Supporting Information

NIR Afterglow Nanosystem for Photodynamic Therapy

Bingxin Shu,[a] Shubi Zhao,[b] Yuling Zhang,[a] Qinchao Sun*[b] and Zhen Huang*[c]

[a] *Shenzhen Clinical Medical College, Guangzhou University of Chinese Medicine, Shenzhen 518172, Guangdong, China.*

[b] *Research Laboratory for Biomedical Optics and Molecular Imaging, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, Guangdong, China. E-mail: qchao.sun@siat.ac.cn*

[c] *Department of Laboratory Medicine of Pingshan District Maternal & Child Healthcare Hospital, Shenzhen 518122, Guangdong, China. E-mail: 635999667@qq.com*

† *Corresponding authors.*

Supplementary Text

1. Introduction

In recent years, much attention has been paid to the research of organic afterglow materials. There are several types of organic afterglow systems that have been developed, such as: Phenylenevinylene(PPV),[1] thioether,[2] Schaap's Dioxetanes,[3] and porphyrins.[4] Characteristically, these organic afterglow materials offer notable benefits, including high biosafety, effective biodegradability, and versatile surface functionalization options.[5, 6] Organic afterglow materials offer great opportunities to overcome the above barriers to inorganic afterglow materials for in vivo bioimaging due to their biocompatible components and flexible designability.

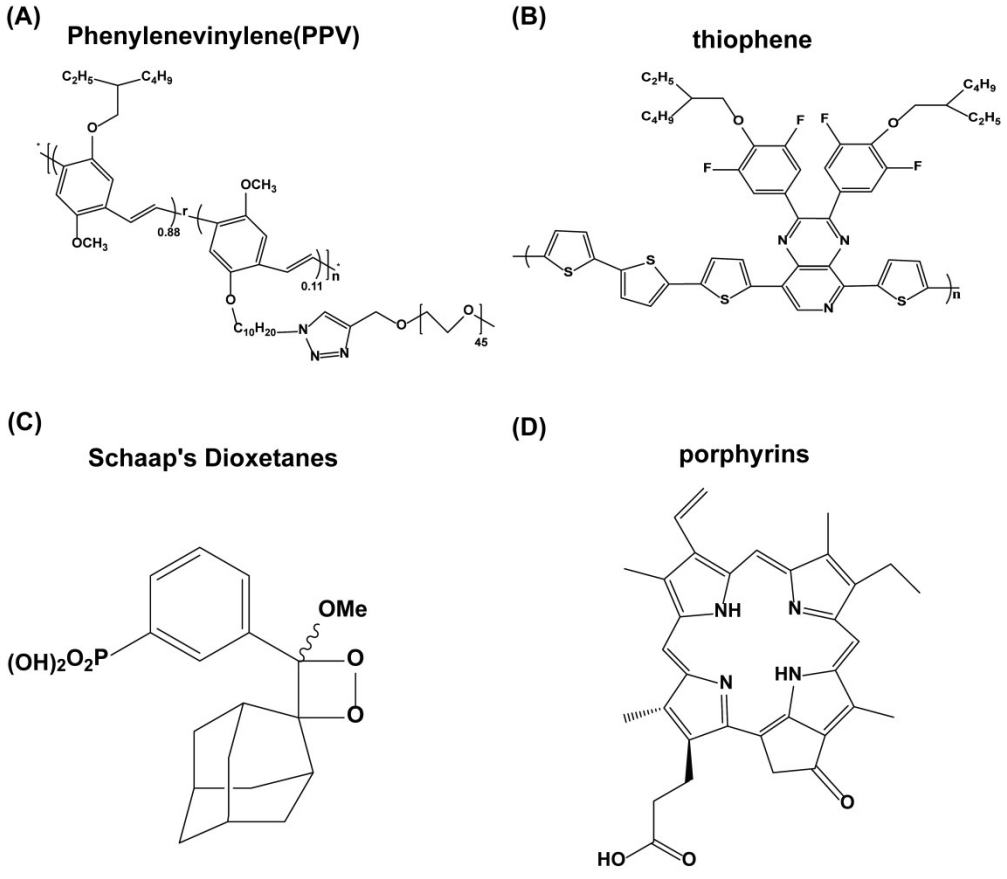

Figure S1. Chemical structures of four organic afterglow materials. (A) Phenylenevinylene(PPV).[1] (B) thioether.[2] (C) Schaap's Dioxetanes.[3] (D) porphyrins.[4]

2. Experimental Section

2.1 Synthesis of CYQ [7].

3-ethyl-1,1,2-trimethyl-1*H*-benzo[*e*]indol-3-ium iodide 1: To a solution of 1,1,2-trimethyl -1*H*-benzo[*e*]indole (1.05 g, 5.0 mmol) in toluene (10 mL), EtI (0.48 mL, 6.0 mmol) was added under argon atmosphere. The reaction mixture was stirred under reflux for overnight, then allowed to cool to room temperature. The solvent was removed under vacuum filtration and the residue was washed with ether to afford the desired product **1** (1.5 g, 82%) as a blue solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16-8.03 (m, 3H), 7.81 (d, J = 8.9 Hz, 1H), 7.74 (t, J = 7.4 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 4.89 (q, J = 7.4 Hz, 2H), 3.24 (s, 3H), 1.88 (s,6H), 1.67 (t, J = 7.4 Hz, 3H).

