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Supporting Information

Photostable BODIPY-Based Molecule with Simultaneous Type I and Type II Photosensitization for Selective Photodynamic Cancer Therapy

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Experiment:

Synthesis

Compound 1: *Meso*-(4-methoxyphenyl)-dipyrromethane

(Compound 1) can be prepared from aldehydes using neat conditions. [1]

Compound 2: 2-(4-methoxyphenyl)-4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaborolane

A high pressure bottle was charged with pinacolborane (3.2 g, 25 mmol), 4-bromoanisole (3.74 g 20 mmol), and bis(triphenylphosphine) palladium(II) dichloride (15 mg), and then the solvent pair (dioxane 30 mL / triethylamine 8 mL) was added. The bottle was then sealed after bubbling with nitrogen for 10 min. After keeping the system refluxing for 24 h, it was cooled to room temperature and then extracted with CH_2Cl_2 / H_2O twice. The organic layer was then dried with $MgSO_4$ and evaporated in vacuum. The crude compound was subjected to silica gel column chromatography and the required intermediate orange powder (yield 90%) was collected using acetone/hexane (1:10, v/v R_f = 0.7) as an eluent.^{1}H NMR (400MHz, $CDCl_3$, δ in ppm): 1.32 (s,12H; CH_3), 3.80 (s, 3H; OCH_3), 6.89 (d, J = 8.0 Hz, 2H; Ar), 7.76 (d, J = 8.8 Hz, 2H; Ar).

Compound 2,6A: 2,6-Dibromo-4,4-difluoro-8-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene

Compound **1** (1.2 g, 5 mmol) was dissolved in dry THF (20 mL) under nitrogen and DDQ (1.25 g, 5.5 mmol) in 10 mL of THF was added dropwise over 10 min. The reaction was monitored by TLC. The solvent was removed on rotary evaporator under vacuum. The crude compound was subjected to flash column chromatography using CH_2Cl_2 to collect the intermediate product. Then, the intermediate was dissolved in Toluene and neutralized with 1mL triethylamine, refluxed for 30 min, then treated with 3 mL of $BF_3{\cdot}Et_2O$ and refluxed for an additional 1 h. The reaction mixture was washed successively with 0.1 M NaOH solution and water. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated. The crude compound was subjected to silica gel column chromatography and the required intermediate orange powder (yield 71%) was collected using acetone/hexane (gradient) as an eluent. ^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm): 4.01 (s, 3H;

OCH_3), 6.50 (m, 2H; py), 6.94 (d, J = 3.6 Hz, 2H; py), 7.01(d, J = 8.8 Hz, 2H; Ar), 7.47 (d, J = 8.8 Hz, 2H; Ar), 7.88 (broad s, 2H; py).

To a solution of this intermediate above (5 mmol) in DMF/CH_2Cl_2 (20 mL/20 mL) was added dropwise a solution of NBS (15 mmol) in 10 mL CH_2Cl_2 over 1 h. The mixture was stirred at room temperature for another 3 h. The reaction was monitored by TLC analysis, which finally indicated the presence of 2,6-dibromo substituted boron-dipyrromethenes as a major product (acetone/hexane. 1:3, v/v R_f = 0.7). The resulting mixture was extracted with H_2O. The organic layer was dried with Mg_2SO_4. After removal of solvents in vacuum, the mixture was purified by silica-gel column chromatography (acetone/hexane, gradient, as an eluent) to afford compound **2,6A** (82% yield). ^{1}H NMR(400MHz, $CDCl_3$, δ in ppm): 3.92 (s, 3H; OCH_3), 6.99 (s, 2H; py), 7.07 (d, J = 8.8 Hz, 2H; Ar), 7.52 (d, J = 8.8 Hz, 2H; Ar), 7.81 (s, 2H; py).

