Electronic Supplementary Information for

Aza-Bodipy Photosensitizer for Photoacoustic and Photothermal Imaging Guided Dual Modal Cancer Phototherapy

Qianyun Tang,^a Weili Si,^a Chuhan Huang,^a Kaikai Ding,^a Wei Huang,^a Peng Chen,^{*b} Qi Zhang,^{*c} Xiaochen Dong^{*a}

^aKey Laboratory of Flexible Electronics (KLOFE) and Institute of Advanced Materials (IAM), Jiangsu National Synergetic Innovation Center for Advanced Materials (SICAM), Nanjing Tech University (NanjingTech), Nanjing 211816, P.R. China. E-mail: iamxcdong@njtech.edu.cn ^bSchool of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, 637459, Singapore E-mail: chenpeng@ntu.edu.sg ^cSchool of Pharmaceutical Sciences, Nanjing Tech University (NanjingTech), Nanjing 211816, P.R. China.

E-mail: zhangqi@njtech.edu.cn

1. Materials and Reagents

4-Methoxybenzaldehyde, 1-(4-bromophenyl)ethanone, diisopropylethylamine, Boron trifluoride diethyl etherate and 1, 3-diphenylisobenzofuran (DPBF) were purchased from Aladdin (Shanghai, China). Potassium hydroxide, diethylamine, hydrochloric acid, chloroform, sodium thiosulphate and sodium bicarbonate were obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Nitromethane, butanol, anhydrous sodium sulfate and ammonium acetate were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). N-iodo-succinimide (NIS) was purchased from Adamas-beta (Shanghai, China). Acetic acid was purchased from Shanghai Shenbo Chemical Co. Ltd. (Shanghai, China). Methylene blue trihydrate (MB) was purchased from Tianjin Chemical Reagent Research Institute Co. Ltd. (Tianjin, China). DSPE-mPEG₂₀₀₀ was purchased from Shanghai ZZBIO. Co. Ltd. (Shanghai, China). All the chemical agents are analytical pure and are used as received.

2. Characterization

Nuclear magnetic resonance (NMR) spectra were measured by using a Bruker Ultra Shield Plus 400 MHz. GC-MS was recorded on a Shimadzu GC-MS-QP 2010 Plus mass spectrometer. MALDI-TOF-MASS (Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry) was performed on a Bruker autoflex speed MALDI-TOF for data acquisition. The UV-visible absorption and fluorescence spectra were recorded an UV-3600 UV-Vis spectrophotometer (Shimadzu, Japan) and an F-4600 spectrofluorophotometer (Hitachi, Japan), respectively. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100 transmission electron microscope. Photoacoustic imaging was conducted with an Endra Nexus128 small animal photoacoustic imaging system (Ann Arbor, MI, USA). The 660nm and 730 nm laser as well as a Xenon lamp were used to carry out the PDT and PTT study. And an FLIR thermal camera (E50, Arlington, VA) was used to monitor the photothermal conversion.

3. Supplemental Methods



3.1. Synthesis and Characterization of IABDP

Scheme S1. Synthesis routes of IABDP.

1-(4-bromophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (1).^{S1} 4-Methoxybenzaldehyde (2.72 g,

20 mmol), 1-(4-bromophenyl)ethanone (3.98 g, 20 mmol) and potassium hydroxide (0.11 g, 2.0 mmol) were dissolved in ethanol / H₂O (85:15 v/v, 40 mL), which were stirred at room temperature overnight. Filtering and collecting the precipitate, followed by washing with cold ethanol. Recrystallization from ethanol to yield the product as Light yellow solid (5.075 g, 80%). ¹H NMR (400 MHz, CDCl₃, δ): 7.88(d, *J* = 8.4 Hz, 2H, Ar-H), 7.79 (d, *J* = 15.6 Hz, 1H, Ar-CH=), 7.68 – 7.53 (m, 4H, Ar-H), 7.35 (d, *J* = 15.6 Hz, 1H, Ar-C=CH), 6.94 (d, *J* = 8.4 Hz, 2H, Ar-H), 3.86 (s, 3H, OCH₃).