2-((*E*)-2-((*E*)-2-chloro-3-((*E*)-2-(3-ethyl-1,1-dimethyl-1,3-dihydro-2*H*-benzo[*e*]indol-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3-ethyl-1,1-dimethyl-1*H*-benzo[*e*]indol-3-ium iodide CYQ: To a solution of compound **1** (182.5 mg, 0.5 mmol) in EtOH (10 mL), sodium acetate (41 mg, 0.5mmol) and *N*-((*E*)-(2-chloro-3-((*E*)-(phenylimino)methyl)cyclohex-2-en-1-ylidene)methyl) aniline hydrochloride (90 mg, 0.25 mmol) was added gradually under argon atmosphere. The reaction mixture was stirred under reflux for 4 h, then allowed to cool to room temperature. After evaporation of solvent, the residue was purified by flash chromatography (MeOH/acetone/CH_2Cl_2, 2.5/12.5/85) to afford CYQ (140 mg, 76%) as a green solid. ^{1}H NMR (400 MHz, MeOD) δ 8.59 (d, J = 14.3 Hz, 2H), 8.30 (d, J = 8.5 Hz, 2H), 8.07 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.2 Hz, 2H), 7.71-7.63 (m, 4H), 7.56-7.50 (m, 2H), 6.37 (d, J = 14.2 Hz, 2H), 4.38 (q, J = 7.2 Hz, 4H), 2.81 (t, J = 6.1 Hz, 4H), 2.07-2.01 (m, 14H), 1.51 (t, J = 7.2 Hz, 6H). HRMS (MALDI-TOF) m/z: $[M-I]^+$ calcd for $C_{42}H_{44}ClN_2^+$: 611.312; Found: 611.271

2.2 Synthesis of CYQI.

1,1,2-trimethyl-7-(tributylstannyl)-1*H*-benzo[*e*]indole 5 [8]**:** A mixture of compound **4** (58 mg, 0.2 mmol), (n-Bu_3Sn)$_2$ (0.4 mL, 0.4 mmol) and $(Ph_3P)_4Pd$ (23 mg, 0.02 mmol) in a mixed solvent (5 ml, 1:1 dioxane/Et_3N) was stirred under reflux for 20 h under argon. The solvent was removed, and the residue was purified by flash chromatography (PE/EA:1/0 to 1/4) to afford compound **5** (72 mg, 72%). H^1 NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1 H), 7.97 (d, J = 8.3 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 2.38 (s, 1H), 1.67-1.50 (m, 12H), 1.40-1.30 (m, 6H), 1.22-1.05 (m, 6 H), 0.91 (t, J = 7.3 Hz, 3H).

7-iodo-1,1,2-trimethyl-1*H*-benzo[*e*]indole 6 [2]**:** To a solution of compound **5** (67 mg, 0.13 mmol) in $CHCl_3$ (13 mL) was added I_2 solution (171 mg, 0.67 mmol in 13 mL $CHCl_3$) dropwise at rt,

then the mixture was stirred for 30 min and quenched with sat. Na_2SO_3, after extraction with $CHCl_3$, the combine organic phase was dried on $MgSO_4$, filtered and concentrated to give crude product which was purified on silica gel (PE/EA: 2/1) to afford compound **6** (19 mg, 44%). H^1 NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1 H), 7.79-7.63 (m, 4H), 2.34 (s, 3H), 1.46 (s, 6H).

3-ethyl-7-iodo-1,1,2-trimethyl-1*H*-benzo[*e*]indol-3-ium iodide 7: To a solution of compound **6** (55 mg, 0.16 mmol) in AcCN (4 mL), EtI (0.04 mL, 0.5 mmol) was added under argon atmosphere. The reaction mixture was stirred under reflux for 36 h, then allowed to cool to room temperature. The solvent was removed, the crude product was purified on basic Al_2O_3 (PE/EA: 50/1) to afford the desired product **7** (25 mg, 31%). ^{1}H NMR (400 MHz, MeOD) δ 8.12 (s, 1 H), 7.74 (d, J = 9.0 Hz, 1H), 7.58 (t, J = 10.1 Hz, 2H), 7.06 (d, J = 8.7 Hz, 1H), 3.68 (q, J = 7.0 Hz, 2H), 1.59 (s, 6H), 1.19 (t, J = 7.0 Hz, 3H).

