Supporting Information

Diversified Photo-Energy Conversion Based on Single-Molecule FRET to Realize Enhanced Phototheranostics

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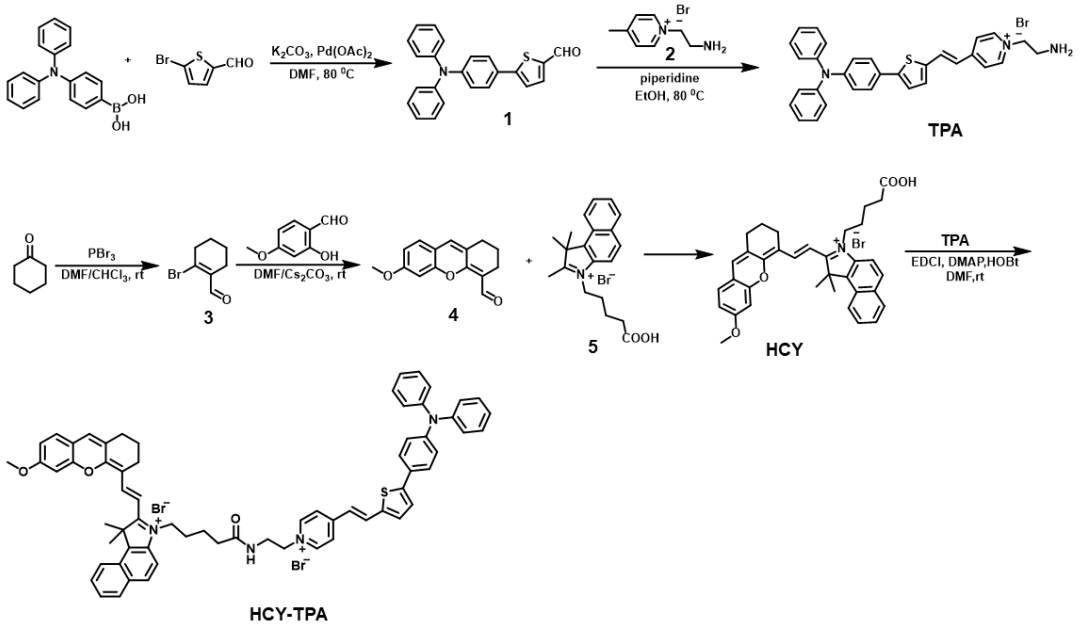
Synthesis and characterization



Scheme S1 Synthetic route to compounds **HCY-TPA**

5-(4-(diphenylamino)phenyl)thiophene-2-carbaldehyde (**1**), 1-(2-aminoethyl)-4-methylpyridin-1-ium (**2**), 2-bromocyclohex-1-ene-1-carbaldehyde (**3**), 6-methoxy-2,3-dihydro-1H-xanthene-4-carbaldehyde (**4**) and 3-(4-carboxybutyl)-1,1,2-trimethyl-1H-benzo[e]indol-3-ium bromide (**5**) were prepared according to the literatures [S1-S4].

Methods

Singlet oxygen ($^1O_2$) detection

9, 10-anthracenedipropanoic acid (ABDA), a singlet oxygen capture agent, was used to measure the singlet oxygen generated by HCY-TPA, HCY or TPA, respectively. Briefly, The absorbance of ABDA (0.049 μM) at 378 nm was recorded in aqueous solution. Then, 10 μM HCY-TPA, HCY or **TPA** was added to this cuvette and irradiated with light for various time (0-60 s), and absorption spectra were measured immediately.

Reactive oxygen species (ROS) detection

2, 7-dichlorodifluorescein diacetate (DCFH-DA) was used as the ROS indicator, which can be converted to 2', 7'-dichlorofluorescein (DCF) in the presence of ROS. **HCY-TPA**, **HCY**, **TPA** and DCFH-DA were prepared as 10 μM in water. Then the cuvette was exposed to light for different time (0-180 s), and the fluorescence spectra with the excitation wavelength at 480 nm were observed immediately after each irradiation.

Detecting $^1O_2$/$O_2^{\cdot-}$ generation via electron paramagnetic resonance (EPR) assay

The EPR assay was carried out with a Bruker Nano x-band spectrometer using 5, 5-dimethyl-1-pyrroline N-oxide (DMPO) and 2, 2, 6, 6-tetramethylpiperidine (TEMP) as a spin-trap agent,

respectively. **HCY-TPA**, **HCY**, **TPA** was dissolved in methanol at a dilution of 10^{-5} M, and then 25 mM DMPO or TEMP was added into methanol without and with LED irradiation (0.5 W/cm^2 for 2 min) for 60 s, respectively. Finally, the EPR signals were recorded at room temperature.

Cell culture

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% of FBS (fetal bovine serum) and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% of CO_2.

Intracellular ROS/$^1O_2$/$O_2^{\cdot-}$ detection

DCFH-DA was applied for the intracellular ROS detection. The HepG2 cells were incubated with 10 μM **HCY-TPA**, **HCY**, **TPA** for 30 min for cell uptake followed by incubation with 10 μM DCFH-DA for 30 min, respectively. After that, the cells were washed with PBS three times and irradiated for 30 min. The green fluorescence was immediately observed using CLSM. (λ_{ex} = 504 nm; λ_{em} = 529 nm).

SOSG was used as the intracellular $^1O_2$ indicator. HepG2 cells were incubated with 10 μM **HCY-TPA**, **HCY**, **TPA** for cell uptake followed by incubation with 2 μM SOSG for 30 min, respectively. After that, cells were washed with PBS, and LED light (0.5 W/cm^2 for 2 min) irradiated for 30 min. The green fluorescence was immediately observed using CLSM. (λ_{ex} = 504 nm; λ_{em} = 525 nm).
DHE was employed as the intracellular $O_2^{\cdot-}$ indicator. HepG2 cells were pretreated with10 μM **HCY-TPA**, **HCY**, **TPA** for 30 min and then co-stained with DHE (10 μM) for another 30 min, respectively. Afterwards, HepG2 cells were irradiated with LED irradiation (0.5 W/cm^2 for 2 min), which were then imaged under CLSM. (λ_{ex} = 488 nm, λ_{em} = 570-620 nm)

Subcellular colocalization assay

HepG2 cells were plated onto 35 mm confocal dishes for 24 h. Next, the cells were firstly incubated with 10 μM **HCY-TPA**, **HCY**, **TPA** for 0.5 h at 37 ℃ under 5 % CO_2 consideration, and then stained by MitoTracker Green (100 nM), respectively. Cells were then visualized with laser confocal microscopy. The excitation wavelength for **TPA** and **HCY-TPA** was 488 nm, **HCY** was 680 nm, MitoTracker Green FM was 488 nm. The emission wavelength of **TPA** was collected from 600 to 630 nm, 700-730 nm for **HCY-TPA** and **HCY**, 500 to 540 nm for Mito Tracker Green.

