

Article

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# Discovery of Monoiodo Aza-BODIPY Near-Infrared Photosensitizer: *in vitro* and *in vivo* Evaluation for Photodynamic Therapy

Zhiliang Yu<sup>#, ac</sup>, Junliang Zhou<sup>#, a</sup>, Xin Ji<sup>a</sup>, Guangyu Lin<sup>a</sup>, Shuang Xu<sup>a</sup>, Xiaochun

Dong<sup>\*a</sup>, and Weili Zhao<sup>\*ab</sup>

<sup>a</sup>School of Pharmacy, Fudan University, Shanghai, 201203, P. R. China.

<sup>b</sup>Key Laboratory for Special Functional Materials of the Ministry of Education, Henan

University, Kaifeng, 475004, P. R. China.

<sup>c</sup>Shanghai Skin Disease Hospital, Shanghai, 200443, P. R. China.

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**ABSTRACT:** Photodynamic therapy as a rising platform of cancer treatment method is receiving increased attention. Through systematic evaluation of halogen substitution on aza-BODIPY, we have found that monoiodo-derived aza-BODIPYs provided greater

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3 efficacy than other halogenated aza-BODIPY PSs. **4** and **15** as monoiodinated aza-  
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6 BODIPY dyes containing *p*-methoxyphenyl moiety were identified to be potent NIR aza-  
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9 BODIPY type PSs with IC<sub>50</sub> values against HeLa cells at a light dose of 54 J/cm<sup>2</sup> as low  
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12 as 76 nM, 81 nM respectively. **4** possessed superior photo-toxicity, low dark-toxicity and  
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15 good thermal/photo stability and distributed majorly in mitochondria in cells. Apoptosis  
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18 was verified to be the main cell death pathway and *in vitro* ROS generation was  
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21 demonstrated. *In vivo* whole-body fluorescence imaging and *ex vivo* organ distribution  
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24 studies suggested that **4** afforded excellent PDT effect with low drug dose under single  
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27 time light irradiation and revealed advantages over known PSs of **ADPM06** and **Ce6**.  
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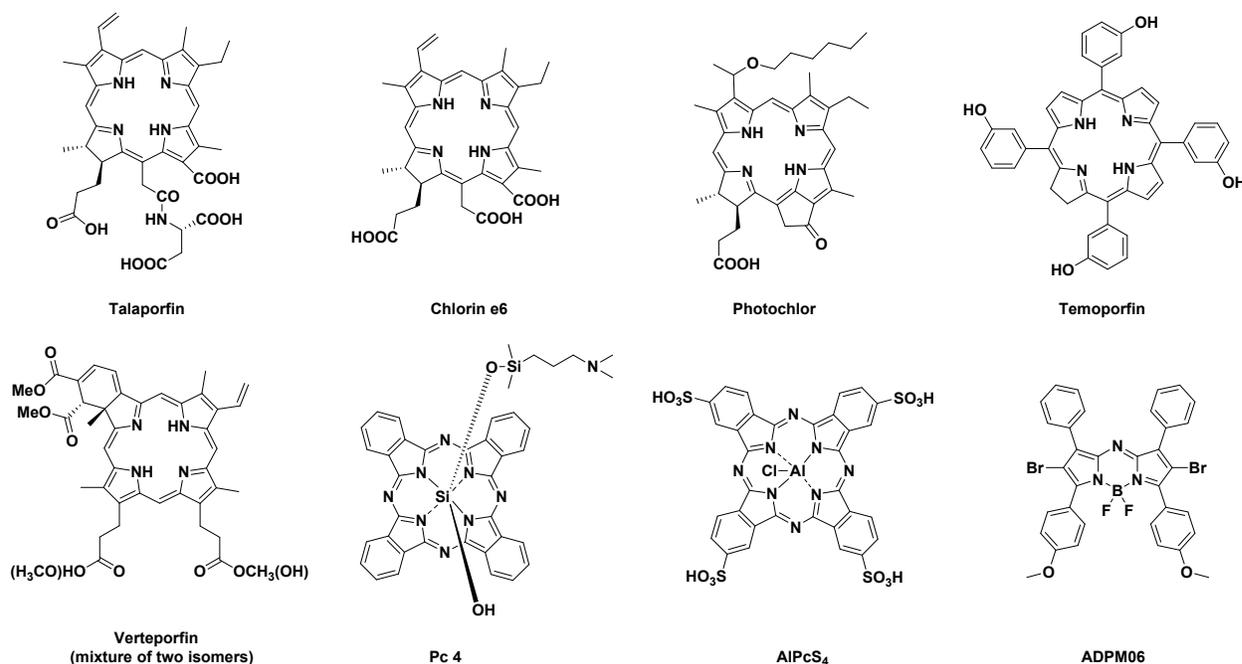
## 36 1. INTRODUCTION

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39 Photodynamic therapy (PDT) is a rising platform of noninvasive and clinically approved  
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42 protocol for the treatment of multiple types of cancers.<sup>1-3</sup> Three pivotal components are  
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45 usually involved in PDT: photosensitizer (PS), light, and oxygen. The light-activated PS  
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48 transfers its excited-state energy to either molecular oxygen (in triplet ground state) to  
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51 form <sup>1</sup>O<sub>2</sub> (type II reaction), or to react with a biological substrate to form superoxide anion  
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3 radical, hydroxyl radical, or hydrogen peroxide (type I reaction). The combined cytotoxic  
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7 reactive oxygen species (ROS) result in damage of tumor cells, tumor-associated  
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10 vasculature infarction, and activation of immune response system.<sup>4-6</sup> Due to different  
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13 microenvironment of tumor tissue from normal tissue, the PS administered intravenously,  
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17 intraperitoneally or locally, may be selectively retained in the tumor tissue after being  
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21 distributed for a period of time in the body.<sup>7-9</sup>  
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24 Since the clinical approval of Porfimer sodium (Photofrin), a complex mixture of  
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27 hematoporphyrin derivatives, as PDT agent for the treatment of early and advanced  
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31 cancers,<sup>10</sup> many efforts have been focused on discovery of new photosensitizers to  
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35 overcome some deficiencies in Photofrin such as low molar absorption coefficient, poor  
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38 bioavailability, and long-lasting cutaneous photosensitivity.<sup>11</sup> Various cyclic tetrapyrrole  
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41 type PSs such as porphyrin, chlorin, bacterial chlorophyll, as well as phthalocyanine were  
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45 developed. Some of them have been widely used as the second-generation of PSs in  
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49 clinical treatments of cancers with improved efficacy and reduced side-effect (Fig. 1).<sup>12-14</sup>  
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52 However, talaporfin and chlorin e6 (**Ce6**) suffered from poor photostability. Temoporfin,  
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56 photochlor and verteporfin are fairly hydrophobic with the defect of serious self-  
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3 aggregation upon administration.<sup>15,16</sup> In addition, these tetrapyrrole-based  
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7 photosensitizers, as well as phthalocyanines (**Pc 4** and **AIPcS<sub>4</sub>**), are difficult to synthesize  
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10 and purify, therefore restricting the fine-tuning of their photophysical and chemical  
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13 properties. Consequently, there is interest to develop non-porphyrin photosensitizers with  
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16 ease of preparation. The focus on non-porphyrin PSs had been primarily examined on  
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19 cationic structures such as methylene blue, Nile blue, Nile red analogues, and the  
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22 chalcogenopyrylium class of photosensitizers. These classes of compounds, however,  
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27 suffer from major drawbacks of inherent dark cytotoxicity and short absorption maxima.  
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31 In recent years, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacenes (BODIPYs) have emerged  
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35 as a new class of PDT agent with many favorable characteristics.<sup>17-25</sup>  
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26 **Figure 1.** Representative photosensitizers.

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30 BODIPYs possess many advantages of ideal photosensitizer characteristics such as  
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32 excellent photostability, high molar extinction coefficient, high photo-dark toxicity ratio,  
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34 and easy structural variation.<sup>20,22,26,27</sup> Since the early discovery of Nagano and coworkers  
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36 through introduction of 2,6-diiodo onto 1,3,5,7-tertramethyl BODIPY to facilitate  
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38 intersystem crossing to afford a potent photosensitizer with higher efficiency of singlet  
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40 oxygen production, as well as favorable photostability,<sup>28</sup> various BODIPY analogues  
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42 carrying two or more heavy atoms have been reported and demonstrated useful  
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44 performances on *in vitro* and *in vivo* anti-tumor studies.<sup>29-34</sup> Some of the non-halogenated  
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4 BODIPYs behaved as PDT agents, unfortunately, their efficacies were fairly weak in polar  
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7 environment.<sup>35</sup> Despite extraordinary efforts on modifications on BODIPY type PSs and  
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10 nano-photosensitizers, they are not featured in clinical photodynamic therapy.<sup>17,18,36</sup> More  
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13 recently, aza-BODIPYs, having the BODIPY *meso*-carbon replaced by nitrogen, attracted  
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16 much attention due to large absorption coefficient and red-shifted absorption to fit  
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19 therapeutic window (650–900 nm) which is clinically favored with improved tissue  
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22 penetration of light.<sup>22</sup> One landmark for aza-BODIPY PS was disclosed by O’Shea and  
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25 coworkers by attaching 2,6-dibromo onto 3,5-di(*p*-methoxyphenyl)-1,7-diphenyl aza-  
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28 BODIPY as efficient NIR PS (ADPM06) as shown in Fig.1.<sup>37-38</sup> It exhibited potent  
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31 efficacies towards a panel of cancer cell lines, as well as effective eradication of tumor  
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34 with comparable to “cure-rates” in mice xenograft models.<sup>37-40</sup> Various examples of aza-  
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37 BODIPY dyes carrying two or more halogen atoms were evaluated as PSs and anti-tumor  
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40 or anti-microbial studies were examined.<sup>41-50</sup> However, incorporation of halogen to  
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43 facilitate intersystem crossing to produce singlet oxygen may lead to increased dark  
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46 toxicity. So far, the bottleneck to limit these aza-BODIPYs moving into clinical stage as  
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49 non-porphyrin PSs for cancer treatment is still unclear. Up to now, there is no systemic  
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3 investigation of the halogen effect and substitution variation on aza-BODIPY as PS for  
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7 anti-tumor study from medicinal chemistry point of view.  
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10 In this paper, a series of bromo and iodo aza-BODIPYs were synthesized based on  
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13 3,5-di(*p*-methoxyphenyl)-1,7-diphenyl aza-BODIPY scaffold and their physical-chemical  
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17 properties were carefully evaluated. We extended our discovery in more broad range and  
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21 came to the conclusion that monoiodo derivatives of aza-BODIPY possessed various  
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25 advantages of ideal PS with excellent photostability, chemical stability and photo  
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28 cytotoxicity. The superior antitumor efficacy was demonstrated both *in vitro* and *in vivo*.  
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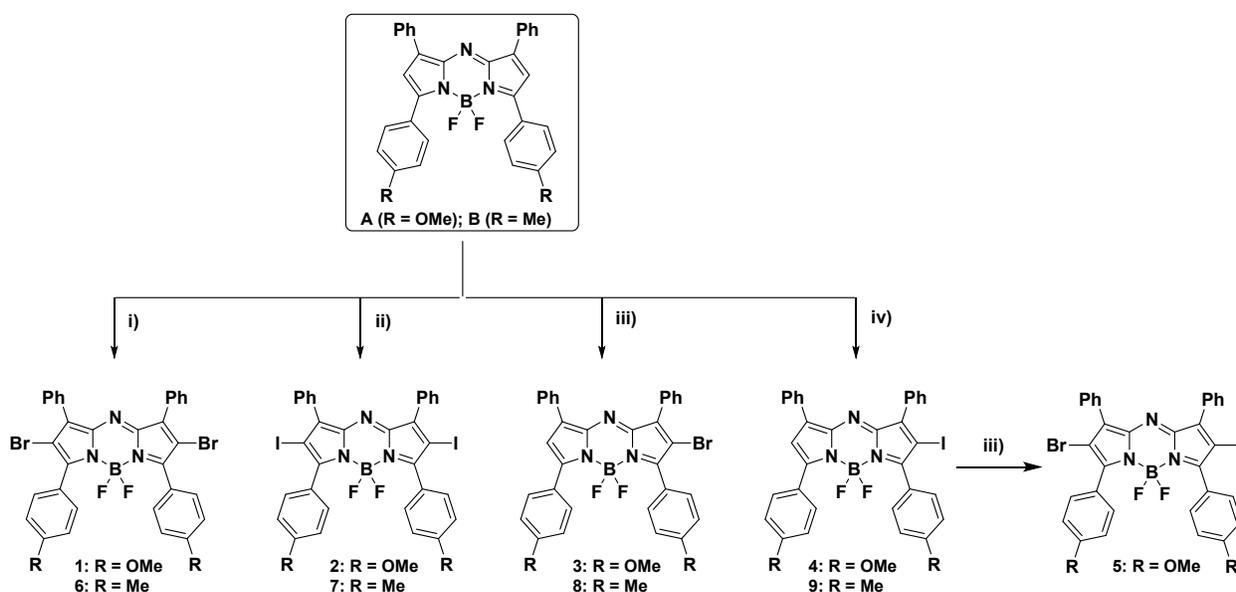
## 31 2. RESULTS AND DISCUSSION