1-(4-bromophenyl)-3-(4-methoxyphenyl)-4-nitrobutan-1-one (2).^{s2} Compound 1 (4.00 g, 12.6 mmol), nitromethane (3.85 g, 63.1 mmol) and diethylamine (4.62 g, 63.1 mmol) were dissolved in methanol (50 mL) and heated under reflux for 24 h. The solution was cooled to room temperature, acidified with 2 M diluted hydrochloric acid and extracted with CH_2Cl_2 , dried over sodium sulfate and evaporated to give the dark green oily crude product. This crude product was used without

further purification (2.039 g, 47%). ¹H NMR (400 MHz, CDCl₃, δ): 7.77 (d, J = 8.7 Hz, 2H, Ar-H), 7.59 (d, J = 8.7 Hz, 2H, Ar-H), 7.18 (d, J = 8.7 Hz, 2H, Ar-H), 6.85 (d, J = 8.7 Hz, 2H, Ar-H), 4.71 (ddd, J = 20.1, 12.3, 7.3 Hz, 2H, NO₂-CH₂) 4.15 (p, J = 7.1 Hz, 1H, CH), 3.77 (s, 3H, OCH₃), 3.38 (dd, J = 7.0, 2.6 Hz, 2H, O=C-CH₂).

[5-(4-bromophenyl)-3-(4-methoxyphenyl)-1H-pyrrol-2-yl][5-(4-bromophenyl)-3-(4-

methoxyphenyl)pyrrol-2-ylidene]amine (3).^{S2} Compound **2** (1.00 g, 2.6 mmol), ammonium acetate (7.13 g, 92.5 mmol), and butanol (40 mL) were added into a 100 mL round-bottomed flask and heated under reflux for 24 h. The reaction was cooled to room temperature and the solvent was concentrated to 10 mL by evaporation. The resulting material was filtered and washed with ethanol resulting in a blue-black solid (0.256 g, 30%). ¹H NMR (400 MHz, CDCl₃, δ): 8.01 (d, J = 8.2 Hz, 4H, Ar-H), 7.77 (d, J = 7.9 Hz, 4H, Ar-H), 7.66 (d, J = 7.3 Hz, 4H, Ar-H), 7.07 (s, 2H, pyrrole-H), 6.97 (d, J = 8.5 Hz, 4H, Ar-H), 3.89 (s, 6H, OCH₃) (NH not observed).

BF₂ **Chelate of [5-(4-bromophenyl)-3-(4-methoxyphenyl)-1H-pyrrol-2-yl][5-(4-bromophenyl) -3-(4-methoxyphenyl)pyrrol-2-ylidene]amine (4).**^{S2} Compound **3** (0.25 g, 0.37 mmol) and diisopropylethylamine (0.54 g, 4.13 mmol) was dissolved in dry CH₂Cl₂ (60 mL) under nitrogen atmosphere. Boron trifluoride diethyl etherate (0.83 g, 5.86 mmol) was dropwise added and the mixture was stirred at room temperature for 24 h. The solution was washed with water. The organic layer was collected, dried over sodium sulfate and evaporated. Purification by column chromatography on silica gel eluting with CH₂Cl₂/hexane (1:1) gave the product as a metallic red solid (0.26 g, 97%). ¹H NMR (400 MHz, CDCl₃, δ): δ 8.04 (d, J = 8.8 Hz, 4H, Ar-H), 7.89 (d, J = 8.6 Hz, 4H, Ar-H), 7.61 (d, J = 8.5 Hz, 4H, Ar-H), 7.00 (d, J = 8.8 Hz, 4H, Ar-H), 6.89 (s, 2H, pyrrole-H), 3.90 (s, 6H, OCH₃).