Dead/Live cell co-staining

For live/dead cells co-staining assay, using Calcein-AM for live cells, PI for dead cells, which could exhibited red fluorescence in nucleus during cell apoptosis. Briefly, HeLa cells were planted onto confocal dishes and incubated for 48 h. Then the cells were further treated with 400 uL dye diluent and 10 uL Calcein-AM stain after 10 uL **HCY-TPA**, **HCY**, **TPA** was added for 30 min, respectively. Co-incubated for 20 min, the cell image was observed immediately using CLSM. (Calcein-AM: λ_{ex} = 490 nm; λ_{ex} = 520 nm, PI: λ_{ex} = 488 nm; λ_{ex} = 650-700 nm)

Confocal imaging of photoinduced cell death

Annexin V-FITC/propidium iodide (PI) Apoptosis Detection Kit was used for detection of **HCY-TPA**, **HCY**, **TPA** mediated photoinduced cell death. Briefly, HeLa cells were planted onto confocal dishes and grew to suitable density (1×10^4 cells). Then **HCY-TPA**, **HCY**, **TPA** (10 μM) was added to co-culture for 30 min, respectively, which was subsequently disposed with 400 μL binding buffer

and stained by 10 μL Annexin V-FITC (λ_{ex} = 488 nm, λ_{em} = 530 nm) and 5 μL PI (λ_{ex} = 488 nm, λ_{em} = 650-700 nm) in a cool and dark atmosphere for 15 min. Lastly, the co-stained HeLa cells were observed by CLSM.

Photothermal conversion efficiency of HCY-TPA/HCY

Photothermal conversion was measured using a custom-built setup. Quartz cuvette walls (path length: 1 cm) comprising 1 mL **HCY-TPA** or **HCY** (100 μM) was placed on a flat platform. A fiber-coupled continuous semiconductor diode laser (720 nm, Shanghai Connect Fiber Optics Co.) with a power density of 1.0 W/cm^2 was used as the laser source. An infrared thermal imaging camera (FOTRIC 220 s) was used to monitor the temperature change. The photothermal conversion efficiency was calculated as follows:

$\theta = (T-T_{surr})/(T_{max}-T_{surr})$

$t=\tau_s\times(-\ln\theta)$

τ_s= 166.91 s

$hS=(\Sigma m_i C_{p,i})/\tau_s$

$=[m(H_2O)\times C(H_2O)+m(quartz)\times C(quartz)]/\tau_s$

=25.03 mW/C(720 nm)

$Q_{Dis}=hS\times(T(H_2O)_{max}-T_{surr})$

=100.12 mW

$\eta_{(HCY\text{-}TPA)}=[hS\times(T_{max}-T_{surr})-Q_{Dis}]/[I\times(1-10^{-A720})]\times100\%$

=58.37 %

$\theta_{(HCY)}=(T-T_{surr})/(T_{max}-T_{surr})$

$t=\tau_s\times(-\ln\theta)$

τ_s= 230.87 s

$hS=(\Sigma m_i C_{p,i})/\tau_s$

$=[m(H_2O)\times C(H_2O)+m(quartz)\times C(quartz)]/\tau_s$

=18.10 mW/C(720 nm)

$Q_{Dis}=hS\times(T(H_2O)_{max}-T_{surr})$

=72.40 mW

$\eta_{(HCY\text{-}TPA)}=[hS\times(T_{max}-T_{surr})-Q_{Dis}]/[I\times(1-10^{-A720})]\times100\%$

=25.72 %

h (mW m^{-2} °C^{-1}): Heat transfer coefficient,

S (m^2): Surface area of the container,

T_{max} (°C): Equilibrium temperature,

T_{surr} (°C): Ambient temperature,

Q_{Dis} (mW): Heat from the light absorbed by the quartz cuvette walls themselves and it was independently measured using a quartz cuvette cell containing aqueous samples without HCY-TPA or HCY,

I: Incident laser power density,

A_{720}: Absorbance of HCY-TPA or HCY at 720 nm.

Animals and tumor model

Animal studies were performed in compliance with the relevant laws and institutional guidelines set by Anhui Committee of Use and Care of Laboratory Animals and the overall project protocols

were approved by the College of Life Science, Anhui University. The H22 tumor-bearing mice were obtained by subcutaneously injecting H22 cells (1×10^6 cells) in subcutaneous injections of female mice.

In vivo fluorescence imaging

Mice with H22 transplanted tumor were subcutaneously injected with PS (HCY-TPA), PBS, and then were anesthetized with 2.5% isofluorane in oxygen at selected time points. Subsequently, the whole-body fluorescence images were captured using an LB983 NC100 imaging system (Berthold *co. Ltd.*). After the 24 h later, mice were sacrificed and their hearts, livers, spleens, lungs, kidneys and tumors were collected. *Ex vivo* fluorescence imaging of these organs was immediately performed on the LB983 NC100 imaging system. The excitation wavelength was 635 nm, and the collected emission wavelength was 680-720 nm.