### 32 2.1. Design and Synthesis.

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35 Various symmetric and non-symmetric aza-BODIPY fluorescent dyes were easily  
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38 achieved using our previous optimized methods.<sup>51-52</sup> The general known strategy to  
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42 transform aza-BODIPY dyes into PSs was to introduce heavy atoms (*e.g.* Br, I) onto both  
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46 2- and 6- positions of aza-BODIPYs.<sup>37-50</sup> The focus of our design was the comparison of  
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52 halogen effect by installation of mono-, di-, or mixed heavy atom(s) onto aza-BODIPY  
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56 core. Therefore, we initially selected the most advanced aza-BODIPY PS of **ADPM06** as  
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4 leading compound. Various selective halogenation methods including monohalogenation,  
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7 dihalogenation, mixed halogenation were successfully explored using 3,5-di(*p*-  
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10 methoxyphenyl)-1,7-diphenyl aza-BODIPY (compound **A**) as the key scaffold (Scheme  
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14 1). Both dibromo PS (**1**, namely **ADPM06** by O'Shea et al.<sup>37</sup>) and diiodo PS (**2**) were  
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16 prepared in excellent yields (91.3%, 90.4% respectively). Monobromo PS (**3**) and  
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18 moniodo PS (**4**) were also obtained nicely (93.5%, 89.2% respectively). Moreover, 2-  
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20 bromo-6-iodo aza-BODIPY (**5**) as mixed halogenated PS was also synthesized in high  
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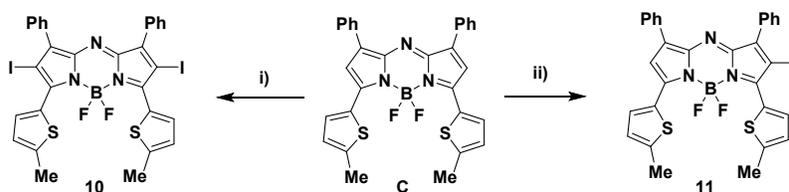
**Scheme 1.** Selective halogenation of aza-BODIPY dyes.



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3 Reagents and conditions: i) NBS (2.2 equiv), AcOH, DCM, r.t., 30 min; ii) NIS (2.2 equiv),  
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5 AcOH, DCM, r.t., 30 min; iii) NBS (1.0 equiv), AcOH, DCM, r.t., 30 min; iv) NIS (1.0 equiv),  
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7 AcOH, DCM, r.t., 30 min.  
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12 We further examined the halogen effect on other NIR aza-BODIPY PSs characterized  
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15 with electron-donation groups in broader range using 3,5-di(*p*-tolyl)-1,7-diphenyl aza-  
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17 BODIPY (compound **B**) as key scaffold and the halogenated PSs were similarly prepared  
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19  
20 BODIPY (compound **B**) as key scaffold and the halogenated PSs were similarly prepared  
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23 as shown in Scheme 1. The generality of halogen effect was also investigated based on  
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26 3,5-di(5'-methylthiophenyl-2-yl)-1,7-diphenyl aza-BODIPY (compound **C**) and the  
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29 halogenated derivatives were prepared as shown in Scheme 2. All PSs were obtained in  
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32 excellent yields.  
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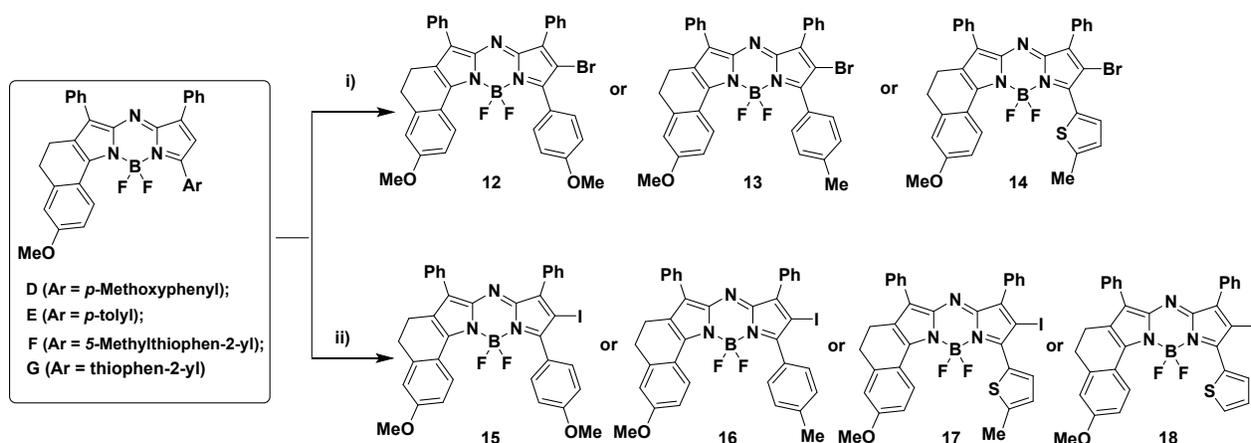
36  
37 **Scheme 2.** Synthesis of mono or dihalogenated aza-BODIPY photosensitizers.  
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Reagents and conditions: i) NIS (2.2 equiv), AcOH, DCM, r.t., 30 min; ii) NIS (1.0 equiv),  
AcOH, DCM, r.t., 30 min.

We finally paid our attention on conformationally restricted aza-BODIPY dyes with favorable long absorption maxima and greater absorption coefficient.<sup>51-52</sup> Various electron-donating aromatic and heteroaromatic groups were included to favor requirement of the deep tissue penetration. The non-symmetric dyes (**D~G**) were easily obtained.<sup>51-52</sup> Mono-brominated derivatives (**12~14**) and mono-iodinated species (**15~18**) were all prepared in excellent yields using the conditions shown in Scheme 3.

**Scheme 3.** Preparation of conformationally restricted aza-BODIPY photosensitizers.



Reagents and conditions: i) NBS (1.0 equiv), AcOH, DCM, r.t., 30 min; ii) NIS (1.0 equiv), AcOH, DCM, r.t., 30 min.

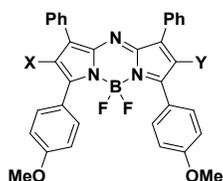
## 2.2. Spectroscopic, Photophysical Properties, and <sup>1</sup>O<sub>2</sub> Generation Efficiencies.

