Supporting Information

A series of water-soluble photosensitizers based on 3-cinnamoylcoumarin for in vitro antimicrobial photodynamic inactivation

Zhiyuan Sun[#a,c], Shaona Zhou[#b], Haixia Qiu[b], Ying Gu[b], Yuxia Zhao[*a]

[a]Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, China;
[b]Department of Laser Medicine, Chinese PLA General Hospital, Beijing,100853, China;
[c]University of Chinese Academy of Sciences, Beijing 100049, China.
* Corresponding author. Tel.: +86 010 8254 3569; fax: +86 010 8254 3569. E-mail: yuxia.zhao@mail.ipc.ac.cn
# These authors contributed equally to this work.

Materials

The synthetic routes towards **M1**–**M5** are shown in Figure S1. 3-acetyl-7-(dimethylamino)-2H-chromen-2-one (**1**) and 3-(ethyl(4-formylphenyl)amino) propanoic acid (**2**) was synthesized according to our previous work. 4-aminobenzaldehyde (**3**), 2-(ethyl(phenyl)amino)ethanol (**6**), 2,2'-(phenylazanediyl) diethanol (**9**) and 4-(dimethylamino)benzaldehyde (**12**) were purchased from Energy Chemical. All targeted compounds were obtained by a high yield aldol reaction between 3-acetyl-7-(dimethylamino)-2H-chromen-2-one and corresponding *p*-aminobenzaldehyde firstly, and then followed by the modification of different anions or cations.

^{1}H NMR spectra were recorded on a Bruker AV400 (400 MHz) spectrometer with deuterated reagents $CDCl_3$ or D_2O using tetramethylsilane as an internal standard. Mass spectra were measured using Bruker APEX 7.0E.

The synthesizes of PSs M1–M5

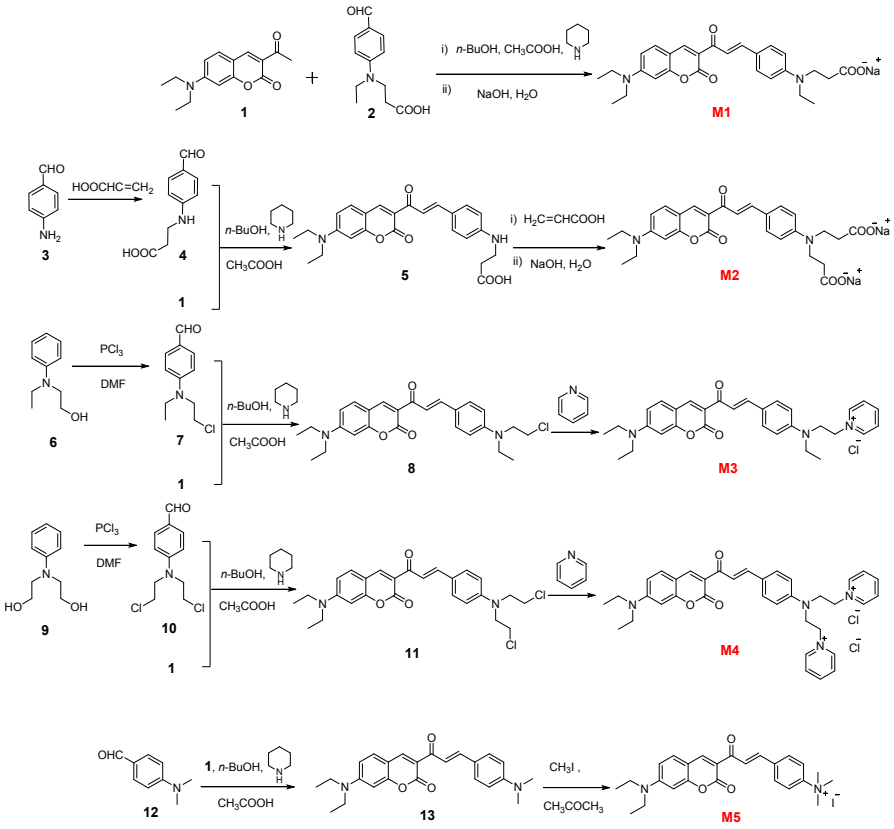

Figure S1. The synthetic routes of targeted PSs **M1**–**M5**.