Antitumor therapy

Cell's suspension (10^8 cells/mL, H22 cells) was obtained and then subcutaneously injected at the female ICR mice (4-5-week old). Then theH22-bearing mice were protected from light and fed and watered freely. After 5-days inoculation, the tumor size was appropriate and the tumor mice were split into 8 groups (3 mice each), treated as below: (a) Control, (b) laser, (c) TPA (10^{-4} M, 100 μL), (d) TPA (10^{-4} M, 100 μL) + 720 nm fs laser and LED light. (e) HCY (10^{-4} M, 100 μL), (f) HCY (10^{-4} M, 100 μL) + 720 nm fs laser and LED light, (g) HCY-TPA (10^{-4} M, 100 μL) (h) HCY-TPA (10^{-4} M, 100 μL) + 720 nm fs laser and LED light. It's worth noting that only a single-dose injection was employed during in vivo treatment process. The changes of tumor volume, tumor weight and body weight were measured every 5 days during treatment. After treatment, major organs and tumors were gathered for H&E staining.

Hematoxylin and eosin (H&E) staining

After 14 days PDT treatment, the mice in different groups were sacrificed and the tissues were collected and fixed in 4 % paraformaldehyde. Subsequently, the obtained tissues were embedded into paraffin, sliced at a thickness of 5 μm, which were stained with hematoxylin and eosin (H&E).

In vitro cytotoxicity assays

HepG2 cells were plated at 10000 cells per well in a 96-well cell-culture plate, followed by incubation at 37 ℃ for 24 h. HCY-TPA, HCY and TPA (100 μL) with varying concentrations were added into each well, and cultured for another 30 min. After that, the cell culture media was replaced with 200 μL fresh medium. Then LED light was employed for treatment, after irradiation 30 min, cells were allowed to continue growing for 24 h. Then, 20 μL MTT solutions (5 mg/mL) in PBS were added to each well. After incubating the cells for 4 h, the medium was removed out carefully, and 200 μL DMSO was added to each well to dissolve blue formazan. Finally, the absorbance of 490 nm was measured with a Bio-Rad microplate reader and the cell viability was calculated by the following equation: cell viability (%) = (mean of abs. value of treatment group/mean abs. Value of control) ×100%. For dark toxicity measurement of HCY-TPA, HCY, TPA, no light irradiation was applied to this experiment, and all other steps were the same. Three groups of parallel experiments were carried out in this experiment, and the survival rate of cells was calculated by averaging the data of each group.

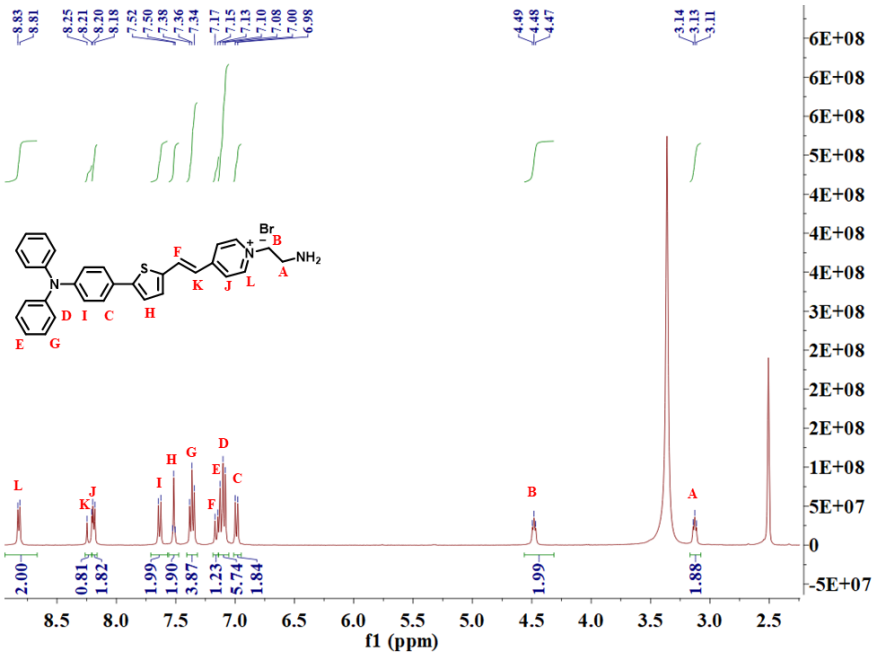


Figure S1 ^{1}H-NMR spectrum of TPA.

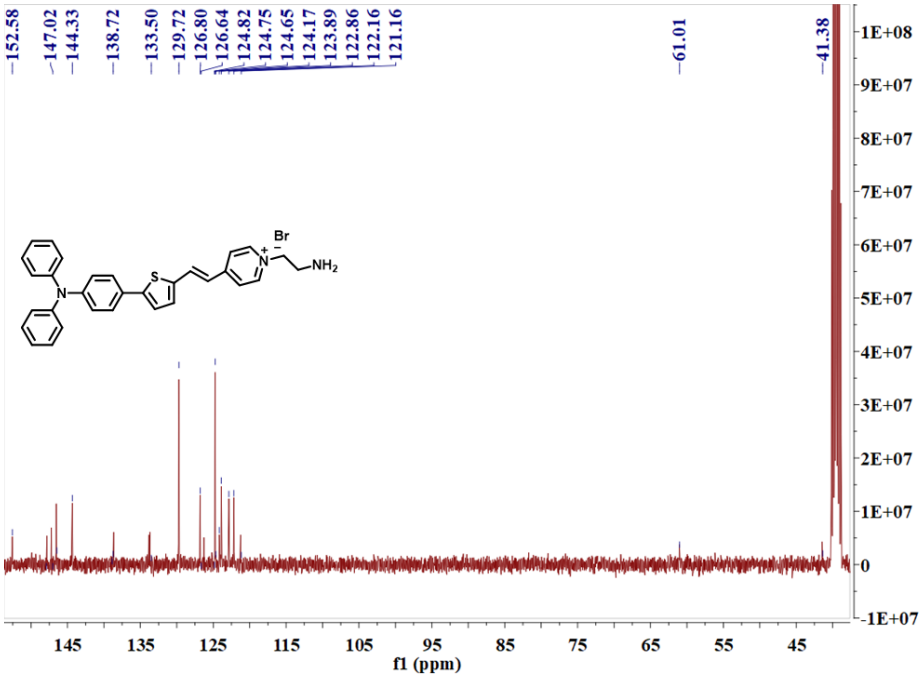


Figure S2 ^{13}C-NMR spectrum of TPA.