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4 The spectroscopic and photophysical properties of aza-BODIPY PSs were frequently  
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7 evaluated in non-polar solvents in literatures.<sup>37-50</sup> The electronic absorption and  
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10 fluorescence spectra of our aza-BODIPY dyes (**1~5**) were initially recorded in chloroform  
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13 using compound **A** as reference dye and the spectroscopic data were collected in Table  
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17 1. The absorption maxima ( $\lambda_{\text{abs}}$ ) of halogenated aza-BODIPY displayed hypsochromic  
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20 shift around 9 nm compared with the parent dye (compound **A** with  $\lambda_{\text{abs}}$  of 688 nm). In  
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23 contrast, the emission maxima ( $\lambda_{\text{em}}$ ) of halogenated aza-BODIPY displayed only minor  
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26 hypsochromic shift (2~5 nm) compared with the parent dye (compound **A** with  $\lambda_{\text{em}}$  of 725  
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29 nm). The monohalogenated aza-BODIPYs presented minor decrease of absorption  
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32 coefficient ( $\epsilon$  of 81,000, 97,000  $\text{M}^{-1}\text{cm}^{-1}$  for **3** and **4**, respectively) comparing with parent  
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35 dye **A** ( $\epsilon$  of 99,000  $\text{M}^{-1}\text{cm}^{-1}$ ), whereas dihalogenated species ( $\epsilon$  of 77,000, 74,000  $\text{M}^{-1}\text{cm}^{-1}$   
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38 for **1** and **2**, respectively) showed much decreased absorption coefficient. The mixed  
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41 halogenated species (**5** with  $\epsilon$  of 76,000  $\text{M}^{-1}\text{cm}^{-1}$ ) behaved as dihalogenated species.  
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44 Interestingly, while monobromination only led to trivial decline of fluorescence quantum  
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47 yield ( $\Phi_f$ ) (comparing **3** with  $\Phi_f$  of 0.34 to **A** with  $\Phi_f$  of 0.36), dibrominated aza-BODIPY  
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50 (**1**) revealed much decreased fluorescence quantum yield ( $\Phi_f = 0.17$ ). In contrast,  
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4 monoiodo derivative (**4**) exhibited even smaller value of  $\Phi_f$  (0.08) than **1**. Diiodination  
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7 resulted in much reduced fluorescence quantum yield ( $\Phi_f = 0.03$ ) as shown from the data  
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10 of **2**. As expected, mixed halogenation (**5**) afforded significantly diminished fluorescence  
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13 emission ( $\Phi_f = 0.07$ ). In PDT application, PSs were usually applied in aqueous  
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16 environments. Due to the hydrophobic nature of aza-BODIPY dye, the photosensitization  
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19 of aza-BODIPY type PS may behave significantly different in polar environment from non-  
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22 polar solvent because of possible aggregation. We therefore examined the spectroscopic  
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25 and photophysical properties of PSs in isopropanol (*i*PrOH), acetonitrile (CH<sub>3</sub>CN), *N,N*-  
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28 dimethylformamide (DMF), as well as PBS buffer containing 1% Cremophor EL  
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31 (CrEL)/1,2-propanediol (10:3, v:v). The absorption spectra of aza-BODIPY PSs in  
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34 emulsified PBS buffer and in other polar solvents were shown in Fig. S1 (supporting  
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37 information). The spectroscopic data in various polar environments were presented in  
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42 Table S1 (supporting information). It can be found that in various solvents, all these  
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45 halogenated compounds revealed intense Q-band with absorption maxima around  
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52 665~685 nm, which falls into the body's therapeutic window (650~900 nm). In consistence  
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56 with the trend observed in chloroform, all PSs showed declined absorption coefficients  
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3 compared with the parent dye compound **A**. Notably, the absorption coefficients ( $\epsilon$ ) of all  
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7 dyes investigated revealed much declined values in PBS (34,000~59,000  $M^{-1}cm^{-1}$ )  
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10 compared with those in organic media (59,300~105,000  $M^{-1}cm^{-1}$ ). Concurrently, the Full-  
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13 Width Half-Maximum (FWHM) values in aqueous environment (87~90 nm) were  
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16 significantly greater than those (56~67 nm) in organic solvents (Table S1).  
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21 **Table 1.** Photophysical data for the first round BODIPY photosensitizers.  
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PS	$\lambda_{\text{abs}}$ (nm) <sup>a</sup>	$\epsilon$ ( $M^{-1} cm^{-1}$ ) <sup>a</sup>	$\lambda_{\text{em}}$	$\Phi_f$ <sup>a</sup>	rel. rate <sup>b</sup>	rel. rate <sup>c</sup>	rel. rate <sup>d</sup>	$\Phi_{\Delta}$ <sup>d</sup>
<b>A</b> (X=Y=H)	688	99000	725	0.36	0.28	0.30	0.04	0.02
<b>1</b> (X=Y=Br)	679	77000	720	0.17	4.5	1.9	0.36	0.14
<b>2</b> (X=Y=I)	679	74000	723	0.03	7.0	2.2	0.66	0.24
<b>3</b> (X=H; Y=Br)	679	81000	720	0.34	1.1	0.83	0.19	0.10
<b>4</b> (X=H; Y=I)	678	97000	721	0.08	7.3	2.4	1.3	0.52
<b>5</b> (X=Br; Y=I)	679	76000	720	0.07	6.8	2.3	0.65	0.29

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<sup>a</sup>In Chloroform; <sup>b</sup>Relative rate of degradation of DPBF to methylene blue in  $\text{PrOH}$ ;

<sup>c</sup>Relative rate of degradation of DPBF to methylene blue in PBS containing 1%

Cremophor EL (CrEL)/1,2-propanediol (10:3, v:v); <sup>d</sup>Measured in DMF using ZnPc ( $\Phi_{\Delta}$  =

0.56 in DMF) as standard.

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3 To evaluate the photosensitizing abilities of these aza-BODIPY dyes, their singlet  
4 oxygen generation efficiencies were studied using 1,3-diphenylisobenzofuran (DPBF) as  
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8 oxygen generation efficiencies were studied using 1,3-diphenylisobenzofuran (DPBF) as  
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11 singlet oxygen scavenger. The rate of photodegradation of this quencher was measured  
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14 by monitoring the decrease in absorbance at 411 nm with time upon irradiation with light.  
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17 Different from previous publications which evaluated photosensitizing efficiency  
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20 frequently in non-polar solvent (*e.g.* toluene), we focused our investigations in polar  
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23 environments (iPrOH, DMF, PBS buffer containing Cremophor EL) in order to find useful  
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26 information for optimal PS. The photosensitizing capabilities of individual PS were carried  
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29 out under irradiation with filtered light ( $\lambda > 590$  nm from halogen lamp with energy power  
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32 of 90 mW/cm<sup>2</sup>). Time-dependent changes of absorption spectra of various aza-BODIPY  
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35 dyes containing DPBF under light irradiation were shown in Fig. S2. The relative rates of  
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38 degradation of DPBF monitored at 411 nm in iPrOH, DMF, PBS buffer containing 1%  
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41 Cremophor EL (CrEL)/1,2-propanediol (10:3, v:v) were illustrated in Fig. S3 and the data  
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44 calculated were collected in Table 1. From Fig. S2 and Fig. S3, it can be found that the  
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52 photosensitizing efficiencies of these PSs were solvent-dependent.  
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4 As expected, the parent fluorescent dye **A** without any heavy atom in the molecule  
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6  
7 exhibited poor  $^1\text{O}_2$  generation ability in all solvents applied. In contrast, all halogenated  
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9  
10 aza-BODIPY PSs (**1**~**5**) revealed potent photosensitizing capability in  $\text{PrOH}$ . In  
11  
12  
13 consistent with previous reports,<sup>37-50</sup> dibrominated aza-BODIPY (**1**) or diiodinated aza-  
14  
15  
16 BODIPY (**2**) afforded potent photosensitization in  $\text{PrOH}$  and **2** was more effective than **1**.  
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18  
19 We also observed that **5** as mixed halogenated PS possessed nice photosensitizing  
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21  
22 ability as well ( $1 < 5 < 2$ ). Though monobromination enabled photosensitizing capability  
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25 (see **3**) similar to the reference PS of methylene blue (**MB**), significant improvement of  
26  
27  
28 singlet oxygen generation was astonishingly found for monoiodo derivative (**4**) with up to  
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30  
31 7-fold rate relative to MB in  $\text{PrOH}$  for degradation of DPBF. It worth to note that monoiodo  
32  
33  
34 PS **4** exhibited much more potent photosensitizing ability than dibromo PS **1** and even  
35  
36  
37 more effective than mixed halogenated PS **5** and diiodo PS **2**. Such trend of halogenation  
38  
39  
40 effects were also observed in PBS buffer containing 1% Cremophor EL (CrEL)/1,2-  
41  
42  
43 propanediol (10:3, v:v) though degradation of DPBF was less efficient due to the  
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45  
46 hydrophobic nature of the aza-BODIPY PSs. Unexpectedly, when evaluation of  
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49 photosensitizing capability was performed in dipolar aprotic solvent such as DMF or  
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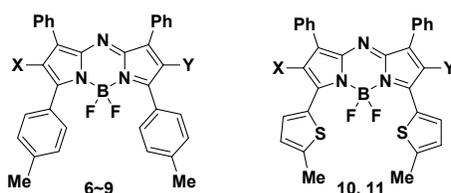
1  
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3 DMSO, it was found that obvious degradation of PS for halogenated aza-BODIPY  
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7 illustrated in the decrease of the absorbance of the NIR band was generally observed as  
8  
9  
10 shown in Fig. S2. To further evaluate the halogenation-related degradation, we selected  
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12  
13 ZnPc ( $\Phi_{\Delta}$  = 0.56 in DMF) as standard to compare the  $^1\text{O}_2$  generation efficiencies of PSs  
14  
15  
16 in DMF. In fact, dihalogenated aza-BODIPY (**1**, **2**) or mixed halogenated species (**5**)  
17  
18  
19 encountered significant degradation and afforded concurrently declined efficiency of  
20  
21  
22 production of  $^1\text{O}_2$  ( $\Phi_{\Delta}$  = 0.14 for **1**; 0.24 for **2**; 0.29 for **5**). While monobromo PS **3** ( $\Phi_{\Delta}$  =  
23  
24  
25 0.10) was weaker than those PSs with two halogen atoms, the monoiodinated aza-  
26  
27  
28 BODIPY (**4**) revealed excellent  $^1\text{O}_2$  production with  $\Phi_{\Delta}$  = 0.52 in DMF. We believed that  
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31 the difference of photosensitizing ability among the aza-BODIPY PSs was amplified in  
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34 DMF and such a solvent was used to evaluate other PSs in our subsequent modifications.  
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41  
42 The next round modification intended to inspect the generality of the trend observed  
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44  
45 above. To enable long absorption maxima, we installed *p*-tolyl moiety or 5-thiophenyl-2-  
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47  
48 yl group onto 3- and 5- positions of aza-BODIPY and the corresponding dyes were termed  
49  
50  
51 **B/C** respectively. The halogenated derivatives were listed in Table 2 and their properties  
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54 were collected as well. It can be found that **B** derived PS series displayed slightly shorter  
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3 absorption maxima than **A** derived series whereas **C** derived PSs possessed longer  
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7 absorption maxima than compound **A** derived PSs as expected based on the electron-  
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10 donating ability of the substituent. Unfortunately, though monoiodination resulted in  
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13  
14 advantageous photosensitizing ability over monobromination, dibromination, or  
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17 diiodination (comparing **9** with **8**, **6**, **7**; comparing **11** with **10**, Table 2), the  $^1\text{O}_2$  production  
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20 quantum yields in DMF were relatively low for either **9** ( $\Phi_{\Delta}$  = 0.14) or **11** ( $\Phi_{\Delta}$  = 0.17). These  
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23  
24 data implied that substituent on 3-/5- position of aza-BODIPY affected the  $^1\text{O}_2$  production  
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26  
27 and *p*-methoxyphenyl group was advantageous over *p*-tolyl or 5-thiophenyl-2-yl.

31 **Table 2.** Photophysical data for aza-BODIPY photosensitizers prepared according to

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35 Scheme 2.



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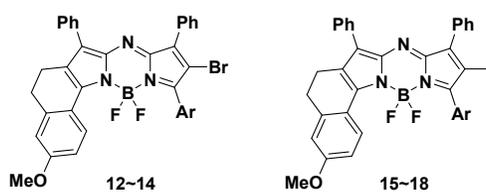
Entry	$\lambda_{\text{abs}}$ (nm) <sup>a</sup>	$\epsilon$ (M <sup>-1</sup> cm <sup>-1</sup> ) <sup>a</sup>	$\lambda_{\text{em}}$ (nm) <sup>a</sup>	$\Phi_{\text{f}}$ <sup>a</sup>	rel. rate <sup>b</sup>	$\Phi_{\Delta}$ <sup>b</sup>
<b>6</b> (X=Y=Br)	658	79900	685	0.04	0.22	0.10
<b>7</b> (X=Y=I)	662	92000	688	0.02	0.28	0.12
<b>8</b> (X=H; Y=Br)	658	83600	685	0.19	0.12	0.05
<b>9</b> (X=H; Y=I)	659	89600	690	0.04	0.26	0.14

10 (X=Y=I)	732	91300	768	0.07	0.36	0.14
11 (X=H; Y=I)	732	71700	766	0.11	0.32	0.17

<sup>a</sup>In chloroform; <sup>b</sup>Relative rate of degradation of DPBF and singlet oxygen quantum yield ( $\Phi_{\Delta}$ ) with reference to ZnPc ( $\Phi_{\Delta}$ = 0.56) in DMF.

