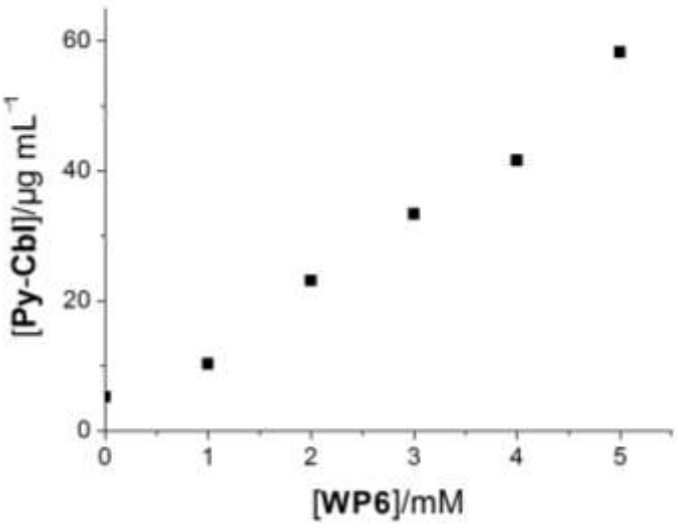


Figure S9. Variations in the solubility of **Py-Cbl** with various concentrations of **WP6**.

*5. Photodegradation of **Py-Cbl** and **WP6⊃Py-Cbl***

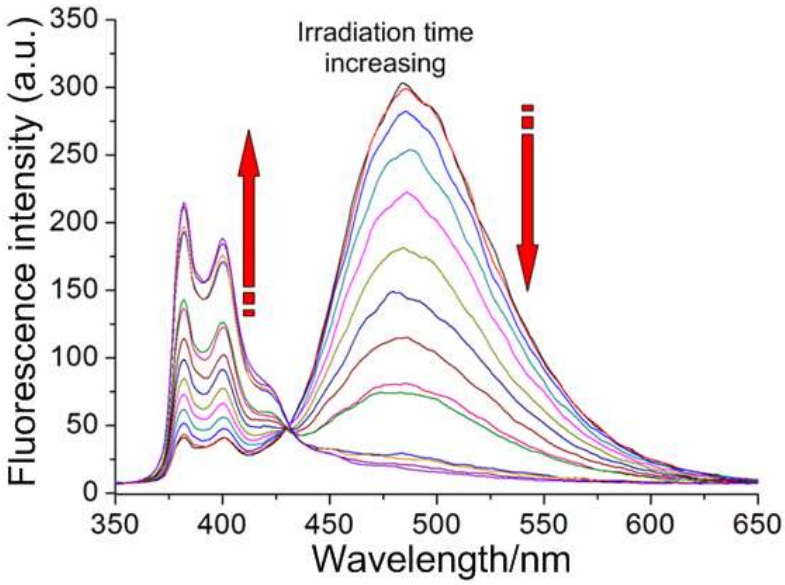


Figure S10. Changes in fluorescence intensity related to **Py-Cbl** (2.00×10^{-5} M) as a function of irradiation time (5 W).

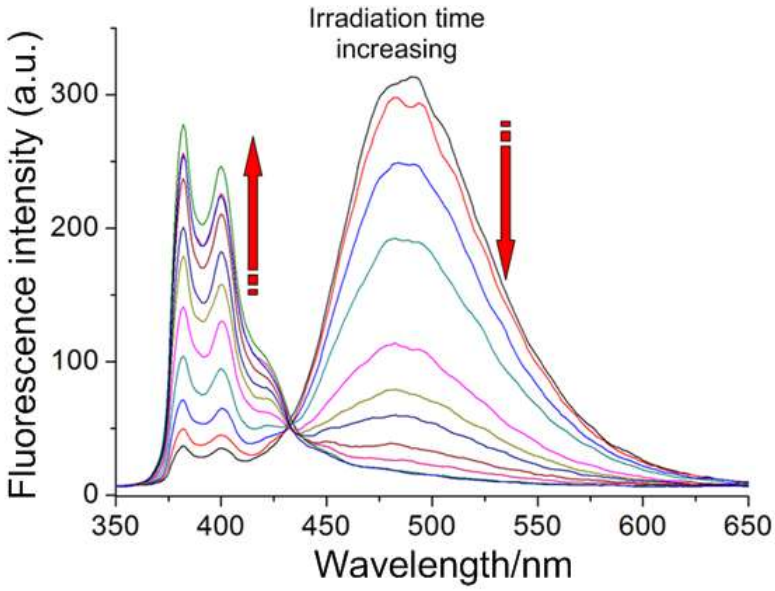


Figure S11. Changes in fluorescence intensity related to **Py-Cbl** (2.00×10^{-5} M) as a function of irradiation time (8 W).