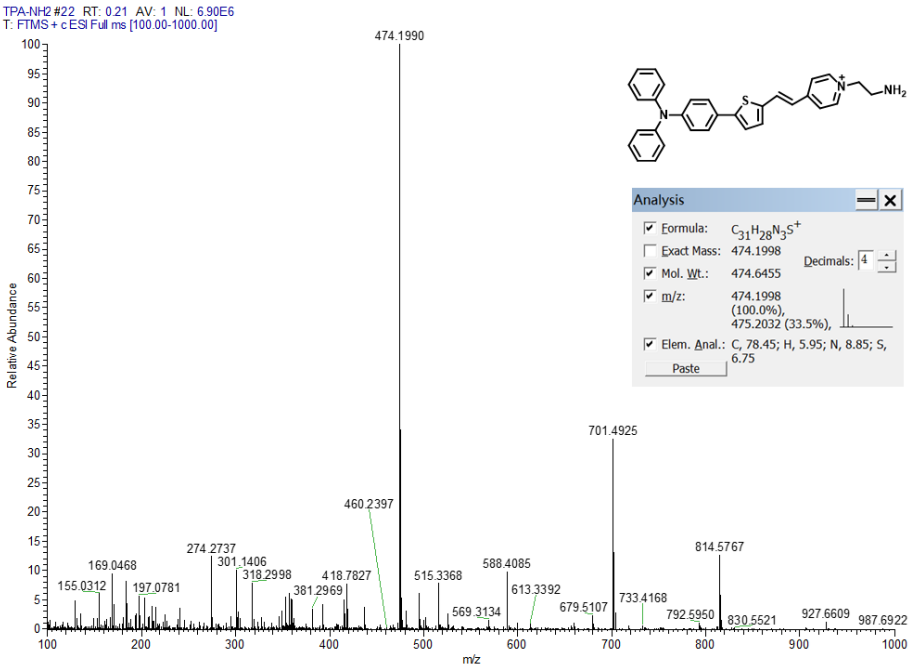


Figure S3 ESI-Mass spectrum of TPA.

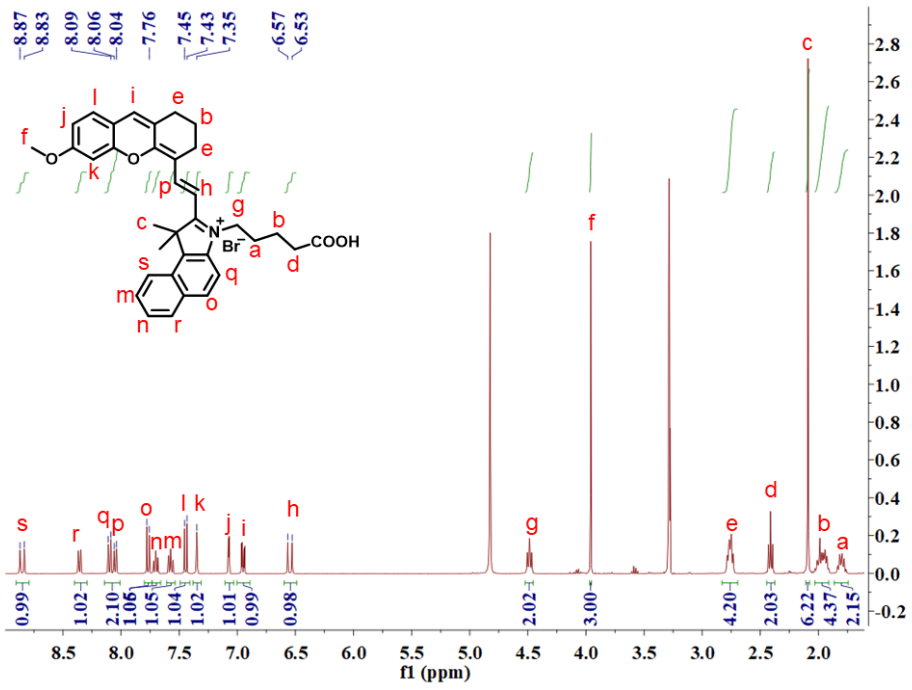


Figure S4 [1]H-NMR spectrum of HCY.

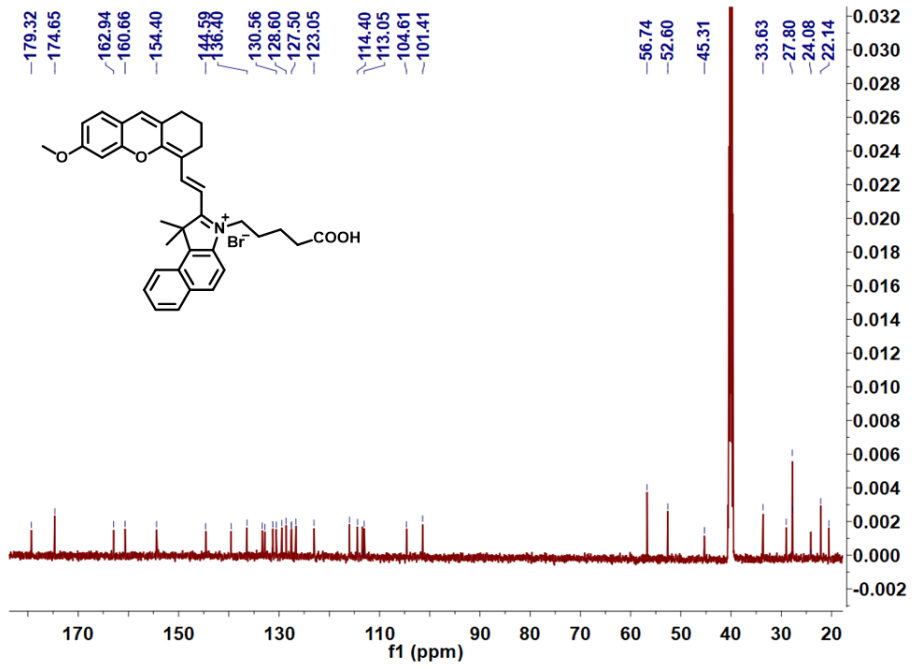


Figure S5 ^{13}C-NMR spectrum of HCY.