**Table 3.** Photophysical data for aza-BODIPY photosensitizers prepared according to

Scheme 3.



Entry	$\lambda_{\text{abs}}(\text{nm})^a$	$\epsilon (\text{M}^{-1} \text{cm}^{-1})^a$	$\lambda_{\text{em}}(\text{nm})^a$	$\Phi_f^a$	rel. rate <sup>b</sup>	$\Phi_{\Delta}^b$
12 (Ar= <i>p</i> -MeOPh)	692	90300	724	0.44	0.17	0.08
13 (Ar= <i>p</i> -MePh)	682	104400	709	0.60	0.14	0.08
14 (Ar=5-Me-thiophen-2-yl)	714	73700	753	0.24	0.14	0.11
15 (Ar= <i>p</i> -MeOPh)	689	101300	725	0.14	1.2	0.51
16 (Ar= <i>p</i> -MePh)	682	110000	709	0.17	0.25	0.16
17 (Ar=5-Me-thiophen-2-yl)	716	76900	756	0.16	0.42	0.25
18 (Ar=thiophen-2-yl)	695	81500	740	0.16	0.37	0.19

<sup>a</sup>In chloroform; <sup>b</sup>Relative rate of degradation of DPBF and singlet oxygen quantum yield ( $\Phi_{\Delta}$ ) with reference to ZnPc ( $\Phi_{\Delta}$ = 0.56) in DMF.

The established advantageous  $^1\text{O}_2$  production with monoiodo aza-BODIPY derivatives shown in Tables 1 and 2 promoted us to apply various monoiodination on

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3 conformationally-restricted aza-BODIPYs using 4,5-dihydro-7-methoxy-3-  
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6 phenylbenzo[g]indole and 2-aryl-4-phenyl pyrrole to constitute the parent aza-BODIPY  
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10 core as shown in Scheme 3 in an attempt to further improve light penetration capability.<sup>51</sup>  
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12  
13  
14 Among the designed PSs, *p*-tolyl, *p*-methoxyphenyl, 5-methyl-thiophene-2-yl, and  
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17 thiophene-2-yl were selected as the candidates of Ar in the pyrrole scaffold (The aza-  
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19  
20 BODIPYs constructed were termed as compounds **D**, **E**, **F**, and **G** respectively). Mono-  
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23 brominated derivatives (**12**~**14**) and mono-iodinated species (**15**~**18**) were all examined  
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27 the photophysical properties and photosensitization capabilities and the results were  
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29  
30 collected in Table 3. As a general rule, the singlet oxygen quantum yields of  
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32  
33 monobrominated PSs (0.08~0.11) were inferior to monoiodinated PSs (0.16~0.51).  
34  
35  
36  
37  
38 Though thienyl (in **18** with  $\Phi_{\Delta}$  of 0.19) and 5-methyl-thiophene-2-yl (**17** with  $\Phi_{\Delta}$  of 0.25)  
39  
40  
41 led to long absorption maxima of the PSs, the photosensitizing capabilities were  
42  
43  
44 unfortunately suboptimal. *p*-Tolyl modified PSs (**13** and **16**) did not provide enough  $^1\text{O}_2$   
45  
46  
47  
48 generation quantum yields ( $\Phi_{\Delta}$  of 0.08, 0.16 respectively) and beneficial absorption  
49  
50  
51 maxima ( $\lambda_{\text{abs}}$  of 682 nm) over other conformationally-restricted PSs. Pleasantly, we  
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55 discovered that **15** carrying *p*-methoxyphenyl moiety possessed nice photosensitization  
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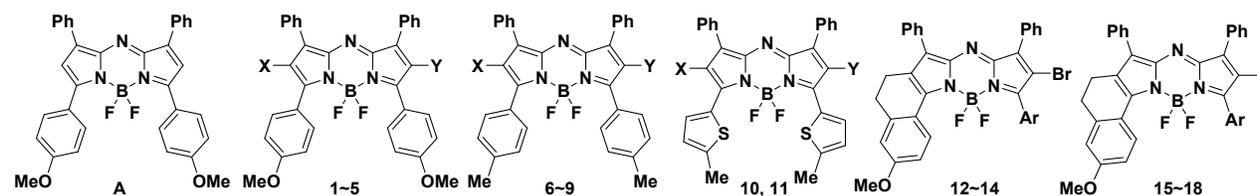
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3 efficiency ( $\Phi_{\Delta}$  of 0.51), favorable absorption maximum ( $\lambda_{\text{abs}}$  of 689 nm), and absorption  
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7 coefficient ( $\epsilon$  of 101,300 M<sup>-1</sup>cm<sup>-1</sup>).  
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### 10 **2.3 Cytotoxicity assays.**

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14 The *in vitro* photodynamic activities of aza-BODIPYs in Cremophor emulsion were  
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17 investigated against cervical carcinoma cell line (HeLa), breast cancer cell line (MCF-7),  
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20 and colon carcinoma cell line (SW480) under irradiation with light > 590 nm using MTT  
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23 assay with **Ce6** as reference PS (Table 4). Parallel assays without light irradiation were  
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26  
27 also performed to determine the dark toxicity. As shown in Table 4, all the PSs have  
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29  
30 negligible dark toxicity up to 50  $\mu\text{M}$ . When irradiated with light dose of 54 J/cm<sup>2</sup>, IC<sub>50</sub>  
31  
32  
33 values of various PSs exhibited broad range of activity from micromolar to nanomolar  
34  
35  
36 level. In the series of aza-BODIPY **A** derived PSs, dibromo species **1** (**ADPM06**)  
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38  
39 possessed good light-induced toxicity (0.13~0.16  $\mu\text{M}$ ) for the investigated cell lines in  
40  
41  
42 consistence with the literature reports.<sup>39</sup> Diiodo PS **2** which efficiently generated high  
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44  
45 singlet oxygen in organic media was found to have unsatisfied potency with light toxicity  
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47  
48 around 1  $\mu\text{M}$ . Similarly, the mixed halogenated PS **5** presented suboptimal light toxicity  
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51 around 0.5  $\mu\text{M}$ . To our surprise, monoiodo PS **4** displayed potent inhibition for all cell lines  
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(up to 76 nM for HeLa cells) in consistent with efficient production of  $^1\text{O}_2$ . For **B** and **C** derived series wherein *p*-tolyl and 5-methylthiophene-2-yl were installed onto the 3,5-position of aza-BODIPY dye respectively, the monoiodo substituted photosensitizers (**9**, **11**) exhibited better efficacy than monobrominated (**8**) and dihalogenated (**6**, **7**, **10**) PSs in cell-based assays, even though the potency was relatively low. Among the conformationally restricted system, we were pleased to find that monoiodo derivatives (**15~18**) were generally more active than monobromo derivatives (**12~14**) and *p*-methoxyphenyl derived monoiodo PS (**15**) showed more potent inhibition than *p*-tolyl, 5-methylthiophene-2-yl, thiophene-2-yl derivatized PSs (**16**, **17**, **18** respectively).

**Table 4.** Photo and dark cytotoxicity of PSs in HeLa, MCF-7 and SW480 tumor cell lines.



PS	$\text{IC}_{50}$ ( $\mu\text{M}$ )					
	HeLa		MCF-7		SW480	
	0 J/cm <sup>2</sup>	54 J/cm <sup>2</sup>	0 J/cm <sup>2</sup>	54 J/cm <sup>2</sup>	0 J/cm <sup>2</sup>	54 J/cm <sup>2</sup>
<b>A (X=Y=H)</b>	>50	11.6±2.0	>50	9.4±1.3	>50	12.1±1.8

1							
2							
3							
4	1 (X=Y=Br)	>50	0.13±0.006	>50	0.15±0.01	>50	0.16±0.01
5							
6	2 (X=Y=I)	>50	0.59±0.12	>50	0.66±0.07	>50	1.3±0.11
7							
8							
9	3 (X=H; Y=Br)	>50	0.27±0.05	>50	0.35±0.06	>50	0.28±0.04
10							
11							
12	4 (X=H; Y=I)	>50	0.076±0.004	>50	0.10±0.01	>50	0.086±0.006
13							
14							
15	5 (X=Br; Y=I)	>50	0.31±0.05	>50	0.42±0.08	>50	0.53±0.06
16							
17							
18	6 (X=Y=Br)	>50	4.3±0.89	>50	1.5±0.20	>50	16.1±2.2
19							
20							
21	7 (X=Y=I)	>50	13.4±2.3	>50	5.3±0.43	>50	>25
22							
23							
24	8 (X=H; Y=Br)	>50	7.2±1.1	>50	2.6±0.18	>50	>25
25							
26							
27	9 (X=H; Y=I)	>50	2.6±0.46	>50	1.2±0.14	>50	12.4±2.0
28							
29							
30	10 (X=Y=I)	>50	>25	>50	16.4±2.9	>50	>25
31							
32							
33	11 (X=H; Y=I)	>50	15.6±3.2	>50	7.7±1.3	>50	20.6±3.2
34							
35							
36	12 (Ar= <i>p</i> -MeOPh)	>50	0.83±0.17	>50	4.5±0.41	>50	1.9±0.26
37							
38	13 (Ar= <i>p</i> -MePh)	>50	1.2±0.31	>50	8.1±1.3	>50	15.6±3.4
39							
40							
41	14 (Ar=5-Me-thiophen-2-yl)	>50	22.6±3.3	>50	>25	>50	>25
42							
43							
44	15 (Ar= <i>p</i> -MeOPh)	>50	0.081±0.01	>50	0.14±0.02	>50	0.12±0.01
45							
46							
47	16 (Ar= <i>p</i> -MePh)	>50	0.11±0.02	>50	0.41±0.06	>50	0.66±0.08
48							
49							
50	17 (Ar=5-Me-thiophen-2-yl)	>50	12.3±1.9	>50	16.6±3.8	>50	21.4±4.6
51							
52							
53	18 (Ar=thiophen-2-yl)	>50	0.46±0.09	>50	0.75±0.11	>50	0.83±0.14
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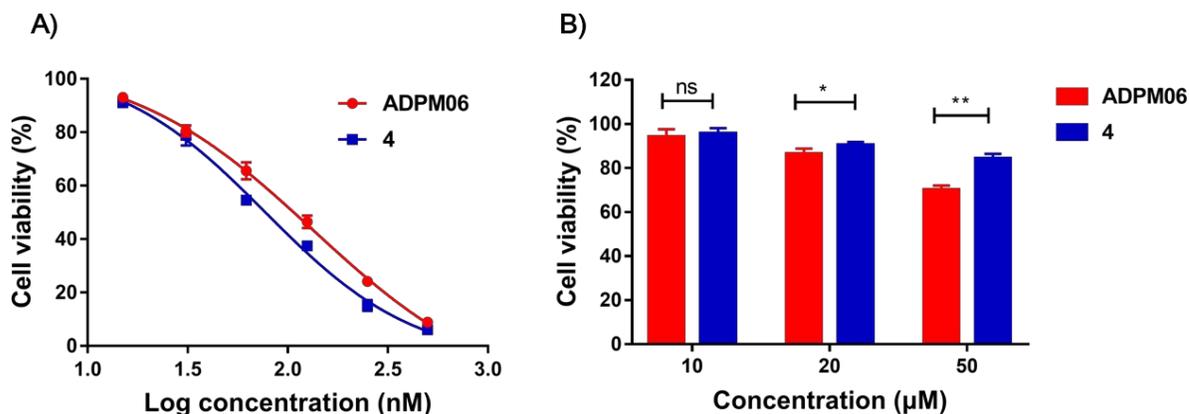
Ce6	>50	1.92±0.22	>50	3.4±0.29	>50	3.1±0.46
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Through comparison of various aza-BODIPY PSs in solution-based and cell-based activities, we came to the conclusion that dihalogenation of aza-BODIPYs did not behave as expected to lead to potent PDT effect according to general trend of heavy atom effect.<sup>17,18,20</sup> Actually, two monoiodinated aza-BODIPY dyes (**4**, **15**) containing *p*-methoxyphenyl moiety among the investigated PSs offered excellent photosensitization and behaved as favorable PSs with intense long absorption, high phototoxicity and low dark toxicity. Therefore, our subsequent efforts focused on detailed studies of **4** as a nice example to confirm the advantage of monoiodination in comparison with known leading standard of **ADPM06** (compound **1**).