PS **M1** (sodium 3-((4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)phenyl)(ethyl)amino)propanoate): 3-acetyl-2H-chromen-2-one (**1**) (1.30 g, 5 mmol) and 3-(ethyl(4-formylphenyl)amino) propanoic acid (**2**) (1.10 g, 5 mmol) were dissolved in *n*-butanol under stirring. Then 0.6 mL of glacial acetic acid and 0.6 mL of piperidine were added to the solution. The reaction mixture was reacted at 100℃ for 4 h under nitrogen atmosphere, and then the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography using petroleum ether: ethyl acetate (1:1) as eluent to give the precursor of PS **M1**. Then the precursor was neutralized using NaOH to obtain the targeted PS **M1** with a yield 82%. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.17 (s, 3H, -CH_3), 1.23-1.26 (m, 6H, -CH_3), 2.67 (s, 2H, -CH_2), 3.43-3.46 (m, 4H, -CH_2), 3.70-3.75 (m, 4H, -CH_2), 6.49 (s, 1H, coumarin-H), 6.61 (d, 1H, J = 8.0 Hz, coumarin-H), 6.67 (d, 2H, J = 8.0 Hz, phenyl-H), 7.41 (d, 1H, J = 8.0 Hz, coumarin-H), 7.57 (d, 2H, J = 8.0 Hz, phenyl-H), 7.81 (d, 1H, J = 16.0 Hz, -C=C-H), 7.94 (d, 1H, J = 12.0 Hz, -C=C-H), 7.51 (m, 1H, coumarin-H), 8.51 (s, 1H,

coumarin-H). MS (ESI): m/z+H Calcd for $C_{27}H_{29}N_2NO_5$ 485.1974; found 485.2035.

3-((4-formylphenyl)amino)propanoic acid (**4**): 4-aminobenzaldehyde (**3**) (1.21 g, 10 mmol) was dissolved in acrylic acid (20 ml) under stirring. The reaction mixture was reacted at 80℃ for 4 h under nitrogen atmosphere, then the mixture was stirred at 5℃for 2 h. Precipitate will appear and the crude product was purified by recrystallization to give **4** (1.42 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.52-2.56 (m, 2H, -CH_2), 3.34-3.39 (m, 2H, -CH_2), 6.68 (d, 2H, J = 8.0 Hz, phenyl-H), 7.62 (d, 2H, J = 8.0 Hz, phenyl-H), 9.62 (s, 1H, -CHO).

3-((4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)phenyl) amino)propanoic acid (**5**): **1** (1.30 g, 5 mmol) and **4** (0.96 g, 5 mmol) were dissolved in *n*-butanol under stirring. Then 0.6 mL of glacial acetic acid and 0.6 mL of piperidine were added to the solution. The reaction mixture was reacted at 100℃ for 4 h under nitrogen atmosphere, and then the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography using petroleum ether: ethyl acetate (1:1) as eluent to give **5** (1.19, 55%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.15-1.18 (m, 6H, -CH_3), 3.24-3.28 (m, 2H, -CH_2), 3.50-3.52 (m, 6H, -CH_2), 6.61-6.63 (m, 3H, coumarin-H, phenyl-H), 6.81 (d, 1H, J = 8.0 Hz, coumarin-H), 7.47 (d, 2H, J = 8.0 Hz, phenyl-H), 7.61 (d, 1H, J = 12.0 Hz, -C=C-H), 7.69 (d, 1H, J = 12.0 Hz, -C=C-H), 7.68-7.71 (m, 1H, coumarin-H), 8.56 (s, 1H, coumarin-H).

PS **M2** (sodium 3,3'-((4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)phenyl)azanediyl)dipropanoate): **5** (2.15 g, 5 mmol) was dissolved in acrylic acid (15 ml) under stirring. The reaction mixture was reacted at 80℃ for 4 h under nitrogen atmosphere, then the mixture was stirred at 5℃ for 2 h. Precipitate will appear and the crude product was purified by recrystallization to give the precursor of PS **M2**. Then the precursor was neutralized using NaOH to obtain the targeted PS **M2** with a yield 74%. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.03-1.05 (m, 6H, -CH_3), 2.37-

2.41 (m, 4H, -CH_2), 3.26-3.29 (m, 4H, -CH_2), 3.55-3.57 (m, 4H, -CH_2), 5.91 (s, 1H, coumarin-H), 6.09 (d, 1H, *J* = 8.0 Hz, coumarin-H), 6.78 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.28 (d, 1H, *J* = 12.0 Hz, -C=C-H), 7.48 (d, 1H, *J* = 12.0 Hz, -C=C-H), 7.51 (m, 1H, coumarin-H), 7.58 (d, 2H, *J* = 8.0 Hz, phenyl-H), 8.06 (s, 1H, coumarin-H). MS (ESI): (m/z-2Na)/2 Calcd for $C_{28}H_{28}N_2Na_2O_7$ 550.1692; found 252.0951.