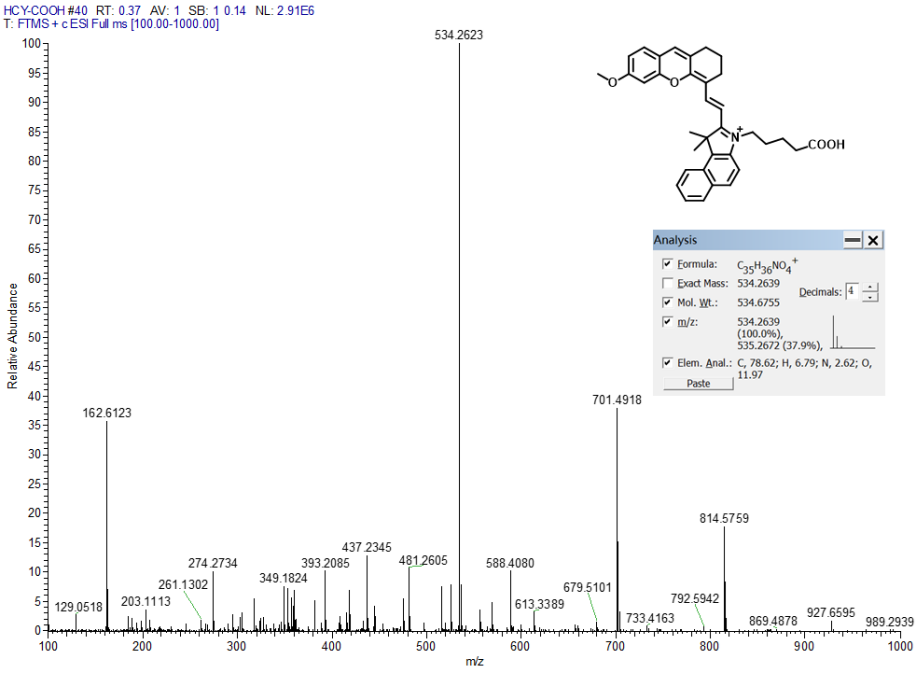


Figure S6 ESI-Mass spectrum of HCY.

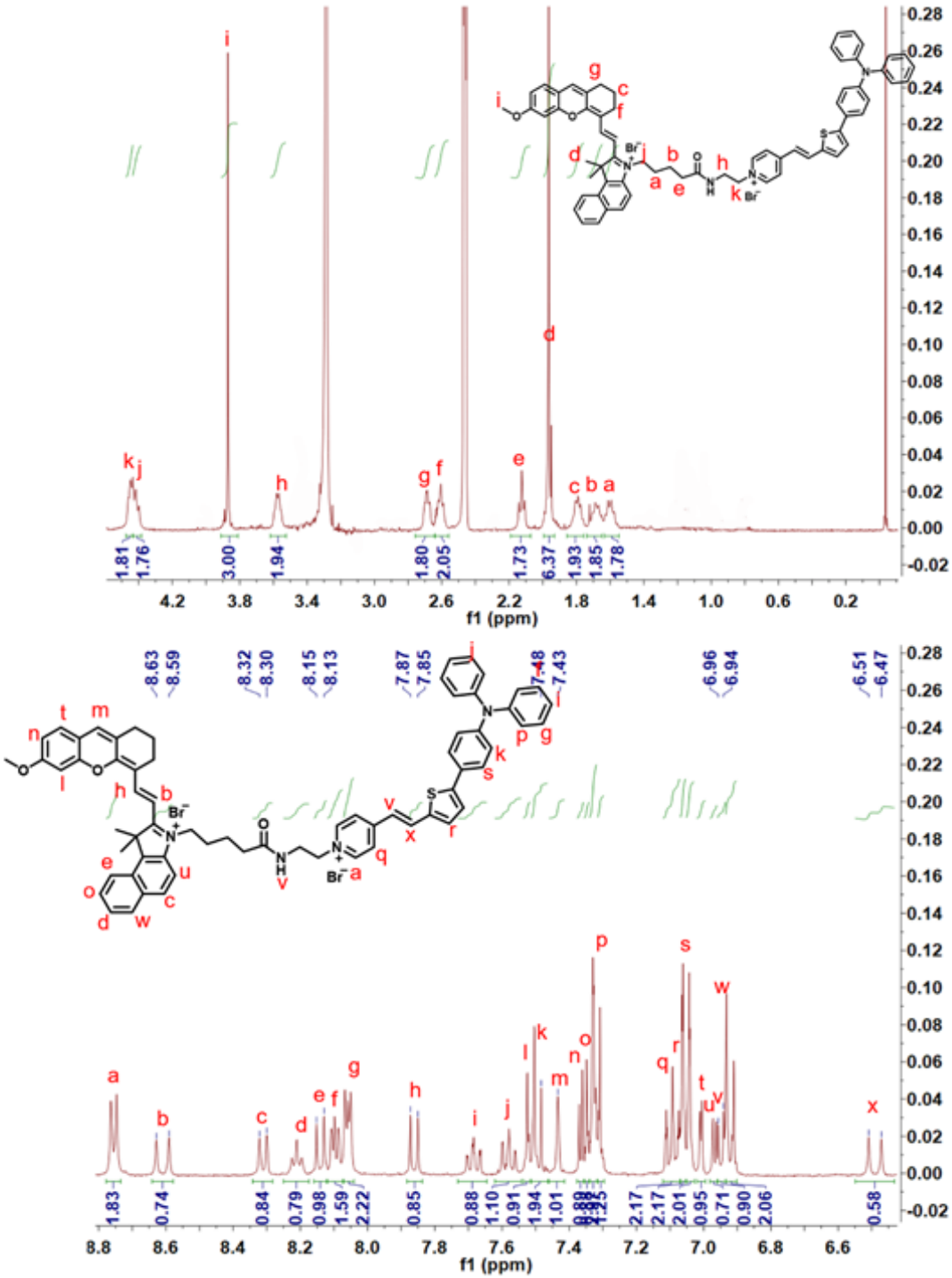


Figure S7 ^{1}H-NMR spectrum of HCY-TPA.