The cytotoxic effects under or without light irradiation for **4** and **1** against HeLa cells were shown in Fig. 2A and Fig. 2B, respectively. From Fig. 2A it can be concluded that **4** displayed better PDT activity than **1** over the investigated concentration range. As shown in Fig. 2B, though both PSs exhibited minimal dark toxicity against HeLa cells without

light irradiation, the cell viability evaluations clearly suggested the dibromination led to greater dark toxicity than monoiodination.



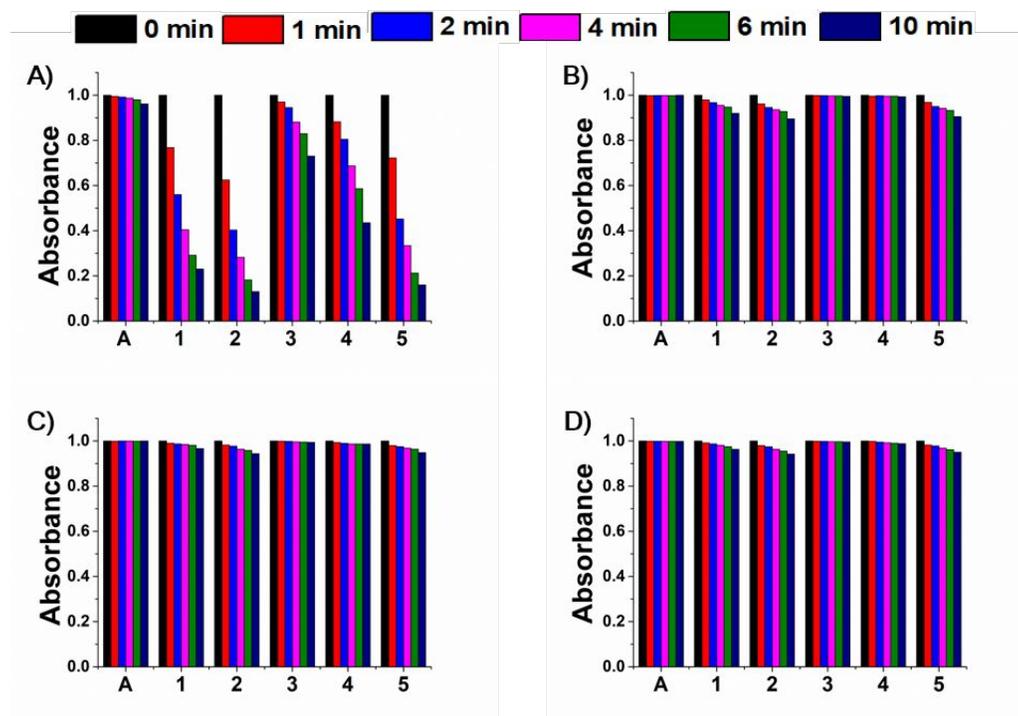
**Figure 2.** Photo toxicity (A) and dark toxicity (B) of photosensitizers **4** and **ADPM06** against HeLa cells. Light dose: 54 J/cm<sup>2</sup>,  $\lambda > 590$  nm. \* $p < 0.05$ , \*\* $p < 0.01$ .

To further investigate the PDT behavior of **4**, light dose-dependent phototoxicity assays were performed and the results were displayed in Fig. S4. From Fig. S4, we were pleased to find that **4** exhibited light dose-dependent antitumor activity against HeLa cells and much greater cell killing ability was achieved by **4** under stronger exposure of light irradiation.

#### 2.4. Photostability and thermal stability studies.

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4 Upon evaluation of photosensitivity, we have noticed that some PSs encountered  
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7 degradation during light irradiation (Fig. S2). We suspected that the observed *in vitro*  
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10 efficacy may significantly rely on the stability of PS. To investigate the photostability  
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13 behavior comprehensively and establish the relationship between the stability and  
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16 potency, we selected A-derived series and monitored the absorbance of PSs in *N,N*-  
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18 dimethylformamide (DMF), acetonitrile (CH<sub>3</sub>CN), isopropanol (iPrOH) and PBS buffer  
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21 containing 1% Cremophor EL (CrEL)/1,2-propanediol (10:3, v:v) respectively under  
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24 irradiation with light ( $\lambda > 590$  nm, light power: 90 mW/cm<sup>2</sup>). As shown in Fig. 3, all PSs  
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26  
27 encountered obvious degradation in DMF under irradiation and followed the trend of  
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29  
30 stability as **A > 3 > 4 > 1 > 5 > 2**. Same trend was also found in other solvent *e.g.* CH<sub>3</sub>CN,  
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33 iPrOH, or emulsified PBS buffer, however, the degradation of PS was insignificant.  
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36 Meanwhile, the thermal stabilities in the dark without irradiation in CH<sub>3</sub>CN and emulsified  
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39 PBS buffer (containing minimum amount of detergent) were investigated as well and the  
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42 results were displayed in Fig. S5. Slow degradation of PS in the dark was discovered for  
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45 all dyes investigated both in CH<sub>3</sub>CN and emulsified PBS buffer and followed the same  
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48 trend as light-induced degradation (Fig. 3). As demonstrated in Fig. 3 and Fig. S5,  
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3 monohalogenated PSs possessed much better stability than dihalogenated and mixed  
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7 halogenated PSs. Therefore, the stability studies may provide a plausible explanation for  
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10 the observed potent PDT effect of 4.  
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12



38 **Figure 3.** Normalized absorbance-irradiation time histogram of dyes and photosensitizers  
39 in different solvents: (A) DMF; (B) CH<sub>3</sub>CN; (C) *i*PrOH; (D) PBS buffer containing 0.01%  
40  
41 Cremophor EL (CrEL)/1,2-propanediol (10:3, v:v). Light power: 90 mW/cm<sup>2</sup> ( $\lambda > 590$  nm).  
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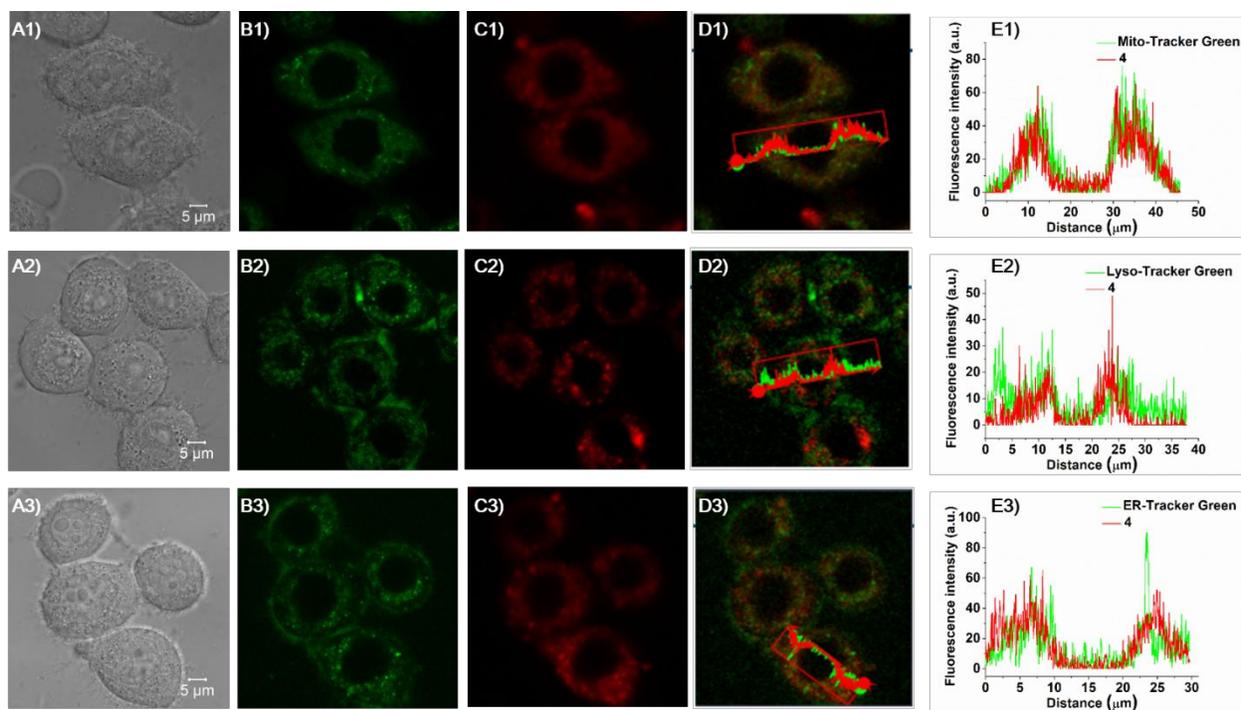
## 46 2.5. Subcellular localization.

