Supporting Information

Tumor-Mitochondria Dual Targeted Aza-BODIPY-based Nanotheranostic Agent for Multimodal Imaging-Guided Phototherapy

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1. Materials and Reagents

3,4-Dimethoxybenzaldehyde, 4-methoxyacetophenone, boron trifluoride etherate, folic acid, (2-carboxyethyl) triphenylphosphonium bromide, N-bromo-succinimide (NBS), 1,3-diphenylisobenzofuran (DPBF), methylene blue (MB) were purchased from Adamas-beta® (Shanghai, China). mPEG-NH$_2$ (molecular weight: 5000) was purchased from Shanghai Yarebio Co. Ltd. Other chemical agents were purchased from Shanghai Lingfeng Chemical Reagent Co. Ltd. All the chemical agents are used as received.
2. Characterization

Nuclear magnetic resonance (NMR) spectra were measured by using a Bruker Ultra Shield Plus. 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 antoflex speed MALDI-TOF for data acquisition. The UV-visible absorption was recorded by UV-3600 Shimadzu UV-vis-NIR spectrometer (Shimadzu, Japan). Fluorescence spectra were recorded by an F-4600 spectrofluorophotometer (Hitachi, Japan). An E50 infrared camera (FLIR, Arlington, VA) was employed to record photothermal images. The confocal fluorescence images were obtained by an Olympus IX 70 inverted microscope (Olympus, Japan).
3. Supplemental Methods

3.1. Synthesis and Characterization of MeOABBr

Scheme S1 Synthetic routes for MeOABBr

1-(4-methoxylphenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (1)

3,4-Dimethoxybenzaldehyde (6.647 g, 0.04 mol), 4-methoxyacetophenone (6.007 g, 0.04 mol) and KOH (0.224 g, 0.004 mol) were dissolved in ethanol/H\textsubscript{2}O (85:15 v/v, 80 mL) and stirred at room temperature for 24 h. The crude product was filtered as yellow precipitate (10.740 g, 90%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) ppm 8.09 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.81 (d, \(J = 6.5\) Hz, 1H, CH-C=O), 7.46 (d, \(J = 15.5\) Hz, 1H, Ar-CH=), 7.20 (d, \(J = 6.5\) Hz, 1H, Ar-H), 7.04 (d, \(J = 9\) Hz, 2H, Ar-H) 6.95 (d, \(J = 8.5\) Hz, 1H, Ar-H), 4.00 (s, 3H, OCH\textsubscript{3}), 3.97 (s, 3H, OCH\textsubscript{3}), 3.93 (s, 3H, OCH\textsubscript{3}), \textsuperscript{13}C NMR (500 MHz, CDCl\textsubscript{3}): \(\delta\) ppm 188.71, 163.28, 151.31, 149.29, 144.06 131.35, 130.68, 128.12, 122.90, 119.90, 113.78, 111.23, 110/28, 55.97, 55.43. ESI MS (m/z): calcd for [C\textsubscript{18}H\textsubscript{18}O\textsubscript{4} + H], 299.1; found 299.1.

1-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl)-4-nitrobutan-1-one (2)

Product 1 (5.971 g, 0.02 mol), nitromethane (6.050 g, 0.1 mol) and diethylamine (7.317 g, 0.1 mol) was dissolved in 100 mL of methanol and heated under reflux for 24 h. After cooling down, acidified with 2 M diluted hydrochloric acid, extracted with CH\textsubscript{2}Cl\textsubscript{2}, and
evaporated to give the dark orange oily crude product. The crude product was further purified by chromatography on silica gel eluting with CH$_2$Cl$_2$/petroleum ether (1:5) to give the white product (3.522 g, 49%). $^1$H NMR (500 MHz, CDCl$_3$): δ ppm 7.94 (d, J = 8.5 Hz, 2H, Ar-H), 6.97 (d, J = 8.5 Hz, 2H, Ar-H), 6.85 (d, J = 14.5 Hz, 3H, Ar-H), 4.86 (dd, J = 6.5, 12 Hz, 1H, CH$_2$-NO$_2$), 4.72 (dd, J = 8.0, 12.0 Hz, 1H, CH$_2$-NO$_2$), 4.2 (m, 1H, CH), 3.90 (s, 6H, OCH$_3$), 3.87 (s, 3H, OCH$_3$), 3.42 (m, 2H, O=C-CH$_2$) $^{13}$C NMR (500 MHz, CDCl$_3$): δ ppm 195.39, 163.74, 149.16, 148.15, 131.72, 130.23, 129.49, 119.18, 113.78, 111.57, 111.08, 79.72, 55.87, 55.77, 55.38, 41.22, 39.16. ESI MS (m/z): calcd for [C$_{19}$H$_{21}$NO$_6$ + Na], 382.1; found 382.1.