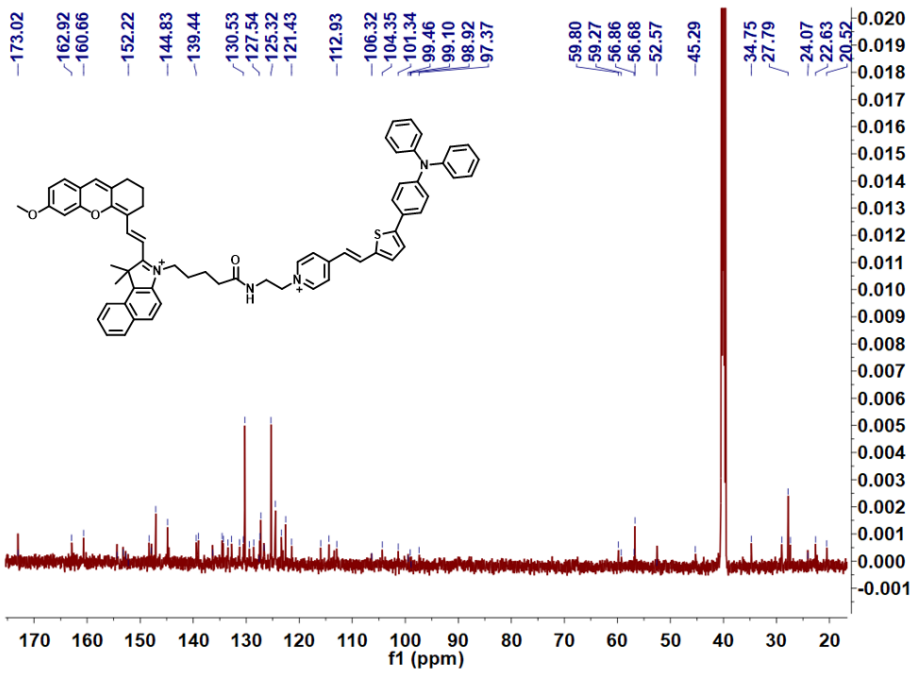


Figure S8 ^{13}C-NMR spectrum of HCY-TPA.

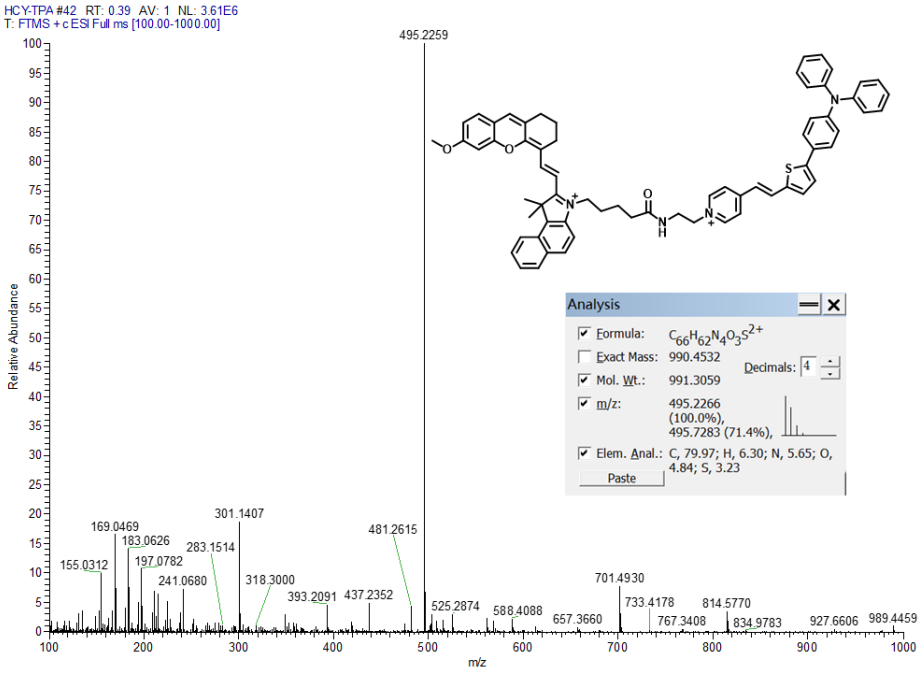


Figure S9 ESI-Mass spectrum of HCY-TPA.

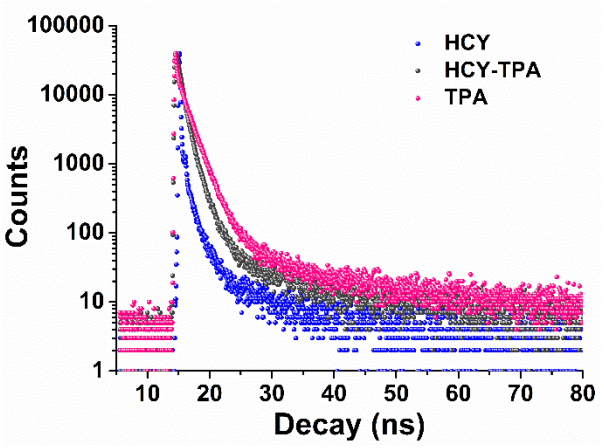


Figure S10 The fluorescence decay curves of TPA, HCY and HCY-TPA.