Compound 3,5A: 3, 5-Dibromo-4,4-difluoro-8-(4-methoxyphenyl)-4-bora-3a,4a-diaza-s-indacene

This compound was prepared in a sequence of steps in one pot reaction. Compound **1** (1.2 g, 5 mmol) in dry THF (20 mL) was added dropwise with 2.2 equivalent of N-bromosuccinimide (11 mmol) in dry THF (25 mL) under nitrogen in an ice bath over 1 h. The reaction mixture was warmed to room temperature after stirring for another 1 h and 2.3-Dichloro-5,6-dicyno-1,4-benzoquinone (DDQ, 1.25 g, 5.5 mmol) in THF (10 mL) was added dropwise over 10 min. The progress of the reaction above can be monitored by thin layer chromatography (TLC). The solvent was removed on a rotary evaporator under vacuum. The crude compound was subjected to flash column chromatography using CH_2Cl_2 to collect the bromination intermediate product. Then, the intermediate was dissolved in Toluene and neutralized with 1mL triethylamine, treated with 3 mL of $BF_3{\cdot}Et_2O$ and refluxed for an additional 1 h. The reaction mixture was washed successively with 0.1 M NaOH solution and water. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated. The TLC analysis of 3,5-dibromo substituted boron-dipyrromethenes was R_f=0.3 (acetone/ hexane = 1/5, v/v,). The crude compound

was subjected to silica gel column chromatography and the required red powder compound **3,5A** (yield 54%) was collected using acetone/ hexane (gradient) as the eluent. ^{1}H NMR (400 MHz, DMSO-d_6, δ in ppm): 3.86 (s, 3H; OCH_3), 6.81 (d, J = 4.4 Hz, 2H; py), 7.00 (d, J = 4.4 Hz, 2H; py), 7.13 (d, J = 8.8 Hz, 2H; Ar), 7.62 (d, J = 8.8 Hz, 2H; Ar).

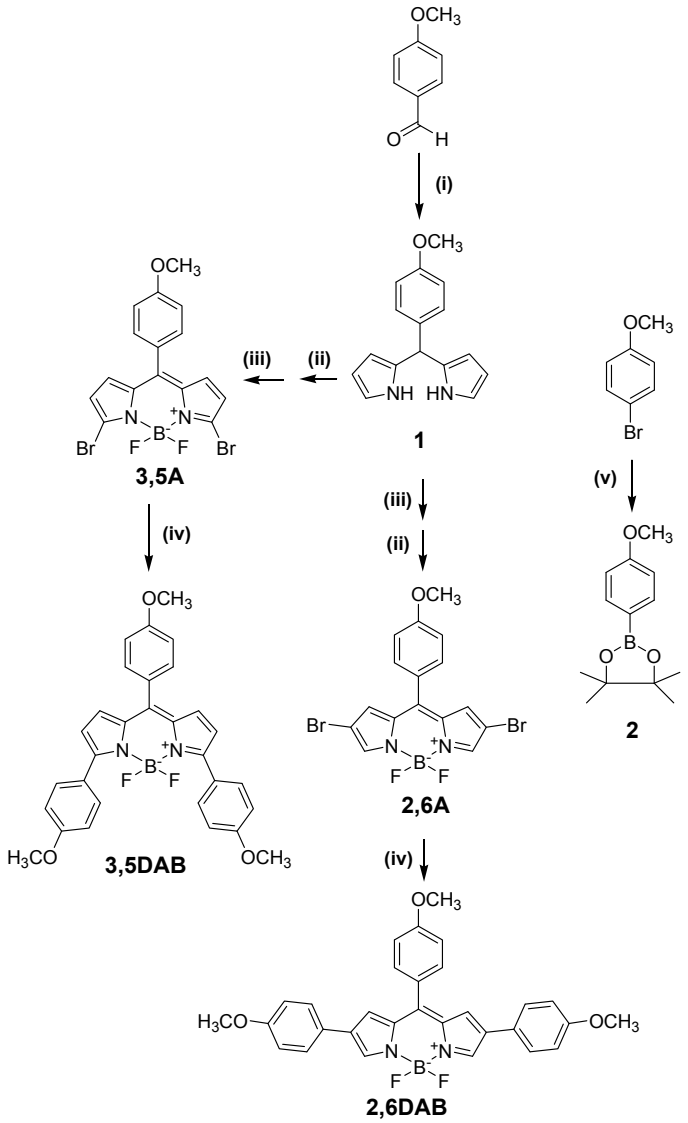
Compound 2,6DAB: 2,6-Bis(4-methoxyphenyl)-4,4-difluoro-8-(4-methoxyphenyl)-4-bora-3a,4a -diaza-s-indacene