2-((*E*)-2-((*E*)-2-ethoxy-3-((*E*)-2-(3-ethyl-7-iodo-1,1-dimethyl-1,3-dihydro-2*H*-benzo[*e*]indol-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-3-ethyl-7-iodo-1,1-dimethyl-1H-benzo[*e*]indol-3-ium iodide CYQI: To a solution of compound **7** (25 mg, 0.05 mmol) in EtOH (2 mL), sodium acetate (4.2 mg, 0.5 mmol) and *N*-((*E*)-(2-chloro-3-((*E*)-(phenylimino)methyl)cyclohex-2-en-1 -ylidene)methyl)aniline hydrochloride (9.3 mg, 0.25 mmol) was added gradually under argon atmosphere. The reaction mixture was stirred under reflux for 20 h, then allowed to cool to room temperature. After evaporation of solvent, the residue was purified by preparative TLC ($MeOH/CH_2Cl_2$, 1/10) to afford CYQI (16.5 mg, 33%) as a green solid. ^{1}H NMR (400 MHz, MeOD) δ 8.32 (s, 2H), 8.18 (d, J = 14.1 Hz, 1H), 7.88-7.78 (m, 6H), 7.45 (d, J = 9.2 Hz, 2H), 6.15 (d, J = 14.1 Hz, 2H), 4.37-4.29 (m, 4H), 4.14 (q, J = 6.9 Hz, 2H), 3.68-3.61 (m, 2H), 2.75-2.60 (m, 4H), 2.22 (t, J = 6.9 Hz, 3H), 1.97 (s, 12H), 1.49 (t, J = 7.0 Hz, 6H).

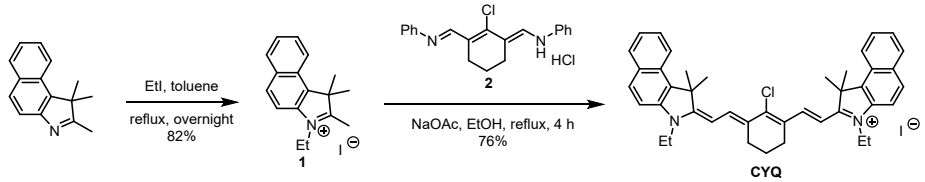

Figure S2. Synthesis of CYQ.

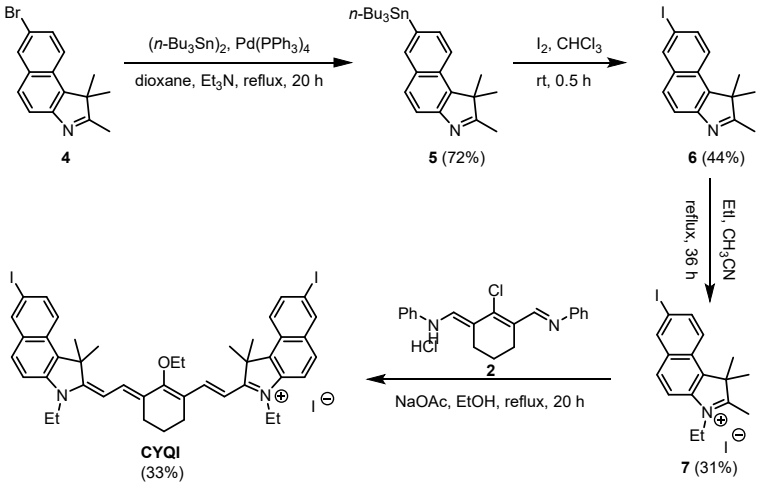

Figure S3. Synthesis of CYQI

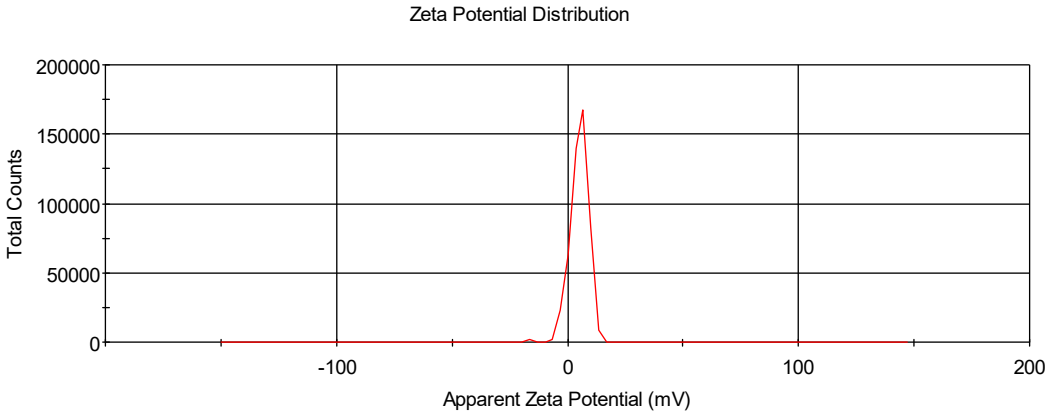

Figure S4. The zeta potentials of Ru/CYQ@CPPO in PBS.

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