Supporting Information

Self-delivery nanoparticles from an amphiphilic covalent drug couple of irinotecan and bendamustine for cancer combination chemotherapy

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1. Schemes and Figures



Figure S1. Synthesis route of amphiphilic covalent drug couple Ir-Bd.



Figure S2. UV/Vis spectra of Bd, Ir, Ir/Bd mixture and Ir-Bd in DMSO.

Compared to the UV/Vis absorption of free Ir at 367 nm, we observe a 4 nm blue-shift in the absorption of the Ir-Bd at 363nm. However, the phenomenon of blue-shift in the absorbance of the Ir/Bd mixture is not observed. Furthermore, the Ir-Bd possesses both UV/Vis absorptions of Bd and Ir. The UV/Vis results further confirm the successful formation of Ir-Bd.



Figure S3. (a) Fluorescence emission spectra of Bd ($\lambda_{ex} = 330 \text{ nm}$, $\lambda_{em} = 424 \text{ nm}$), Ir , ($\lambda_{ex} = 360 \text{ nm}$, $\lambda_{em} = 430 \text{ nm}$) and Ir-Bd ($\lambda_{ex} = 360 \text{ nm}$, $\lambda_{em} = 426 \text{ nm}$) in DMSO. (b) Fluorescent emission spectrum of Ir-Bd NPs in water ($\lambda_{ex} = 360 \text{ nm}$, $\lambda_{em} = 423 \text{ nm}$).



Figure S4. Influence of storage time on diameter (a) and PDI (b) of Ir-Bd NPs. The solution of Ir-Bd NPs was stored at 4 °C in refrigerator for 30 days. At different time intervals (0, 1, 5, 10, 15, 20, 25 and 30 d), the average size and PDI were determined. Samples were measured in triplicates. The values are the mean \pm SD.

In the Figure S4, no significant change in diameter of Ir-Bd NPs is observed even after 30 days storage. And the value of PDI is under 0.2 at every measurement. The results demonstrate that Ir-Bd NPs are extremely stable for 30 days under storage.



Figure S5. (a) Total ion chromatography (TIC) of Ir and Bd. (b,c) Extracted ion chromatography (EIC) of Ir (m/z = 587.2870, (M+H⁺)) and Bd (m/z = 358.1079, (M+H⁺)). The retention time of Ir (M+H⁺, m/z = 587.2857) and Bd (M+H⁺, m/z = 358.1072) is 3.21, and 3.59 min.

In order to confirm Ir-Bd is converted into free Ir and Bd through ester degradation in cells, the mixed standard with Bd and Ir is used as a control for the LC-MS measurements. Figure S5 gives the molecular weight and retention time of standard Ir $(M+H^+, m/z = 587.2857, at 3.21 min)$ and Bd $(M+H^+, m/z = 358.10, at 3.59 min)$.



Figure S6. (a) Total ion chromatography (TIC) of the cell extracts. (b,d) Extracted ion chromatography (EIC) of Ir (m/z = 587.2870, ($M+H^+$)) and Bd (m/z = 358.1079, ($M+H^+$)). (c,e) The retention time of Ir ($M+H^+$, m/z = 587.2892) and Bd ($M+H^+$, m/z = 358.1087) is at 3.18, and 3.60 min.

As expected, Ir-Bd can be converted to free Ir and Bd via ester hydrolysis in cells. Compared to the molecular weight and retention time of standard Ir (M+H⁺, m/z = 587.2857) and Bd (M+H⁺, m/z = 358.1072) at 3.21, and 3.59 min (Figure S5), the molecular weight and retention time of cell extracts are 587.2892 (M+H⁺, m/z) and 358.1087 (M+H⁺, m/z) at 3.18, and 3.60 min, confirming the existence of free Ir and Bd in the cell extracts. The LC-MS results clarify that Ir and Bd are released after internalization of Ir-Bd NPs by tumor cells.



Figure S7. *In vitro* cytotoxicity of Ir-Bd NPs to CIK cells at various concentrations from 0.1 to 50 μ M for 72 h. Cell viability was measured using a CCK-8 assay. The cells without the treatment were used as a control. The data are presented as average \pm standard deviation (n = 5).

Figure S7 gives the viability of immune cells (CIK cells) after incubation with Ir-Bd NPs for 72 h. Clearly, no obvious cell proliferation inhibition of Ir-Bd NPs is found at the test concentration range after 72 h incubation to confirm the low immunotoxicity of Ir-Bd NPs.