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51 The subcellular localization of photosensitizer is critical for photodynamic effect and  
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54 plays critical role for the mode of cell death.<sup>5</sup> Since singlet oxygen has short half-life (~3.5  
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3  $\mu\text{s}$ ) and limited diffusion distance ( $< 0.02 \mu\text{m}$ ),<sup>53-55</sup> the sites where the photosensitizers  
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7 accumulated in tumor cells coincide with the photo-damaged cellular structures. Actually,  
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9  
10 some examples of subcellular-targeted photosensitizers were reported and it was well-  
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12  
13 known that subcellular localization of photosensitizers could strongly affect the cell death  
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16  
17 pathways.<sup>56</sup> Mitochondria perform critical cellular functions in regulating multiple signaling  
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20  
21 cascade reactions, such as energy production, intrinsic apoptotic mediated by caspase  
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23  
24 9/caspase 3 pathway and cell cycle regulation, thus acting as a crucial target for PDT.<sup>57</sup>  
25  
26  
27  
28 Localization of photosensitizers in mitochondria can cause *in situ* damage, maximize the  
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30  
31 killing effect of the photosensitizer, lead to the destruction of the cells' energy supply  
32  
33  
34  
35 system and ultimately cell death.<sup>58-60</sup>  
36  
37

38 To investigate the subcellular localization behavior of **4**, laser confocal microscopy  
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40  
41 studies were performed on HeLa cells incubated with **4** together with commercially  
42  
43  
44 available organelle-targeted dyes (Mito Tracker Green FM, Lyso Tracker Green, or ER  
45  
46  
47 Tracker Green) and their co-localization patterns were displayed in Fig. 4. The overlapped  
48  
49  
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51 images indicated that **4** revealed good overlap with Mito Tracker Green FM (Fig. 4D1/E1),  
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54  
55 as well as partial colocalization with Lyso Tracker Green (Fig. 4D2/E2) and ER Tracker  
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4 Green (Fig. 4D3/E3), indicating that **4** was mainly localized in mitochondria, and  
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6 distributed a part in endoplasmic reticulum and lysosomes. The subcellular localization  
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10 studies of **4** may afford reasonable explanation for its excellent phototoxicity.  
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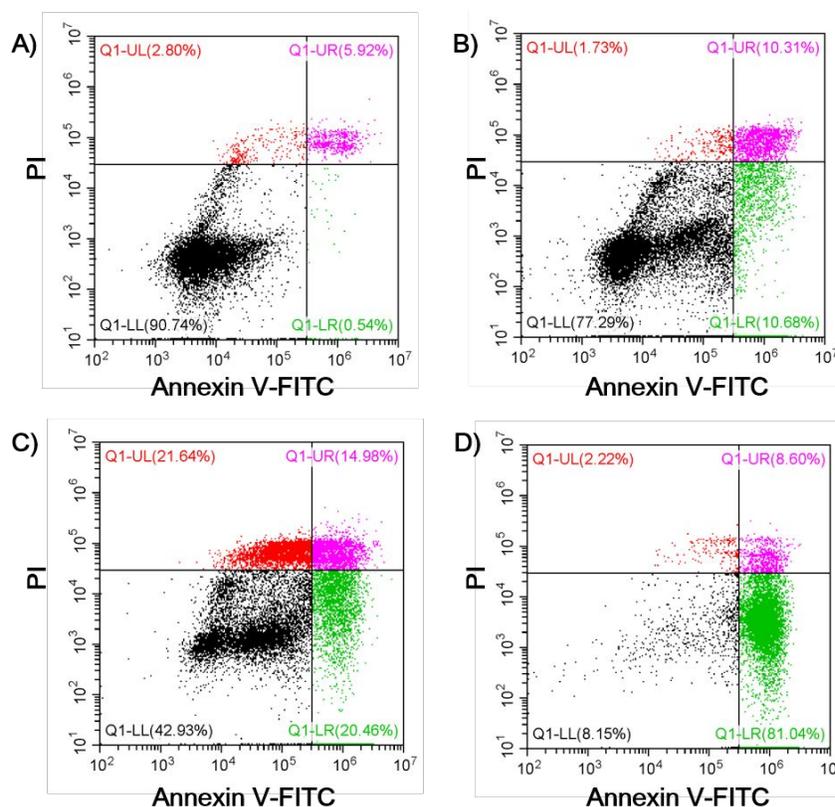


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38 **Figure 4.** Subcellular location of **4** in HeLa cells at 10  $\mu\text{M}$  for 2 h: (A1, A2, A3) bright field;  
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40 (B1) fluorescence of Mito Tracker Green (250 nM); (B2) fluorescence of Lyso Tracker  
41  
42 Green (160 nM); (B3) fluorescence of ER Tracker Green (2  $\mu\text{M}$ ); (C1, C2, C3) the  
43  
44 corresponding red fluorescence of **4**; (D1, D2, D3) the corresponding superimposed  
45  
46 images of organelle trackers and **4**; (E1, E2, E3) Luminescence intensity profiles of  
47  
48 regions of interest (ROI) across HeLa cells in (D1, D2, D3) respectively. Scale bar: 5  $\mu\text{m}$ .  
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## 54 2.6. Investigation on cell death pathway.

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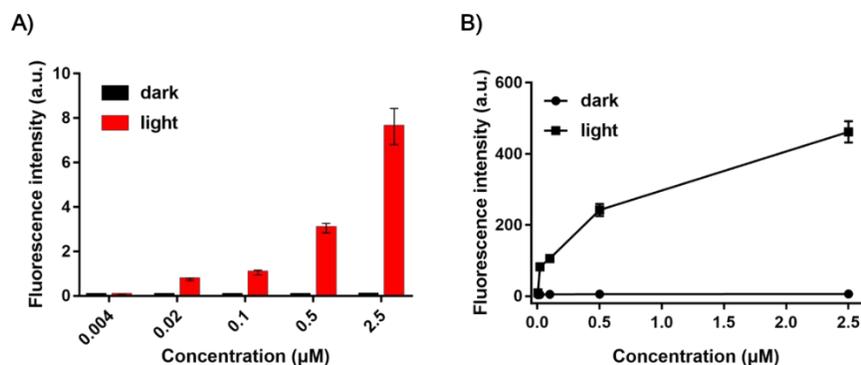
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3 Apoptosis is an important manner of cell death after illumination during photodynamic  
4 therapy.<sup>61,62</sup> In early apoptotic cells, phosphatidylserine exposes to the outside of the cell  
5  
6 membrane, which can bind to Annexin-V with high affinity. Propidium iodide (PI) is able  
7  
8 to stain nucleus of late apoptotic and necrotic cells. Since **4** located majorly in  
9  
10 mitochondria, we were looking forward to know whether apoptosis is the major way of cell  
11  
12 death. Therefore, Annexin V-FITC/PI double staining flow cytometry assay was  
13  
14 conducted to evaluate the effect caused by **4** in HeLa cells. As is shown in Fig. 5, no  
15  
16 obvious apoptotic cells was detected in control experiment wherein photosensitizers and  
17  
18 light irradiation were not treated (Fig. 5A). When the cells were incubated with 40 nM of  
19  
20 **4** and irradiated with 54 J/cm<sup>2</sup> of light ( $\lambda > 590$  nm), the proportion of apoptotic cells was  
21  
22 about 20.99% (Fig. 5B). Using 200 nM of PS and exposed to 27 J/cm<sup>2</sup> of light increased  
23  
24 the apoptosis to 35.44% (Fig. 5C). Upon increment of light dose to 54 J/cm<sup>2</sup>, the apoptosis  
25  
26 reached to 89.64% (Fig. 5D). These results demonstrated that **4** induced major death of  
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28 cells through early/late apoptosis and also resulted in some necrosis under light  
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30 irradiation.  
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**Figure 5.** Flow cytometric evaluations of **4** on cellular apoptosis in HeLa cells: (A) Control without PS and light irradiation; (B) Treatment with 40 nM of **4** for 2 h and irradiated with light dose of 54 J/cm<sup>2</sup>; (C) Incubation with 200 nM of **4** for 2 h and irradiated with light dose of 27 J/cm<sup>2</sup>; (D) Incubation with 200 nM of **4** for 2 h and irradiated with light dose of 54 J/cm<sup>2</sup>; LL (lower left quadrant): Annexin V (-) PI (-), survival cell; LR (lower right quadrant): Annexin V (+) PI (-), early apoptotic cells; UR (upper right quadrant): Annexin V (+) PI (+), late apoptotic or necrotic cells; UL (upper left quadrant): Annexin V (-) PI (+), dead cells.

## 2.7. Intracellular ROS production.

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4        Photosensitizers eradicate tumor cells by reactive oxygen species (ROS) produced by  
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7 type I and/or type II reactions, thus the produced ROS level can reflect the therapeutic  
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10 effect in photodynamic reactions.<sup>63,64</sup> We measured the production of ROS inside HeLa  
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12  
13 cells using commercially available 2',7'-dichlorodihydrofluorescein diacetate (H<sub>2</sub>DCFDA)  
14  
15  
16 as a probe. HeLa cells were incubated with various concentrations of **4** followed by  
17  
18  
19 H<sub>2</sub>DCFDA and then subjected to light irradiation. As shown in Fig. S6A–E, while  
20  
21  
22 intracellular ROS generation reflected by the green fluorescence emission exhibited a  
23  
24  
25 concentration-dependent manner ranging from 0.004 to 2.5  $\mu$ M, the ROS level in the dark  
26  
27  
28 remained low. The clear difference between light irradiation and dark conditions was  
29  
30  
31 easily caught in Fig. 6A wherein relative intensities of fluorescence emission were  
32  
33  
34 compared. Moreover, to verify the accuracy of the results, the ROS levels were also  
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36  
37 measured using the microplate reader method (Fig. 6B). The increment of fluorescence  
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39  
40 was in consistent with the results observed from confocal fluorescence studies. At a  
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42  
43 concentration of 2.5  $\mu$ M of **4**, the ROS level inside HeLa cells was about 77-fold than that  
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45  
46 of the dark control measured by microplate reader.  
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**Figure 6.** Determination of **4** induced intracellular reactive oxygen species. (A) Relative fluorescence intensities in Fig.S6. (B) Relative fluorescence intensities measured by microplate reader. Light dose: 54 J/cm<sup>2</sup> ( $\lambda > 590$  nm).