Compound **2,6A** (0.5 mmol), compound **2** (2.5 mmol) and K_2CO_3 (0.18g) were added into to a high pressure bottle containing a mixture of palladium (II) acetate (50 mg) and tri-*o*-tolyl phosphine (100 mg), then to which was added the solvent pair (1,2-dimethoxyethane (DME) 15 ml / H_2O 3 mL). The bottle was then sealed after bubbling with nitrogen for 10 min. After keeping the system refluxing for 12 h, the system was cooled to room temperature and then extracted with CH_2Cl_2 / H_2O twice. The organic layer was then dried with $MgSO_4$ and evaporated in vacuum. The residue was subjected to chromatography on a silica gel by using acetone / hexane (1/1, Rf = 0.4). The dark-green solid was then obtained by recrystallizing with acetone / hexane (50% yield). Data for compound **2,6DAB**: ^{1}H NMR (400MHz, $CDCl_3$, δ in ppm): 3.83 (s, 6H; OCH_3), 3.94 (s, 3H; OCH_3), 6.91 (d, J = 8.0 Hz, 4H; Ar), 7.04 (s, 2H; py), 7.09 (d, J = 8.0 Hz, 2H; Ar), 7.46 (d, J = 8.0 Hz, 4H; Ar), 7.62 (d, J = 8.0 Hz, 2H; Ar), 8.20 (s, 2H; py). $[M+H]^+$ 511.24; found, 511.8878. Anal. calcd for $C_{30}H_{25}BF_2N_2O_3$ (**2,6DAB·H_2O**: **$C_{30}H_{27}BF_2N_2O_4$**): C, 70.60 (**68.20**); H, 4.94 (**5.15**); N, 5.49 (**5.30**); found, C, 68.13; H, 5.15; N, 5.28.

Compound 3,5DAB: 3,5-Bis(4-methoxyphenyl)-4,4-difluoro-8-(4-methoxyphenyl)-4-bora-3a,4a -diaza-s- indacene

Compound **3,5A** (0.6 mmol), compound **2** (3.0 mmol) and K_2CO_3 (0.18 g) were added into to a high pressure bottle containing a mixture of palladium (II) acetate (50 mg) and tri-*o*-tolyl phosphine (100 mg), then to which was added the solvent pair (1,2-dimethoxyethane (DME) 10 mL / H_2O 2 mL). The bottle was then sealed after bubbling with nitrogen for 10 min. After keeping the system refluxing for 12 h, the system was cooled to room temperature and then

extracted with CH_2Cl_2 / H_2O twice. The organic layer was then dried with $MgSO_4$ and evaporated in vacuum. The residue was subjected to chromatography on a silica gel by using acetone / hexane (1/1, R_f = 0.6). The tan colour solid was then obtained by recrystallizing with acetone / hexane (58% yield). Data for compound **3,5DAB**: ^{1}H NMR (400MHz, $CDCl_3$, δ in ppm): 3.85(s, 6H; OCH_3), 3.92 (s, 3H; OCH_3), 6.61 (d, J = 4.4 Hz, 2H; py), 6.89 (d, J = 4.4 Hz, 2H; py), 6.95 (d, J = 8.4 Hz, 4H; Ar), 7.05 (d, J = 8.4 Hz, 2H; Ar), 7.53 (d, J = 8.4 Hz, 2H; Ar), 7.87 (d, J = 8.4 Hz, 4H; Ar). $[M+H]^+$ 511.24; found, 511.3215. Anal. calcd for $C_{30}H_{25}BF_2N_2O_3$: C, 70.60; H, 4.94; N, 5.49; found, C, 70.51; H, 4.96; N, 5.41.