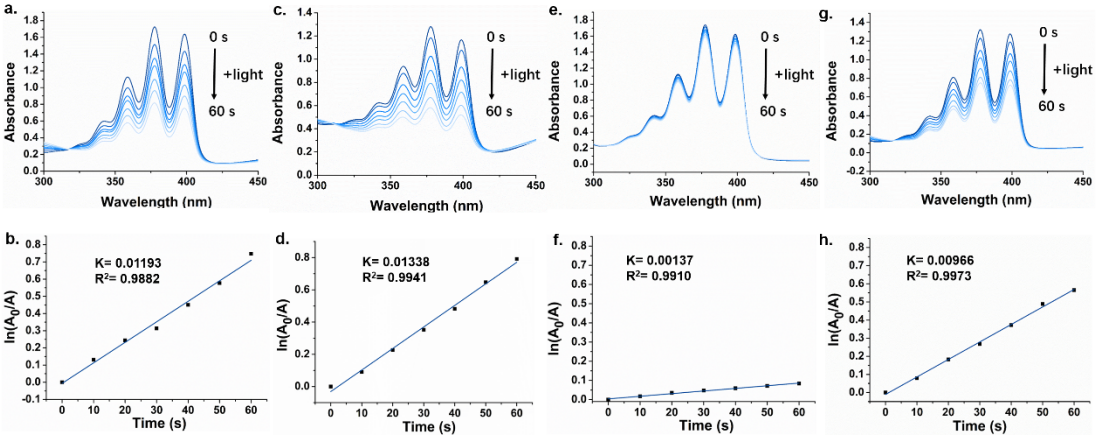


Figure S11 The $^1O_2$ generation contents of HCY-TPA (a and b), TPA (c and d), HCY (e and f), RB (g and h) in PBS: (a, c, e, g). UV-visible absorption spectra of ABDA added to PSs at 0-60 s illumination; (b).The linear relation of absorbance value at 378 nm in under different illumination time.

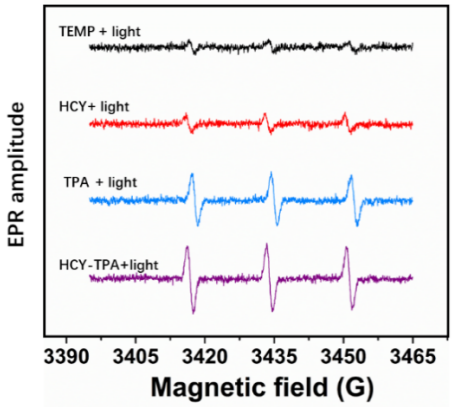


Figure S12 The EPR signals of TEMP for $^1O_2$ characterization in the presence of HCY-TPA, HCY, TPA in H_2O (10 μM) with white light irradiation (20 mW/cm^2 for 2 min), respectively.

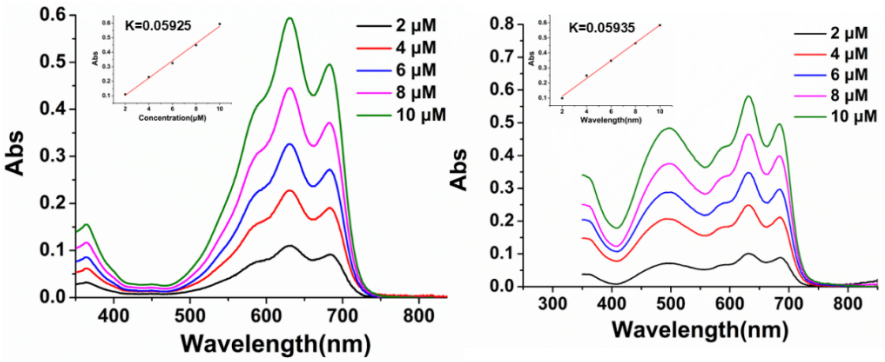


Figure S13 The molar absorption coefficients (ε) of HCY, HCY-TPA in aqueous solutions (containing 1% DMSO). Insert: absorption spectra of PSs with different concentrations (2 μM~10 μM).

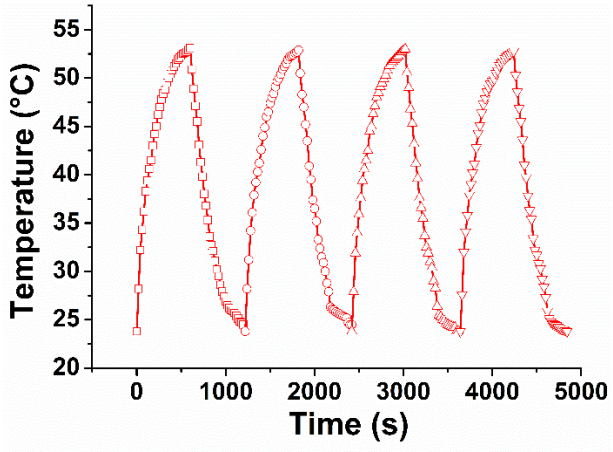


Figure S14 The heating cycling curves of HCY-TPA over four on-off cycles of 720 nm laser and white light irradiation (20 mW/cm^2).

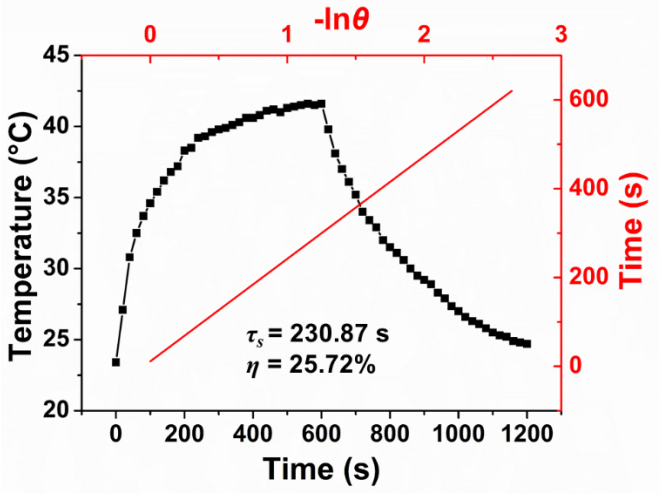


Figure S15 Photothermal conversion efficiency of HCY (100 μM) under 720 nm laser (1.0 W/cm^2)

and white light irradiation (20 mW/cm^2).

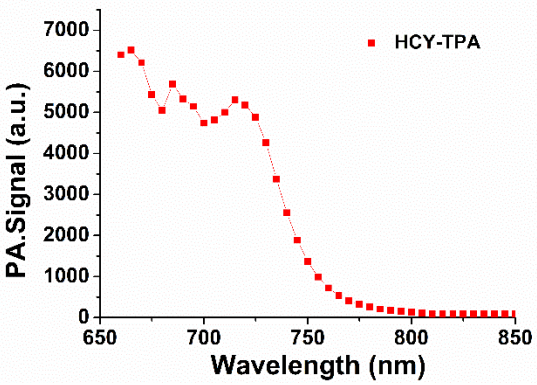


Figure S16 *In vitro* PA signal of HCY-TPA (100 μM).