## 2.8 *In vivo* fluorescence imaging and distribution in organs for **4**.

One of the advantages of our **4** is that the PS possesses NIR fluorescence and allows *in vivo* imaging as a convenient tool to study the distribution and clearance of the PS. The *in vivo* fluorescence images of **4** were collected after intravenous injection through the tail vein at a dose of 2 mg/kg. As displayed in Fig. S7A, **4** quickly distributed to the whole-body inside living mice and gradually cleared from the major organs over 10 h, which indicated the fast uptake and reasonable clearance rate. To further characterize the distribution of **4**, mice injected with **4** were executed at various time period and imaging of major organs dissected were performed (Fig. S7B). The organ distribution studies

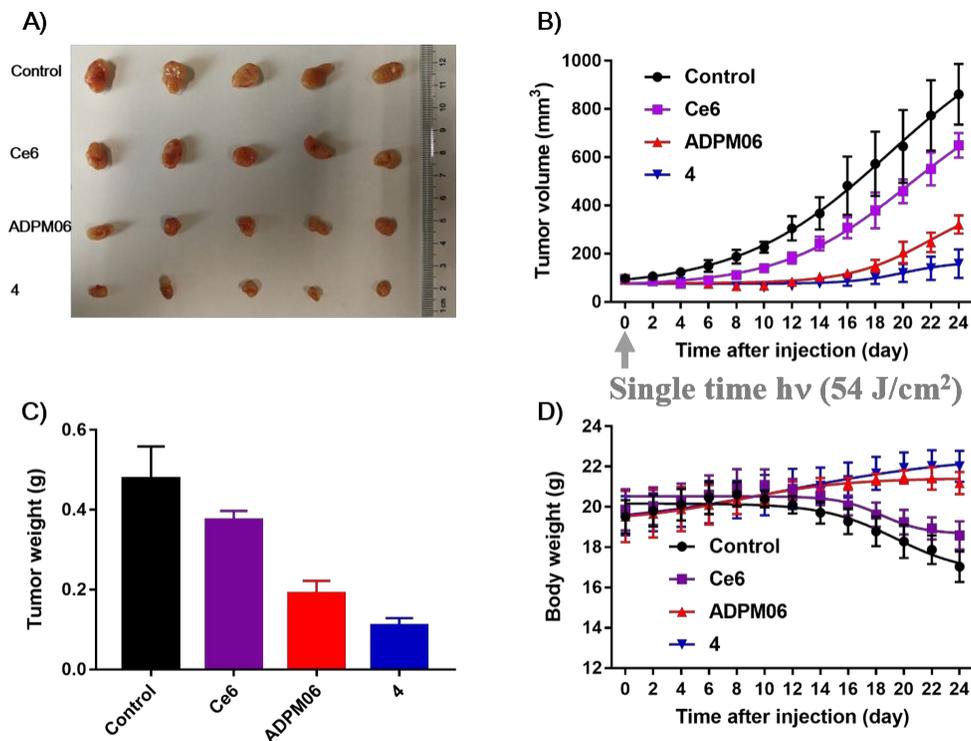
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4 turned out that the peak fluorescence intensities for liver, lungs, spleen, and kidneys were  
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6  
7 within 1 h, while the tumor and heart accumulated PS to the maximum degree around 3  
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9  
10 h. The clearance in major organs was found to be faster than that in tumor and reasonable  
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12  
13 amount of PS was retained in tumor site (Fig. S7C).  
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### 17 **2.9. *In vivo* response to 4-mediated PDT under single time light irradiation.**

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21 To further validate the anti-tumor effect of **4** in animals, HeLa tumor-bearing nude mice  
22  
23 were used for *in vivo* efficacy studies. **ADPM06** (**1** in this study) as an advanced PS of  
24  
25 aza-BODIPY type was selected for comparison and commercially **Ce6** was used as  
26  
27 porphyrin type PS standard. In most reported cases, the good *in vivo* anti-tumor  
28  
29 treatments were achieved through multiple PDT treatments.<sup>65</sup> In this paper, to testify the  
30  
31 effectiveness of PSs and reveal anti-tumor effect of our PS, only a single time irradiation  
32  
33 with light from a 75 W halogen lamp ( $\lambda > 590$  nm) was adopted after initial investigation.  
34  
35 All photosensitizers were dosed with 2 mg/kg and light energy of 54 J/cm<sup>2</sup> ( $\lambda > 590$  nm)  
36  
37 were applied for irradiation. The control groups were also exposed to 54 J/ cm<sup>2</sup> ( $\lambda > 590$   
38  
39 nm) light irradiation wherein physiological saline was used as control. As shown in Fig. 7  
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41 (A–C), the control group faced relative fast tumor growth with extend time period. The  
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3 commercial PS of **Ce6** exhibited insignificant tumor-inhibition after irradiation with 54  
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7 J/cm<sup>2</sup> of light. In contrast, **ADPM06** retarded tumor growth significantly in accordance with  
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9  
10 the reported anti-tumor ability.<sup>40</sup> To our delight, **4** was discovered to have the best efficacy  
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12  
13 with dramatically delayed tumor growth ( $p < 0.001$  compared with **ADPM06** group) under  
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15  
16 the situation wherein only a single light irradiation was performed at beginning of the  
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18  
19 investigation and no other treatment was provided over the investigated time period. Body  
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21  
22 weight measurements suggested that **4** and **ADPM06** group all performed steady  
23  
24  
25 increase which is better than control group. However, **Ce6** group showed body loss at the  
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28 late stage of the investigated period and the control group encountered great body weight  
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32 decrease along with time extended (Fig. 7D).  
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**Figure 7.** Evaluation of tumor growth inhibition in HeLa-tumor bearing xenograft model mediated by PDT. (A) Dissected tumor images after 24 days; (B) Tumor growth curves; (C) Isolated tumor weight; (D) Nude mice body weight change curves. Light irradiation was only performed at day 0 for all groups with light dose of 54 J/cm<sup>2</sup> ( $\lambda > 590$  nm from a 75 W halogen lamp with energy power of 90 mW/cm<sup>2</sup> for 10 min) on the tumor site without anaesthetization.

### 3. CONCLUSION

In summary, through systematic evaluation of halogen substitution on aza-BODIPY, we have found that moniodo-derived aza-BODIPYs possess better efficacy than

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3 monobromo, dibromo-, diiodo-, or mixed halogenated aza-BODIPY PSs. We have  
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6 identified monoiodinated aza-BODIPY dyes (**4**, **15**) containing *p*-methoxyphenyl moiety  
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8  
9 as efficient NIR aza-BODIPY type PSs. **4** with intense NIR absorption, high  $^1\text{O}_2$   
10  
11 generation capability, low dark-toxicity and good thermal/photo stability was selected to  
12  
13 investigate cell-death pathway, subcellular distribution, *in vitro* ROS generation to provide  
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15 the key information for such a NIR PS. *In vivo* whole-body fluorescence imaging and *ex*  
16  
17  
18 *vivo* organ distribution studies suggested that **4** behaved as very nice NIR PS with  
19  
20 reasonable clearance and tumor accumulation. *In vivo* efficacy studies indicated that even  
21  
22 with low drug dose of 2 mg/kg and light dose of 54 J/cm<sup>2</sup> with single time irradiation, the  
23  
24 tumor growth was dramatically inhibited without causing body weight loss. Our non-  
25  
26 porphyrin PS **4** possessed advantages of ideal PS and led to better efficacy than **ADPM06**  
27  
28 and **Ce6**. We hope that through systemic investigations examined, we have demonstrated  
29  
30 that monoiodo aza-BODIPY PS provides overall better PDT behavior and improved  
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32 stability and dark toxicity over dibromo, diiodo, and mixed halogenated PS. Our studies  
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34 may encourage scientific community to open a new way to develop powerful NIR PS.  
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#### 55 56 **4. EXPERIMENTAL SECTION**

#### 4.1. Chemistry and experimental instruments.

All chemical reagents were of analytical grade and purchased from commercial sources and used without further purification. Anhydrous solvents were acquired by standard methods prior to use. TLC analysis was performed on silica gel plates GF254 and chromatography was carried out on 200–300 mesh silica gel (Qingdao Haiyang Chemical, China).  $^1\text{H}$ -NMR spectra were measured on a Varian Model Mercury 400 MHz or 600 MHz spectrometer.  $^{13}\text{C}$ -NMR spectra were performed on a Varian Model Mercury 150 MHz spectrometer. Chemical shifts ( $\delta$ ) were expressed in ppm (parts per million) and coupling constant ( $J$ ) were recorded in hertz (Hz). HRMS (high resolution mass spectrometry, DART positive) spectra were obtained on Thermo Fisher Scientific LTQ FT Ultra. Purity of the compounds were analyzed using high-performance liquid chromatography (HPLC) (Agilent Technologies 1200 Infinity) at 1 mL/min on a C18 column (Agilent 5 HC-C18, 250  $\times$  4.6 mm). Compounds were first dissolved in THF/ $\text{CH}_3\text{CN}$  (1:9, volume ratio) and pure  $\text{CH}_3\text{CN}$  was used as the mobile phase. All compounds displayed purity of more than 95%.

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3 The pyrrole derivatives and aza-BODIPY dyes (**A~G**) were synthesized according to  
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7 the procedure reported.<sup>51</sup>  
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10 *General procedure to synthesize non-symmetric aza-BODIPY dyes:*

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14 The first molecular pyrrole derivative (0.1 mmol) was added to glacial acetic acid (1  
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17 mL), then sodium nitrite (6.9 mg, 0.1 mmol) was slowly added and stirred for 15 min. The  
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21 color of the solution changed from colorless to brown, then to green, and finally brown  
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23  
24 was observed. Then, the second pyrrole derivative (0.1 mmol) was added, followed by  
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28 acetic anhydride (0.4 ml), the reaction mixture immediately turned green, stirring was  
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31 continued for 30 min at room temperature, then warmed to 80 °C for 30 min. The reaction  
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35 was monitored by TLC until the complete consumption of the starting material. The  
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39 reaction solution was cooled to room temperature, quenched by the addition of ice water,  
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42 the precipitated blue dye was filtered, and the filter cake was rinsed with water and dried.  
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46 The filter cake was chromatographed on a neutral alumina column, dichloromethane was  
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49 used as an eluent, and the solvent was evaporated to dryness.  
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52 The resulted residue was dissolved in 1,2-dichloroethane, triethylamine (0.24 ml) was  
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56 added, then a solution of boron trifluoride diethyl ether (0.24 ml) was added and reacted  
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3 at room temperature for 30 min and then 80 °C for 30 min. The reaction solution was  
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6 cooled to room temperature, crushed ice was added, extracted with CH<sub>2</sub>Cl<sub>2</sub> and water,  
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9  
10 and the organic layer was combined, washed with saturated brine, dried over anhydrous  
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12  
13 sodium sulfate. The obtained organic phase was concentrated and purified by column  
14  
15  
16 chromatography with CH<sub>2</sub>Cl<sub>2</sub> as eluent. The product was recrystallized from  
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21 CH<sub>2</sub>Cl<sub>2</sub>/hexane.  
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23  
24 *General procedure to synthesize dihalogenated aza-BODIPY photosensitizers:*  
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27  
28 Aza-BODIPY dye (0.036 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 ml), acetic acid (2 ml) was  
29  
30  
31 added, followed by the addition of NBS or NIS (0.079 mmol) and allowed to react at room  
32  
33  
34 temperature for 30 min. The mixture were washed with sodium sulfate aqueous and  
35  
36  
37 CH<sub>2</sub>Cl<sub>2</sub> for three times, and the organic phases were combined and separated by column  
38  
39  
40 chromatography with CH<sub>2</sub>Cl<sub>2</sub> as eluent. The product was recrystallized from  
41  
42  
43  
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45 CH<sub>2</sub>Cl<sub>2</sub>/petroleum ether (1/1) to give the final product. For **5**, **4** was used as starting  
46  
47  
48 material.  
49

