Supplementary information

Y₁ receptor ligand synergized with P-glycoprotein inhibitor improves therapeutic efficacy of multidrug resistant breast cancer

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**Materials:** Ethanol, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), N-hydroxysuccinimide (NHS), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-tetrazolium bromide (MTT), HPLC grade acetonitrile and trifluoroacetic acid (TFA) were purchased from Aladdin Industrial Inc (Shanghai, China). Penicillin, and streptomycin were purchased from Invitrogen™ (Carlsbad, USA). [Asn<sup>6</sup>, Pro<sup>34</sup>]-NPY (AP) (YPSKPNPNGEDAPAEGLARYYSALRHYINLI TRPRY-NH<sub>2</sub>) were synthesized by the Dechi Biosciences Co, Ltd (Shanghai, China). Doxorubicin (DOX) hydrochloride and IRDye780 iodide were purchased from Sigma-Aldrich Co. LLC (Shanghai, China). Tariquidar (Tar) was purchased from ApixBio (Hangzhou, China). DPSE-PEG2000 (50:50) and PLGA-PEG-2000 (50:50) were purchased from A.V.T. Pharmaceutical Ltd. (Shanghai, China). All reagents were used as received.

**Characterization:** Particle size, size distribution, and zeta potential of the nanomicelle dispersions were measured at room temperature by dynamic light scattering (DLS) using a Zeta particle size analyzer (Nano-ZS, Malvern, England). The data was collected on an autocorrelator with a detection angle of 173°. To obtain detailed structural and morphological information, ~1 μL of the diluted micelle dispersion was dropped onto a copper grid coated with a thin layer of carbon film and then dried at room temperature. High-resolution transmission electron microscopy (HRTEM) images were recorded from a JEOL-2100 (JEOL, Japan) instrument, which was operated at 200 kV.

**Cell culture:** Human multidrug resistant breast cancer line MCF-7/ADR was cultured in Dulbecco’s modified Eagle’s medium (DMEM). The medium contains fetal bovine serum (FBS, 20 wt%), penicillin (100 units/mL), and streptomycin (100 mg/mL). The cells were maintained in a 37 °C incubator with 5% CO<sub>2</sub>. Origin cells human breast cancer cells MCF-7 were bought from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), then incubated into MCF-7/ADR cell line in our lab.¹
**Figure S1** Western blot analysis of Y₁R expression on MCF-7/ADR cells.
**Figure S2** TEM images of NM-DOX, AP-NM-DOX, NM-DOX&Tar and AP-NM-DOX&Tar.
Figure S3 LC-MS images of Tar standards, NM-DOX&Tar and AP-NM-DOX&Tar.
Figure S4 Mass spectrometry images of Tar.
Figure S5 Standard curve of Tar. Tar concentration is varied from 50 to 400 nM.