BF₂ Chelate of [4-iodo-5-(4-bromophenyl)-3-(4-methoxyphenyl)-1H-pyrrol-2-yl][4-iodo-5- (4-bromophenyl)-3-(4-methoxyphenyl)pyrrol-2-ylidene]amine (IABDP). Compound 4 (0.10g, 0.14 mmol), N-iodo-succinimide (79 mg, 0.35 mmol) were dissolved in 24 mL mixture (3:1) of

chloroform and acetic acid, and stirred at 30 °C for 10 h . The reaction mixture was washed with sodium thiosulphate and sodium bicarbonate solution, and then extracted with chloroform. The organic layer was dried over sodium sulfate and evaporated under reduced pressure to give the product as red brown solid (0.13 g, 95%). ¹H NMR (400 MHz, CDCl₃, δ): 7.83 (d, J = 8.9 Hz, 4H, Ar-H), 7.60 (d, J = 8.5 Hz, 4H, Ar-H), 7.51 (d, J = 8.5 Hz, 4H, Ar-H), 6.98 (d, J = 8.9 Hz, 4H, Ar-H), 3.89 (s, 6H, OCH₃). ¹³C NMR (125 MHz, CDCl₃, δ): 161.15, 132.41, 131.84, 131.29, 129.94, 125.49, 124.33, 113.60, 55.38. MALDI-TOF (m/z): calcd for C₃₄H₂₂BBr₂F₂I₂N₃O₂, 966.9821; found, 966.9775.

3.2. Singlet oxygen detection of PS. The ${}^{1}O_{2}$ quantum yields (Φ_{Δ}) of IABDP were detected with methylene blue trihydrate (MB) as the standard (Φ_{Δ} = 0.57 in DCM).^{S3} The absorbance of DPBF was adjusted around 1.3 in air saturated DCM while that of PS was adjusted around 0.3. Air saturated solution of IABDP and DPBF in DCM were irradiated with a 660 nm laser for 30 s. The decrease of the absorption of DPBF at 414 nm was monitored by UV-VIS-NIR spectrophotometer. Singlet oxygen quantum yield (Φ_{Δ}) was calculated according to the following equation:

$$\Phi_{\Delta(sam)} = \Phi_{\Delta(std)} \times \frac{S_{sam}}{S_{std}} \times \frac{F_{std}}{F_{sam}}$$
(1)

where 'sam' and 'std' designate IABDP and MB, respectively. S denotes the slope of the plots of the absorbance of DPBF (at 414 nm) vs. the irradiation time, F represents the absorption correction factor, which is given by $F = 1-10^{-O.D.}$ (O. D. is the optical density of the samples at 660 nm).

3.3. Photothermal effect of IABDP NPs. Both the solution of IABDP NPs in saline (0.94 μ M) and the control saline were irradiated with a 730 nm laser (0.944 W cm⁻²) or a Xenon lamp (20 mW cm⁻²) for 20 min. The temperature was collected by an FLIR thermal camera.

Photothermal conversion efficiency of IABDP NPs aqueous solutions were determined under the irradiation of 730 nm laser at power density of 0.944 W cm⁻² for 9 min. DI water was used as the control group. The real-time temperature change was recorded using a FLIR thermal camera. The

photothermal conversion efficiency (η) was calculated according to the following equation:

$$\eta = \frac{hS\Delta T_{max} - Q_s}{I(1 - 10^{-A_{730}})}$$
(2)
$$\tau_s = \frac{m_D C_D}{hS}$$
(3)

where h is the heat transfer coefficient, S is the surface area of the container, and the value of hS is obtained from the Figure S3b. The ΔT_{max} is the temperature change of IABDP NP aqueous solution at the maximum steady-state temperature, I is the laser power, A₇₃₀ is the absorbance of the IABDP NPs at 730 nm, and Q_s expresses the heat associated with light absorption by the solvent. The variable τ_s is the sample-system time constant, and m_D and C_D are the mass and heat capacity (4.2 J g⁻¹) of the deionized water used as the solvent.

3.4. Photostability of IABDP NPs. The aqueous solution of IABDP NPs was irradiated by a 730 nm laser (0.944 W cm⁻²) for 2 h. The absorbance of IABDP NPs solution was measured by UV-Vis spectrophotometer.