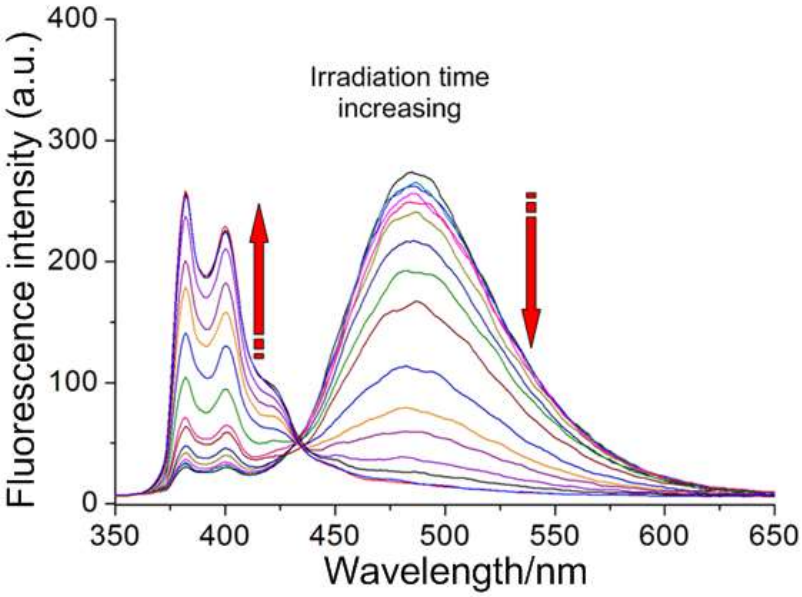


Figure S12. Changes in fluorescence intensity related to **WP6⊃Py-Cbl** (2.00×10^{-5} M) as a function of irradiation time (5 W).

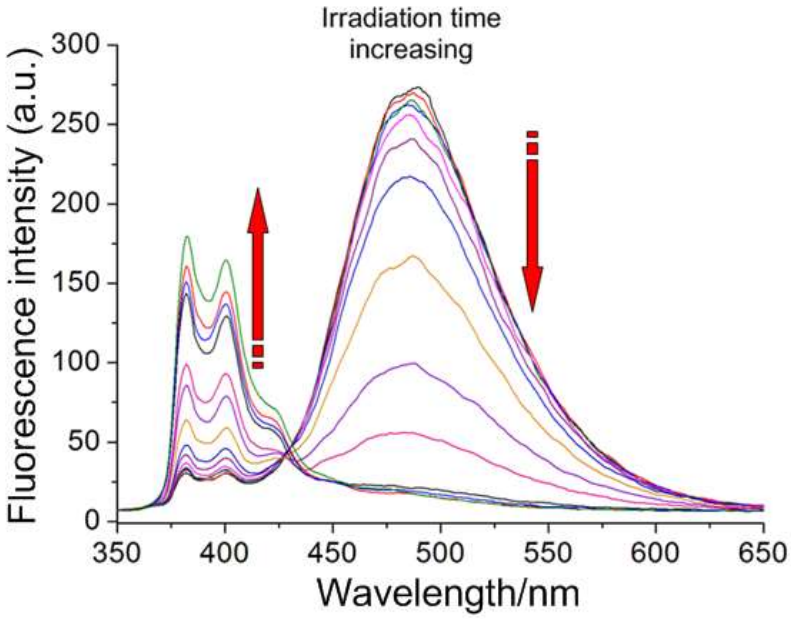


Figure S13. Changes in fluorescence intensity related to **WP6⊃Py-Cbl** (2.00×10^{-5} M) as a function of irradiation time (8 W).

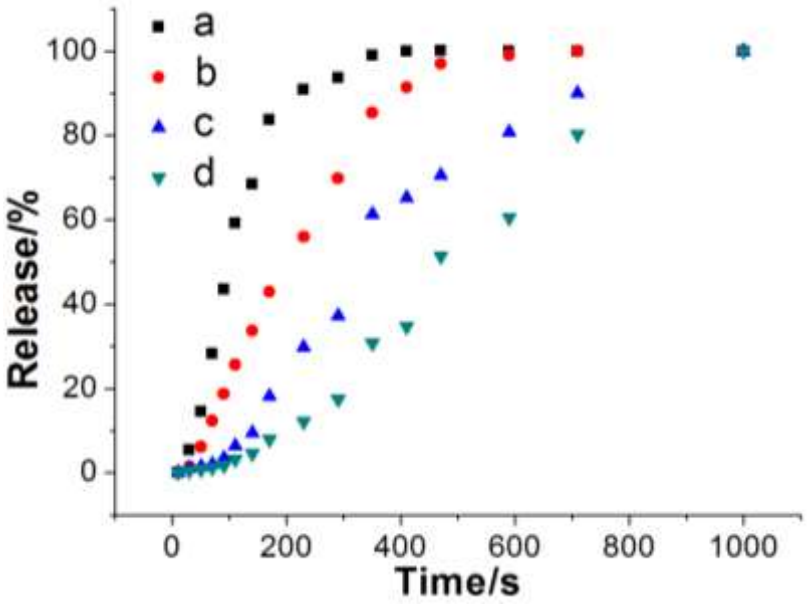


Figure S14. Progress of the release of chlorambucil from **Py−Cbl** nanoparticles under UV light irradiation: a. **Py-Cbl** (8 W); b. **WP6⊃Py-Cbl** (8 W); c. **Py-Cbl** (5 W); d. **WP6⊃Py-Cbl** (5 W).