Scheme 1. Synthetic route to BODIPY-based photosensitizers **2,6DAB** and **3,5DAB**: (i) pyrrole, TFA, rt. (ii) NBS, THF, ice bath. (iii) DDQ, THF, rt. then Et_3N/BF_3OEt_2, Toluene, reflux (iv) K_2CO_3, (*o*-tol)$_3$P, $Pd(OAc)_2$, DME/H_2O=5/1, compound **2**, reflux. (v) $Pd(PPh_3)_2Cl_2$, pinacolborane, dioxane/Et_3N, reflux.

Reference:
[1] Wagner, R. W.; Lindsey, J. S. Pure Appl. Chem. 1996, 68, 1373-1380.

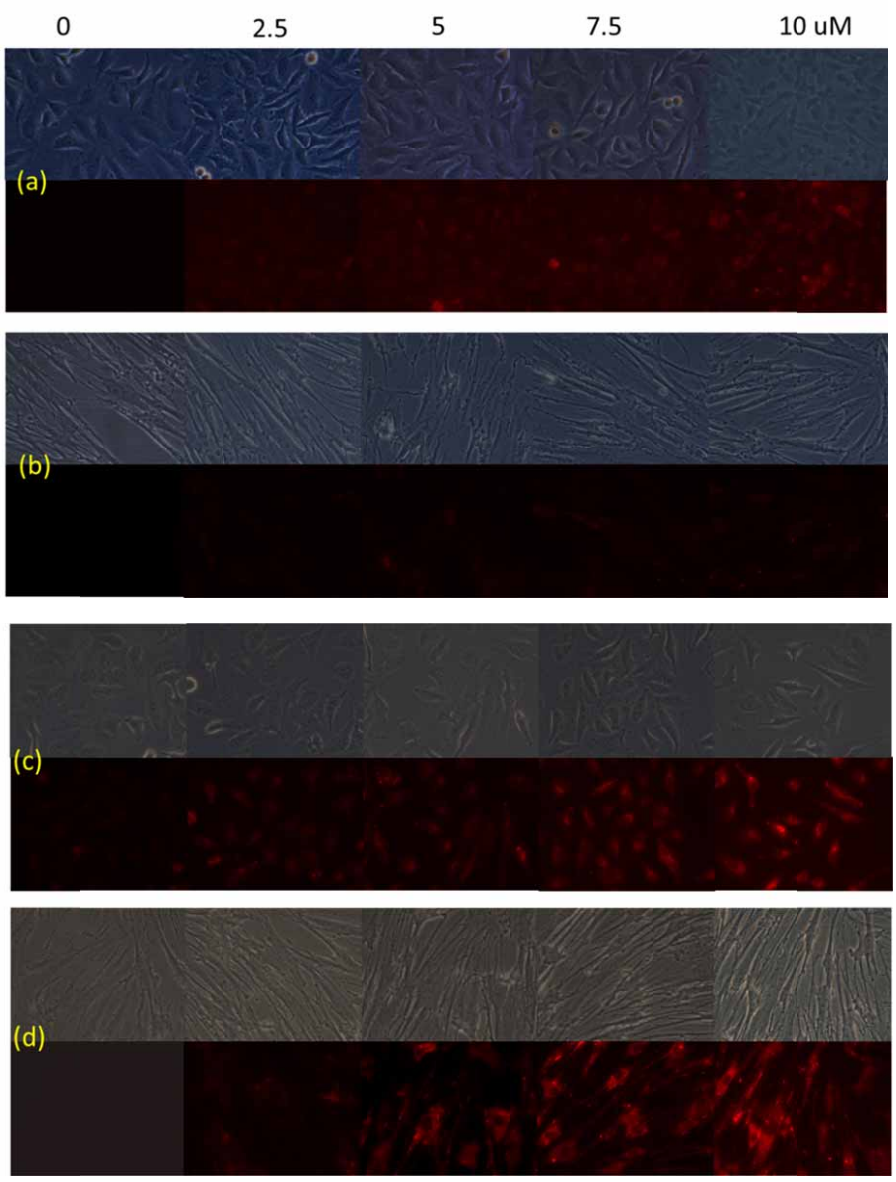


Figure S1. The concentration-dependent accumulation images of compound 2,6 DAB in the (a) HeLa cancer cell and (b) MRC-5 cell. Compound 3,5 DAB in the (c) HeLa cancer cell and (d) MRC-5 cell. The red images of compound were excited by a green light cube that passed light through a 535±25 nm bp filter, and emission was collected through a 590 nm lp filter.