3.5. Cellular uptake. 2×10^5 HeLa cells were seeded into 6 cm petri dish and incubated in the culture media (5 mL) at 37 °C under 5% CO₂ for 24 h, and then in media containing IABDP NPs (50 μ M, 2 mL) for additional 24 h. In the control group, cells were just incubated in the culture media without IABDP NPs. After washing with PBS solution, the cells were harvested by 0.25% trypsin (Invitrogen, 500 μ L) for 2 min, followed by centrifugation at 1000 rpm. Finally, cells were washed thrice with PBS solution and suspended in 100 μ L PBS solution, which were applied to PAI.

4. Statistical analysis

Statistical analysis was performed by Student's t-test for two groups, and one-way analysis of variance for multiple groups. All results were expressed as the mean \pm s.d. unless otherwise noted. A value of p < 0.05 was considered statistically significant.

5. Supplemental Figures



Scheme S1 Molecular structure of IABDP and preparation of IABDP NPs with different morphology.



Fig. S1 TEM images of spherical IABDP NPs.



Fig. S2 Fluorescence spectra of IABDP in dichloromethane, as well as cubic and spherical IABDP NPs in PBS solution.



Fig. S3 (a) Absorption spectra of DPBF solution incubated with free IABDP molecules in dichloromethane upon irradiation with a Xenon lamp for different time. (b) Absorbance decrease of DPBF with increasing photoirradiation time in the presence of IABDP and MB.

	$\lambda_{abs}(nm)$	$\lambda_{em}(nm)$	Φ_{Δ} (%)	Ref
IABDP	658	714	92	this work
Ir(III) complexes Ir-1	473	607	80.3	S4
(BODIPY)2-diiodo-aza-BODIPY triad (B-2)	504/683	520/714	69	S3
ZnPc	347/682/691	697	62	S5
Thalocyanine derivative LIN-Br-C ₁	615	-	80	S6
lutetium(III) acetate phthalocyanines LuPc-5	685	721	35	S7

Table S1. Singlet oxygen quantum yield (Φ_{Δ}) of IABDP compared with other photosensitizers reported in the literatures.



Fig. S4 (a) Photothermal effect of the irradiation of the aqueous solution of IABDP NP with NIR laser (730 nm, 0.944 W cm⁻²), in which the irradiation lasted for 9 min and then turned off the laser. (b) Time constant for heat transfer from the system is determined to be $\tau_s = 168.3$ s by applying the linear time data from the cooling period (after 540 s) versus negative natural logarithm of driving force temperature, which is obtained from the cooling stage of panel (a).

Material	Irradiation	Power (W) x	Efficiency	Ref
	wavelength (nm)	Time (s)	η (%)	
IABDP	730	0.944 x 540	37.9	this work
Cu ₉ S ₅ nanoparticles	980	0.51 x 600	25.7	S 8
Gold nanovesicles (BGVs)	808	2 x 300	37	S9
Polymer Nanobioconjugates SPN1	808	1 x 360	30	S10
Thiadiazole TPT-NPs	808	2 x 600	32	S11
polymer PPDS nanoassembly	808	0.5/0.2 x	31.4	S12
		1080		

Table S2. Photothermal conversion efficiency (η) of IABDP NPs compared with other photothermal agents reported in the literatures.



Fig. S5 Absorption spectra of IABDP NPs aqueous solution before and after irradiation of NIR laser (730 nm, 0.944 W cm⁻²) for 2 h.



Fig. S6 Tumor weight of three groups after 14 times treatment (5 mice per group). Error bars represent the standard deviations (S.D.). (*p < 0.05, **p < 0.01).



Fig. S7 Body weight of three groups of mice before and after treatment. The body weight of post-treatment mice was the weight removed of tumor weight.



Fig. S8 H&E staining images of the major organs (heart, lung, liver, spleen, and kidney) of each group after treatment.

6. Supplemental References

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