[5-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrrol-2-yl][5-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl) pyrrol-2-ylidene] amine (3)

Product 2 (1.797 g, 0.005 mol), acemonium acetate (15.42, 0.2 mol) and butanol (100 mL) were added into a 250 mL round-bottom flask and heated under reflux for 48 h. After cooling down, the precipitate was purified by chromatography on silica gel eluting with CH$_2$Cl$_2$/petroleum ether (1:1.5) to give the dark brown product (1.390 g, 44%). $^1$H NMR (500 MHz, CDCl$_3$): δ ppm 7.79 (d, J = 10.5 Hz, 4H, Ar-H), 7.54 (dd, J = 2.5, 10.5 Hz, 2H, Ar-H), 7.47 (d, J = 2.5 Hz, 2H, Ar-H), 6.96 (s, 2H, pyrrole-H), 6.94 (d, J = 6.5 Hz, 4H, Ar-H), 6.85 (d, J = 10.5 Hz, 2H, Ar-H), 3.92 (s, 6H, OCH$_3$), 3.84 (s, 6H, OCH$_3$), 3.73 (s, 6H, OCH$_3$). NH was not observed. $^{13}$C NMR (500 MHz, CDCl$_3$): δ ppm 161.18, 153.96, 149.30, 149.16, 148.83, 142.13, 128.03, 127.31, 125.15, 121.90, 114.59, 113.41, 112.50, 111.10, 56.00, 55.83, 55.44. ESI MS (m/z): calcd for [C$_{38}$H$_{37}$N$_3$O$_6$], 631.3; found 631.2.
BF$_2$ Chelate of [5-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrrol-2-yl] [5-(4-methoxyphenyl)-3-(3,4-dimethoxyphenyl) pyrrol-2-ylidene] amine (4)

Compound 3 (0.632 g, 1 mmol) and diisopropylethylamine (1.16 g, 9 mmol) was dissolved in dried CH$_2$Cl$_2$ (100 mL) under nitrogen atmosphere. Boron trifluoride diethyl etherate (1.7 g, 12 mmol) was added and stirred at room temperature for 24 h. The solution was washed with deionized water. Finally, the organic layer was collected, dried, evaporated, and purified to give the purple product (0.556 g, 82%). $^1$H NMR (500 MHz, CDCl$_3$): δ ppm 8.08(d, J = 11 Hz, 4H, Ar-H), 7.65 (d, J = 2.5, 10.5 Hz, 2H, Ar-H), 7.54 (d, J = 2 Hz, 2H, Ar-H), 7.00 (s, 2H, pyrrole-H), 6.98 (s, 2H, Ar-H), 6.94 (d, J = 11 Hz, 4H, Ar-H), 3.95 (s, 6H, OCH$_3$), 3.87 (s, 6H, OCH$_3$), 3.81 (s, 6H, OCH$_3$). $^{13}$C NMR (500 MHz, CDCl$_3$): δ ppm 161.78, 157.74, 150.41, 149.10, 145.22, 143.408, 143.17, 131.50, 125.92, 124.33, 122.61, 117.65, 114.17, 112.47, 111.24, 56.03, 55.94, 55.37. ESI MS (m/z): calcd for [C$_{38}$H$_{34}$BF$_2$N$_3$O$_6$ + H]/2, 339.1; found 339.1.

BF$_2$ Chelate of [4-bromo-5-(4-methoxyphenyl)-3-(3,4-dimethoxy phenyl)-1H-pyrrol-2-yl] [5-(4- methoxyphenyl)-3-(3,4-dimethoxyphenyl) pyrrol-2-ylidene] amine (MeOA BBr)

Compound 4 (0.271 g, 0.40 mmol) and N-bromo-succinimide (178 mg, 1 mmol) were dissolved in chloroform and acetic acid solution and stirred for 12 h. The reaction mixture was washed with sodium thiosulphate and sodium bicarbonate solution, and then extracted with chloroform. The organic layer was dried over Na$_2$SO$_4$ and evaporated under reduced pressure to obtain the product as dark blue solid (0.301 g, 90%). $^1$H NMR (500 MHz, CDCl$_3$): δ ppm 7.71(d, J = 11.5 Hz, 4H, Ar-H), 7.53 (d, J = 13 Hz, 2H, Ar-H), 7.38 (s, 2H,
Ar-H), 7.02 (m, 6H, Ar-H), 3.99 (s, 6H, OCH$_3$), 3.90 (s, 6H, OCH$_3$), 3.74 (s, 6H, OCH$_3$).