3-(3-(4-((2-chloroethyl)(ethyl)amino)phenyl)acryloyl)-7-(diethylamino)-2H-chromen-2-one (**8**): **1** (2.60 g, 10 mmol) and 4-((2-chloroethyl)(ethyl)amino)benzaldehyde (**7**) (2.10 g, 10 mmol) were dissolved in *n*-butanol under stirring. Then 1 mL of glacial acetic acid and 1 mL of piperidine were added to the solution. The reaction mixture was reacted at 100℃ for 4 h under nitrogen atmosphere, and then the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography using petroleum ether: ethyl acetate (4:1) as eluent to give **8** (2.12, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.10-1.17 (m, 9H, -CH_3), 3.50-3.52 (m, 6H, -CH_2), 3.71-3.76 (m, 4H, -CH_2), 6.61 (s, 1H, coumarin-H), 6.77-6.81 (m, 3H, coumarin-H, phenyl-H), 7.55 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.62 (d, 1H, *J* = 16.0 Hz, -C=C-H), 7.68 (d, 1H, *J* = 8.0 Hz, coumarin-H), 7.74 (d, 1H, *J* = 12.0 Hz, -C=C-H), 8.52 (s, 1H, coumarin-H).

PS **M3** (1-(2-((4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)phenyl)(ethyl)amino)ethyl)pyridin-1-ium chloride): **8** (0.45 g, 1 mmol) was dissolved in pyridine (10 ml) under stirring. The reaction mixture was reacted at 100℃ for 24 h under nitrogen atmosphere. Precipitate will appear and the crude product was purified by recrystallization to give PS **M3** with a yield 67%, ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.02-1.06 (m, 3H, -CH_3), 1.15-1.19 (m, 6H, -CH_3), 3.51-3.53 (m, 4H, -CH_2), 3.99 (s, 2H, -CH_2), 4.83 (s, 2H, -CH_2), 6.61 (s, 1H, coumarin-H), 6.71 (d, 1H, *J* = 8.0 Hz, coumarin-H), 6.83 (d, 1H, *J* = 8.0 Hz, phenyl-H), 7.23-7.27 (m, 2H, phenyl-H), 7.51 (d, 1H, *J* = 8.0 Hz, coumarin-H), 7.66 (m, 1H, coumarin-H), 7.66 (d, 1H, *J* = 16.0 Hz, -C=C-H), 7.69 (d, 1H, *J* = 8.0 Hz, coumarin-H), 7.73 (d, 1H, *J* = 12.0 Hz, -C=C-H), 8.13-8.17 (m, 2H, pyridyl-H), 8.57-8.61 (m, 2H, coumarin-H,

pyridyl-H), 9.06 (d, 2H, *J* = 4.0 Hz, pyridyl-H). MS (ESI): m/z-Cl Calcd for $C_{31}H_{34}ClN_3O_3$ 496.2595; found 496.2596.

3-(3-(4-(bis(2-chloroethyl)amino)phenyl)acryloyl)-7-(diethylamino)-2H-chromen-2-one (**11**): **1** (2.60 g, 10 mmol) and 4-(bis(2-chloroethyl)amino)benzaldehyde (**10**) (2.48 g, 10 mmol) were dissolved in *n*-butanol under stirring. Then 1 mL of glacial acetic acid and 1 mL of piperidine were added to the solution. The reaction mixture was reacted at 100℃ for 4 h under nitrogen atmosphere, and then the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography using petroleum ether: ethyl acetate (4:1) as eluent to give **11** (1.92, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.24-1.27 (m, 6H, -CH_3), 3.46-3.48 (m, 4H, -CH_2), 3.67-3.68 (m, 4H, -CH_2), 3.78-3.82 (m, 4H, -CH_2), 6.56 (s, 1H, coumarin-H), 6.68-6.70 (m, 3H, coumarin-H, phenyl-H), 7.47 (d, 1H, *J* = 8.0 Hz, coumarin-H), 7.62 (d, 2H, *J* = 8.0 Hz, phenyl-H), 7.91 (d, 1H, *J* = 16.0 Hz, -C=C-H), 7.99 (d, 1H, *J* = 12.0 Hz, -C=C-H), 8.56 (s, 1H, coumarin-H).