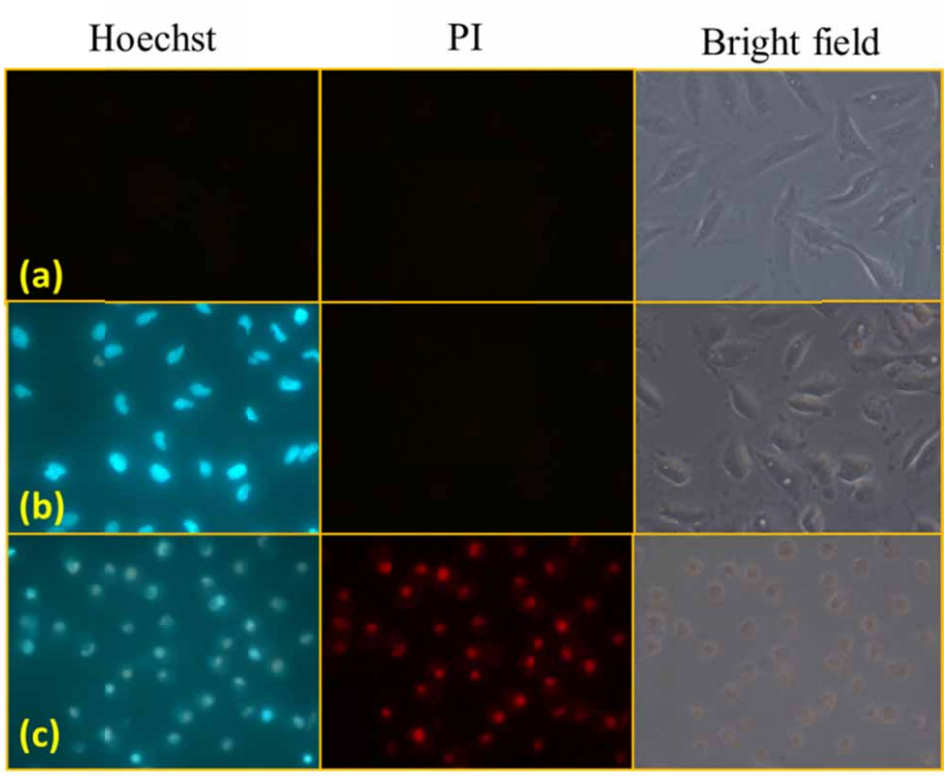


Figure S2. Following the experimental condition of Figure 6: (a) Before irradiation. (b) System was treated with Hoechst and PI once after irradiation. (c) Cultured (b) overnight. The images of Hoechst were collected by exciting with a blue light cube that passed light through a 370±10 nm bp filter, and emission was collected through a 450 nm lp filter. The PI images were excited by a green light cube that passed light through a 535±25 nm bp filter and emission was collected through a 590 nm lp filter. (Under these conditions, the red emission is from PI, not **2,6-DAB**).