$^{13}$C NMR (500 MHz, CDCl$_3$): $\delta$ ppm 168.48, 161.63, 160.33, 157.27, 150.54, 148.55, 144.06, 132.37, 127.17, 125.52, 124.23, 122.00, 114.20, 113.07, 110.65, 55.98, 55.77, 55.29. MALDI-TOF MS (m/z): calcd for C$_{38}$H$_{32}$BBr$_2$F$_2$N$_3$O$_6$, 833.07; found 833.47.

3.2. Singlet oxygen detection of MeOABBr

Singlet oxygen quantum yields ($\Phi_\Delta$) of MeOABBr were measured with MB as standard ($\Phi_\Delta = 0.57$ in DCM). The solution containing MeOABBr and DPBF were irradiated with 660 nm laser for 30 s. The oxidation decrease of DPBF’s absorption at 418 nm was monitored by UV-vis-NIR spectrophotometer. Thereafter, $\Phi_\Delta$ was calculated according to Equation S1:

$$\Phi_\Delta = \Phi_\Delta^{(std)} \times \frac{S_{sam}}{S_{std}} \times \frac{F_{std}}{F_{sam}} \quad (S1)$$

where ‘sam’ and ‘std’ represent MeOABBr and MB, respectively. $S$ represents the slope of the absorbance of DPBF at 418 nm versus irradiation time, $F$ represents the absorption correction factor, which is given by $F = 1 - 10^{-O.D.}$.

3.3. Photothermal effect of FMAB NPs

The solution of FMAB NPs with different concentration (10, 20 and 50 µg/mL, equivalent to the concentration of MeOABBr) in PBS solution were irradiated with 730-nm laser (1 W cm$^{-2}$) for 12 min. The temperature was recorded by FLIR thermal camera. Photothermal conversion efficiency ($\eta$) of FMAB NPs solution was measured under the irradiation of 730-nm laser at power density of 1 W cm$^{-2}$. DI water was used as the control group. The real-time temperature change was recorded using a FLIR thermal camera. Then $\eta$ was calculated according to Equation S2 and S3:
\[ \eta = \frac{hS\Delta T_{\text{max}} - Q_s}{I(1 - 10^{-A_{730}})} \]  
(S2)

\[ \tau_s = \frac{m_D C_D}{hS} \]  
(S3)

where \( h \) denotes the coefficient of heat transfer, \( S \) is container’s surface area, and the value of \( hS \) is calculated from the Figure S4b. \( \Delta T_{\text{max}} \) represents temperature change of FMAB NPs aqueous solution at the maximal steady-state temperature, and \( I \) is laser power. \( A_{730} \) is the absorbance intensity of FMAB NPs at 730 nm and \( Q_s \) expresses the heat associated with light absorption by the solvent. \( \tau_s \) represents the time constant of sample-system. \( C_D \) and \( m_D \) are heat capacity and mass (4.2 J g\(^{-1}\)) of the solvent H\(_2\)O, respectively.
4. Supplemental figures

(a)  
[Image of 1H NMR spectrum of PEG-FA]

(b)  
[Image of 1H spectrum of PEG-TPP]

Figure S1 (a) 1H NMR spectrum of PEG-FA; (b) 1H spectrum of PEG-TPP.
Figure S2 (a) Fluorescence spectrum of FMAB NPs. (b) UV-vis absorption spectra of DPBF solution incubated with MB in dichloromethane upon irradiation with 660-nm laser for different time. (c) UV-vis absorption spectra of DPBF solution incubated with MeOABBr in dichloromethane upon irradiation with 660-nm laser for different time.

Figure S3 (a) Photographs of FMAB NPs in PBS and serum solution for different days. (b) Size change of FMAB NPs in PBS for different days. (c) Zeta potential of FMAB NPs.
Figure S4 (a) Photothermal effect of FMAB NPs solution with 730-nm laser (1 W cm\(^{-2}\)).

(b) Time constant is measured to be \(\tau_s = 164.988\) s, in which the linear time data from cooling period was applied versus negative natural logarithm of driving force temperature.

Figure S5 (a) Fluorescence intensity changes of FMAB NPs in tumor sites at different time. (b) Fluorescence intensity of FMAB NPs in tumor and normal organs.
**Figure S6** Photo images of tumors collected from mice treated with PBS (black dash line, FMAB NPs (red dash line), FMAB NPs + irradiation (blue dash line).
**Figure S7** H&E staining examination of tumors for different groups after 18 days of treatments.
Supplemental References