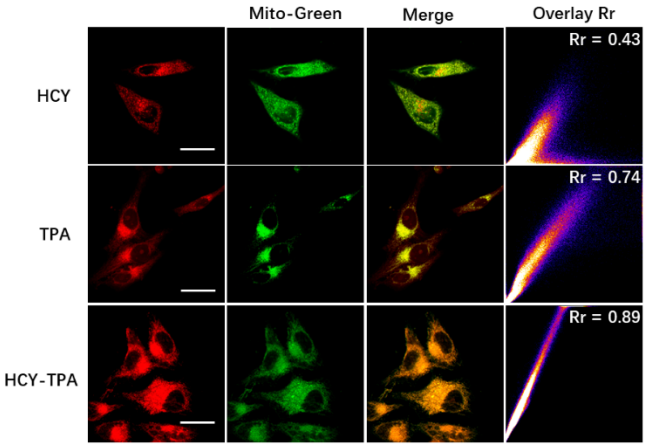


Figure S17 Colocalization imaging of HepG2 cells stained with TPA, HCY, HCY-TPA and Mito-Green after 3 h incubation. All scale bars: 20 μm.

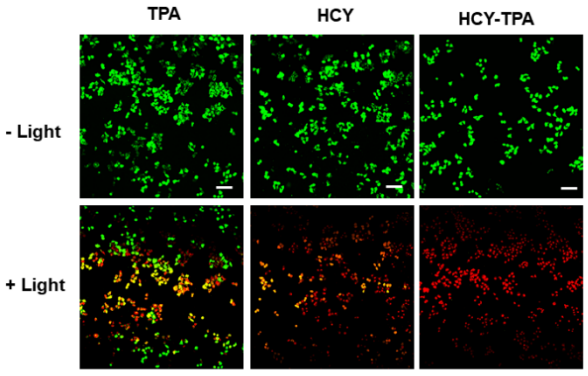


Figure S18 PDT and PTT of cell apoptosis *in vitro* of HCY-TPA, HCY, TPA (10 μM) under 720 nm laser and LED light irradiation, scale bars: 100 μm.

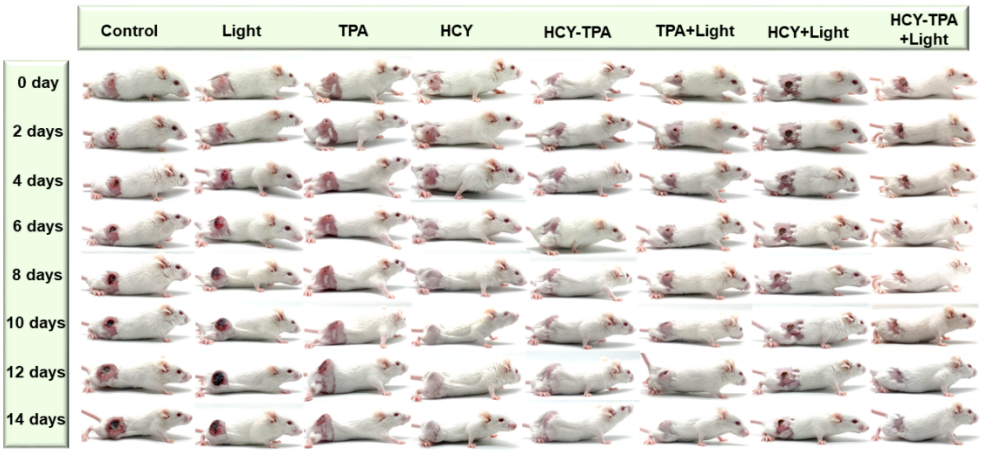


Figure S19 Photographs of H22 tumor mice after the control and different treatments in 14 days' period.

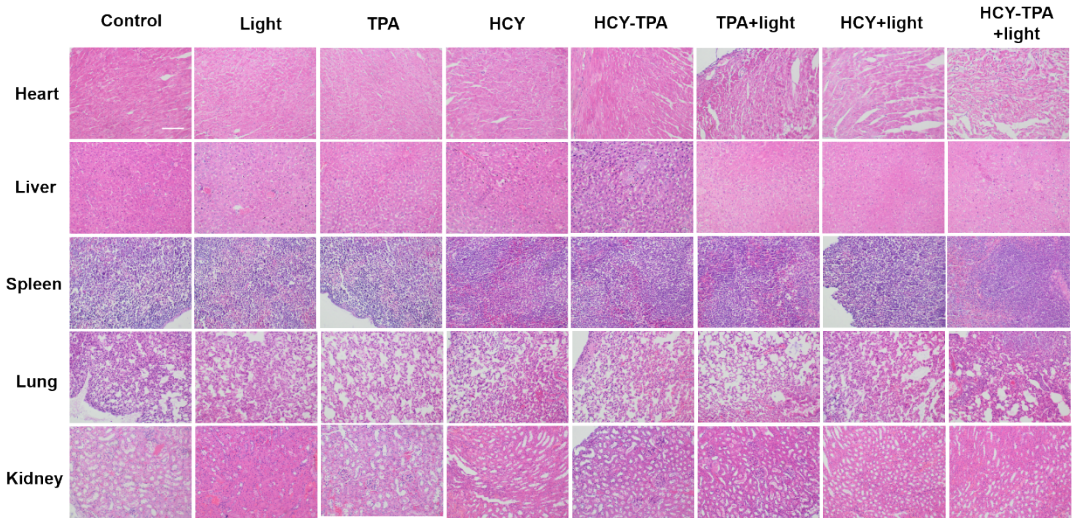


Figure S20 The H&E staining of main organs (heart, liver, spleen, lung, and kidney) of eight groups (control, light, TPA, HCY, HCY-TPA, TPA+light, HCY+light and HCY-TPA+ light) mice after treatment, scale bar, 100 μm.

References

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[S4] D. M. Mizrahi, O. Ziv-Polat, B. Perlstein, E. Gluz and S. Margel, *Eur. J. Med. Chem.*, 2011, **46**, 5175-5183.