6. Characterizations of the PIC micelles

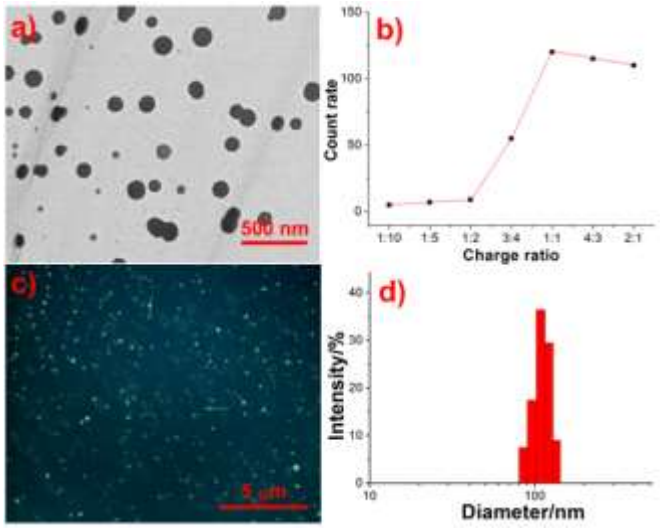


Figure 15. a) DLS count rates of the ternary PIC complex at different charge ratios. b) TEM image of the ternary PIC complex micelles. c) Fluorescence microscopy images of the ternary PIC complex micelles. d) DLS data of the ternary PIC complex. The concentration of the polymer was fixed at 0.75 mg mL^{-1}.

7. Cellular uptake of the anticancer prodrug

A549 cells were seeded at a density of 1.00×10^4 cells/well in a 96-well plate, and incubated for 24 h for attachment. The cells were incubated with fresh serum-supplemented DMEM with **Py-Cbl**, **WP6⊃Py-Cbl**, and the ternary PIC micelles, respectively, for 4 h. The concentration of **Py-Cbl** was kept at 5×10^{-5} M. After 4 h, the medium was removed and washed with PBS for three times. The cells were disrupted by adding water and further sonication for 0.5 h. After centrifugation, the supernatant was employed to monitor their absorption intensity, corresponding to the cellular uptake amount of **Py-Cbl**.

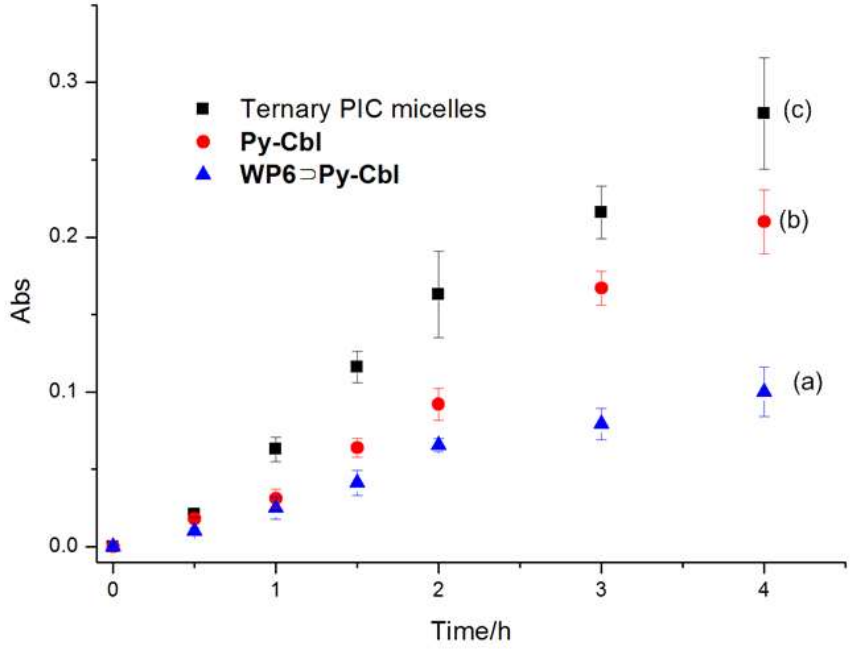


Figure S16. Cellular uptake of the anticancer prodrug determined by UV-vis spectroscopy: (a) **WP6⊃Py-Cbl**; (b) **Py-Cbl**; (c) ternary PIC micelles.

References:

S1. T. M. Guardado-Alvarez, L. S. Devi, M. M. Russell, B. J. Schwartz, J. I. Zink, *J. Am. Chem. Soc*. **2013**, *135*, 14000.

S2. D. Ma, G. Hettiarachchi, D. Nguyen, B. Zhang, J. B. Wittenberg, P. Y. Zavalij, V. Briken, L. Isaacs, *Nature Chem*. **2012**, *4*, 503.