The equation of the line is $y = 0.0959x + 0.6807$ with $R^2 = 0.9975$. 
Figure S6 Pyrene excitation spectra of PEG-PLGA in aqueous solution. Emission wavelength is at 302 nm.
Figure S7 Intensity ratio of $I_{335}/I_{333}$ versus $\lg C$ for PEG-PLGA in water. The critical micelle concentration of PEG-PLGA is 8.073 $\mu$g/mL.
**Figure S8** DOX concentration of AP-NM-DOX before and after the demulsification by acetonitrile.
**Figure S9** XY-Z series of MCF-7/ADR cells incubated with AP-NM-DOX&Tar for 8 h. Z-axis is from 0 to 3 μm.
Figure S10 Effect of different proportions of PEG-PLGA to AP-DSPE-PEG (w/w) on particle size and zeta potential. The proportion of PEG-PLGA to AP-DSPE-PEG (w/w) varies from 20:1 to 100:1. Mean ± SD (n = 3).
**Figure S11** Inhibitory effect of different proportions of PEG-PLGA to AP-DSPE-PEG (w/w) on MCF-7/ADR cells. The proportion of PEG-PLGA to AP-DSPE-PEG (w/w) varies from 20:1 to 100:1. DOX concentration is varied from 0.3125 to 80 μg/mL. Mean ± SD (n = 3).
Figure S12 - Inhibition effect of NM-DOX, AP-NM-DOX, NM-DOX&Tar, and AP-NM-DOX&Tar on MCF-7/ADR cells after 24 h incubation. DOX concentration is varied from 0.3125 to 80 μg/mL. Mean ± SD (n = 3).
**Figure S13** Inhibition effect of NM-DOX, AP-NM-DOX, NM-DOX&Tar, and AP-NM-DOX&Tar on MCF-7 cells after 24 h incubation. DOX concentration is varied from 0.3125 to 80 μg/mL. Mean ± SD (n = 3).
**Figure S14** IC\textsubscript{50} value of different micelles on MCF-7 cells after 24 h incubation. Mean ± SD (n = 3). **p < 0.01, * p < 0.05
Figure S15 Inhibition effect of NM-DOX, AP-NM-DOX, NM-DOX + Antagonist, and AP-NM-DOX+Antagonist on MCF-7/ADR cells after 24 h incubation. DOX concentration is varied from 0.3125 to 80 $\mu$g/mL. Mean ± SD (n = 3), Y$_1$R antagonist (CAS: 221697-09-2) was used at a dose of 10 $\mu$M.
Figure S16 IC$_{50}$ value of different micelles of NM-DOX, AP-NM-DOX, NM-DOX+Antagonist, and AP-NM-DOX+Antagonist on MCF-7/ADR cells after 24 h incubation. DOX concentration is varied from 0.3125 to 80 μg/mL. Mean ± SD (n = 3), an antagonist of Y$_1$R (CAS: 221697-09-2) was used at a dose of 10 μM. ** $p < 0.01$
Figure S17 Mean fluorescence intensity (MFI) of MCF-7/ADR cells incubated with different IRDye780 loaded micelles following by flow cytometry analysis. All micelles were incubated with MCF-7/ADR cells for 8 h.
Figure S18 *In vivo* fluorescence imaging of MCF-7/ADR tumor bearing mice were taken before and after intravenous injection of NM-IRDye780 at 0, 2, 4, 6, 12, 24 h (IRDye780: 0.25 mg/kg).
Figure S19 *In vivo* fluorescence imaging of MCF-7/ADR tumor bearing mice were taken before and after intravenous injection of AP-NM-IRDye780 at 0, 2, 4, 6, 12, 24 h (IRDye780: 0.25 mg/kg).
Figure S20 *In vivo* fluorescence imaging of MCF-7/ADR tumor bearing mice were taken before and after intravenous injection of NM-IRDye780&Tar at 0, 2, 4, 6, 12, 24 h (IRDye780: 0.25 mg/kg).
Figure S21 *In vivo* fluorescence imaging of MCF-7/ADR tumor bearing mice were taken before and after intravenous injection AP-NM-IRDye780&Tar at 0, 2, 4, 6, 12, 24 h (IRDye780: 0.25 mg/kg).
**Figure S22** Photos of tumor-bearing mice after i.v. injection of PBS, free DOX, NM-DOX, AP-NM-DOX, NM-DOX&Tar, and AP-NM-DOX&Tar from 0 to 14 days.
Figure S23 Hematological analysis of the mice after i.v. injection of PBS, free DOX, NM-DOX, AP-NM-DOX, NM-DOX&Tar, and AP-NM-DOX&Tar. Mean ± SD (n = 3).
Figure S24 H&E staining of MCF-7/ADR tumor bearing nude mice liver after intravenous injection of Free DOX, NM-DOX, AP-NM-DOX, NM-DOX&Tar, and AP-NM-DOX &Tar. The mice were killed at 28th day after six times of tail vein injection.
Table S1 Characterization of nanomicelles

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>DOX loading efficiency (%)</th>
<th>Tar loading efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM-DOX</td>
<td>71.4 ± 3.1</td>
<td>0.189 ± 0.017</td>
<td>-9.8 ± 0.9</td>
<td>81.4 ± 1.1</td>
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<tr>
<td>AP-NM-DOX</td>
<td>60.9 ± 2.4</td>
<td>0.217 ± 0.014</td>
<td>-7.9 ± 0.8</td>
<td>82.3 ± 2.4</td>
<td>--</td>
</tr>
<tr>
<td>NM-DOX&amp;Tar</td>
<td>74.5 ± 4.2</td>
<td>0.201 ± 0.009</td>
<td>-10.1 ± 0.6</td>
<td>86.9 ± 1.7</td>
<td>67.8 ± 2.3</td>
</tr>
<tr>
<td>AP-NM-DOX&amp;Tar</td>
<td>82.1 ± 3.9</td>
<td>0.196 ± 0.008</td>
<td>-9.1 ± 1.3</td>
<td>87.1 ± 1.4</td>
<td>65.9 ± 1.8</td>
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</tbody>
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