PS **M4** (1,1'-(((4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)phenyl)azanediyl)bis(ethane-2,1-diyl))bis(pyridin-1-ium) chloride): **11** (0.45 g, 1 mmol) was dissolved in pyridine (10 ml) under stirring. The reaction mixture was reacted at 100℃ for 24 h under nitrogen atmosphere. Precipitate will appear and the crude product was purified by recrystallization to give PS **M4** with a yield 40%, ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.12-1.19 (m, 6H, -CH_3), 3.41 (s, 4H, -CH_2), 4.03 (s, 4H, -CH_2), 4.83 (s, 4H, -CH_2), 6.47 (s, 1H, coumarin-H), 6.69 (d, 1H, *J* = 8.0 Hz, coumarin-H), 7.41-7.43 (m, 1H, coumarin-H), 7.42 (d, 1H, *J* = 16.0 Hz, -C=C-H), 7.50 (d, 1H, *J* = 16.0 Hz, -C=C-H), 7.94-7.98 (m, 4H, pyridyl-H), 8.32 (s, 1H, coumarin-H), 8.46-8.50 (m, 2H, pyridyl-H), 8.74-8.75 (m, 4H, pyridyl-H). MS (ESI): m/z-2Cl Calcd for $C_{36}H_{38}Cl_2N_4O_3$ 574.2933; found 574.2935.

7-(diethylamino)-3-(3-(4-(dimethylamino)phenyl)acryloyl)-2H-chromen-2-one (**13**): **1** (2.60 g, 10 mmol) and 4-(dimethylamino)benzaldehyde (**12**) (1.49 g, 10 mmol) were

dissolved in *n*-butanol under stirring. Then 1 mL of glacial acetic acid and 1 mL of piperidine were added to the solution. The reaction mixture was reacted at 100℃ for 8 h under nitrogen atmosphere, and then the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography using petroleum ether: ethyl acetate (3:1) as eluent to give the precursor **13** (2.20 g, yield 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.24-1.28 (m, 6H, -CH_3), 3.10 (s, 6H, -NCH_3), 3.45-3.51 (m, 6H, -CH_2), 6.56 (s, 1H, coumarin-H), 6.70 (d, 1H, J = 8.0 Hz, coumarin-H), 7.20 (d, 1H, J = 8.0 Hz, coumarin-H), 7.46-7.48 (m, 2H, phenyl-H), 7.85-7.88 (m, 2H, phenyl-H), 8.02 (d, 1H, J = 16.0 Hz, -C=C-H), 8.14 (d, 1H, J = 16.0 Hz, -C=C-H), 8.57 (s, 1H, coumarin-H).

PS **M5** (4-(3-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)-3-oxoprop-1-en-1-yl)-N,N,N-trimethylbenzenaminium): The precursor **13** (0.39 g, 1 mmol) was dissolved in acetone. Methyl iodide (3 mL, 50 mmol) was added dropwise to the solution. The mixture was continued stirring 12 h at 40℃. The target compound **M5** was obtained with a yield 60%. ^{1}H NMR (400 MHz, D_2O) δ (ppm) 1.07-1.10 (m, 6H, -CH_3), 3.31 (s, 4H, -CH_2), 3.59 (s, 9H, -NCH_3), 6.44 (s, 1H, coumarin-H), 6.69 (d, 1H, J = 8.0 Hz, coumarin-H), 7.42-7.44 (m, 1H, coumarin-H), 7.46 (d, 1H, J = 16.0 Hz, -C=C-H), 7.70 (d, 1H, J = 16.0 Hz, -C=C-H), 7.79-7.83 (m, 4H, phenyl-H), 8.35 (s, 1H, coumarin-H). MS (ESI): m/z-I Calcd for $C_{25}H_{29}IN_2O_3$ 405.2173; found 405.2173.