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52 *BF<sub>2</sub> Chelate of [4-bromo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][4-bromo-5-(4-*  
53  
54  
55 *methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine (1)*,<sup>38</sup> coppery solid, yield 91.3%. <sup>1</sup>H  
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57

1  
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3  
4 NMR (400 MHz, CDCl<sub>3</sub>) δ 7.84 (dd, *J* = 6.9, 2.8 Hz, 4H), 7.80 – 7.73 (m, 4H), 7.43 (dd, *J*  
5  
6  
7 = 5.2, 2.0 Hz, 6H), 7.01 – 6.94 (m, 4H), 3.85 (s, 6H).  
8  
9

10  
11 *BF<sub>2</sub> Chelate of [4-iodo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][4-iodo-5-(4-*  
12  
13 *methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine (2)*, coppery solid, yield 90.4%. <sup>1</sup>H NMR  
14  
15 (400 MHz, CDCl<sub>3</sub>) δ 7.82 – 7.76 (m, 4H), 7.70 (d, *J* = 8.5 Hz, 4H), 7.43 (t, *J* = 3.3 Hz, 6H),  
16  
17  
18 7.01 – 6.95 (m, 4H), 3.86 (s, 6H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 161.60, 160.47, 147.92,  
19  
20  
21 145.02, 132.41, 131.96, 130.73, 129.40, 127.86, 123.31, 113.47, 83.04, 55.29. HRMS  
22  
23  
24 (DART) *m/z*. 809.0121 [M + H]<sup>+</sup>, calcd for C<sub>34</sub>H<sub>25</sub>BF<sub>2</sub>I<sub>2</sub>N<sub>3</sub>O<sub>2</sub> 809.0128.  
25  
26  
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31  
32 *BF<sub>2</sub> Chelate of [4-bromo-3-phenyl-5-(4-tolyl)-1H-pyrrol-2-yl][4-bromo-3-phenyl-5-(4-*  
33  
34 *tolyl)-pyrrol-2-ylidene]amine (6)*, coppery solid, yield 93.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  
35  
36 δ 7.90 – 7.83 (m, 4H), 7.66 (d, *J* = 8.0 Hz, 4H), 7.45 (dd, *J* = 5.0, 1.9 Hz, 6H), 7.28 (d, *J*  
37  
38 = 8.4 Hz, 4H), 2.41 (s, 6H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 157.73, 143.61, 142.10, 140.71,  
39  
40  
41  
42 130.13, 130.01, 129.72, 128.91, 128.16, 127.37, 125.96, 109.64, 21.08. HRMS (DART)  
43  
44  
45  
46  
47  
48  
49 *m/z*. 681.0495 [M + H]<sup>+</sup>, calcd for C<sub>34</sub>H<sub>25</sub>BBr<sub>2</sub>F<sub>2</sub>N<sub>3</sub> 681.0507.  
50  
51

52  
53 *BF<sub>2</sub> Chelate of [4-iodo-3-phenyl-5-(4-tolyl)-1H-pyrrol-2-yl][4-iodo-3-phenyl-5-(4-tolyl)-*  
54  
55 *pyrrol-2-ylidene]amine (7)*, coppery solid, yield 87.1%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78  
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57  
58  
59  
60

1  
2  
3 (dd,  $J = 6.8, 2.9$  Hz, 4H), 7.56 (d,  $J = 7.9$  Hz, 4H), 7.42 (t,  $J = 3.3$  Hz, 6H), 7.25 (s, 4H),  
4  
5  
6  
7 2.39 (s, 6H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  148.05, 141.09, 131.88, 130.71, 130.31,  
8  
9  
10 129.47, 128.69, 128.14, 127.88, 21.71. HRMS (DART)  $m/z$ : 777.0237  $[\text{M} + \text{H}]^+$ , calcd for  
11  
12  
13  
14  $\text{C}_{34}\text{H}_{25}\text{BF}_2\text{I}_2\text{N}_3$  777.0230.

15  
16  
17 *BF<sub>2</sub> Chelate of [4-iodo-5-(5-methylthiophen-2-yl)-3-phenyl-1H-pyrrol-2-yl][4-iodo-5-(5-*  
18  
19  
20 *methylthiophen-2-yl)-3-phenylpyrrol-2-ylidene]amine (10)*, coppery solid, yield 91.5%.  $^1\text{H}$   
21  
22  
23  
24 NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.86 (d,  $J = 3.8$  Hz, 2H), 7.69 (dd,  $J = 6.6, 3.0$  Hz, 4H), 7.42 –  
25  
26  
27 7.35 (m, 6H), 6.90 (d,  $J = 3.0$  Hz, 2H), 2.58 (s, 6H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  151.87,  
28  
29  
30  
31 147.77, 147.43, 144.62, 135.25, 131.43, 130.19, 128.63, 128.45, 127.12, 126.22, 82.30,  
32  
33  
34  
35 15.09. HRMS (DART)  $m/z$ : 788.9361  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{30}\text{H}_{21}\text{BF}_2\text{I}_2\text{N}_3\text{S}_2$  788.9358.

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37  
38 *General procedure to synthesize monohalogenated aza-BODIPY photosensitizers:*  
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41  
42 Aza-BODIPY dye (0.036 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (6 ml), acetic acid (2 ml) was  
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44  
45 added, followed by the addition of NBS or NIS (0.036 mmol) and allowed to react at room  
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48 temperature for 30 min. The mixture were extracted with sodium sulfate aqueous and  
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52  $\text{CH}_2\text{Cl}_2$  for three times, and the organic phases were combined and separated by column  
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1  
2  
3 chromatography with CH<sub>2</sub>Cl<sub>2</sub> as eluent. The product was recrystallized from  
4  
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6  
7 CH<sub>2</sub>Cl<sub>2</sub>/petroleum ether (1/1) to give the final product.  
8  
9

10 *BF<sub>2</sub> Chelate of [4-bromo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(4-*  
11  
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13  
14 *methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine (3)*, coppery solid, yield 93.5%. <sup>1</sup>H NMR  
15  
16  
17 (400 MHz, CDCl<sub>3</sub>) δ 8.10 – 8.00 (m, 4H), 7.89 (d, *J* = 7.0 Hz, 2H), 7.77 (d, *J* = 8.7 Hz,  
18  
19  
20 2H), 7.48 (dt, *J* = 13.7, 6.9 Hz, 3H), 7.41 – 7.35 (m, 3H), 7.11 (s, 1H), 7.02 (d, *J* = 8.8 Hz,  
21  
22  
23 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 3.87 (s, 3H), 3.85 (s, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ  
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25  
26 162.26, 161.24, 160.44, 152.67, 146.29, 144.69, 141.90, 139.11, 131.76, 131.66, 131.62,  
27  
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29 131.01, 130.83, 130.21, 129.23, 128.64, 128.28, 127.99, 127.28, 122.55, 121.98, 119.11,  
30  
31  
32 113.86, 112.82, 107.67, 54.88, 54.64, 28.70. HRMS (DART) *m/z*: 635.1300 [M + H]<sup>+</sup>,  
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37  
38 calcd for C<sub>34</sub>H<sub>26</sub>BBrF<sub>2</sub>N<sub>3</sub>O<sub>2</sub> 635.1300.  
39  
40

41 *BF<sub>2</sub> Chelate of [4-iodo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(4-*  
42  
43  
44  
45 *methoxyphenyl)-3-phenylpyrrol-2-ylidene]amine (4)*, coppery solid, yield 89.2%. <sup>1</sup>H NMR  
46  
47  
48 (400 MHz, CDCl<sub>3</sub>) δ 8.09 – 7.99 (m, 4H), 7.86 – 7.80 (m, 2H), 7.70 (d, *J* = 8.6 Hz, 2H),  
49  
50  
51 7.54 – 7.42 (m, 3H), 7.37 (dd, *J* = 5.2, 1.9 Hz, 3H), 7.12 (s, 1H), 7.04 – 6.99 (m, 2H), 6.98  
52  
53  
54 – 6.93 (m, 2H), 3.88 (s, 3H), 3.85 (s, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 162.28, 161.28,  
55  
56  
57  
58  
59  
60

1  
2  
3 160.34, 155.70, 146.19, 144.80, 143.95, 142.83, 132.08, 131.81, 131.67, 131.63, 131.60,  
4  
5  
6  
7 130.99, 130.23, 129.23, 128.62, 128.25, 127.98, 127.17, 123.49, 122.53, 119.24, 113.87,  
8  
9  
10 112.69, 80.03, 54.87, 54.63, 29.07, 28.70. HRMS (DART)  $m/z$ : 683.1158 [M + H]<sup>+</sup>, calcd  
11  
12  
13  
14 for C<sub>34</sub>H<sub>26</sub>BF<sub>2</sub>IN<sub>3</sub>O<sub>2</sub> 683.1162.

15  
16  
17 *BF<sub>2</sub> Chelate of [4-bromo-3-phenyl-5-(4-tolyl)-1H-pyrrol-2-yl][3-phenyl-5-(4-tolyl)-pyrrol-*  
18  
19  
20  
21 *2-ylidene]amine (8)*, coppery solid, yield 90.4%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.05 (dd, *J*  
22  
23 = 6.7, 3.0 Hz, 2H), 7.93 (dd, *J* = 13.3, 7.6 Hz, 4H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.49 (dd, *J* =  
24  
25 = 6.7, 3.0 Hz, 2H), 7.93 (dd, *J* = 13.3, 7.6 Hz, 4H), 7.69 (d, *J* = 7.6 Hz, 2H), 7.49 (dd, *J* =  
26  
27 12.2, 6.9 Hz, 3H), 7.44 – 7.36 (m, 3H), 7.34 – 7.23 (m, 4H), 7.10 (s, 1H), 2.44 (s, 3H),  
28  
29 2.40 (s, 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 161.94, 154.08, 146.19, 145.00, 142.23,  
30  
31 142.08, 139.90, 139.83, 131.02, 130.69, 130.22, 129.85, 129.30, 129.27, 128.96, 128.67,  
32  
33 128.45, 128.08, 128.02, 127.46, 127.32, 126.62, 119.19, 108.02, 29.08, 28.71, 21.08.  
34  
35  
36  
37  
38  
39  
40  
41  
42 HRMS (DART)  $m/z$ : 603.1395 [M + H]<sup>+</sup>, calcd for C<sub>34</sub>H<sub>26</sub>BBrF<sub>2</sub>N<sub>3</sub> 603.1402.

43  
44  
45 *BF<sub>2</sub> Chelate of [4-iodo-3-phenyl-5-(4-tolyl)-1H-pyrrol-2-yl][3-phenyl-5-(4-tolyl)-pyrrol-2-*  
46  
47  
48  
49 *ylidene]amine (9)*, coppery solid, yield 87.5%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.05 – 7.97  
50  
51 (m, 2H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.86 – 7.80 (m, 2H), 7.60 (d, *J* = 7.9 Hz, 2H), 7.53 –  
52  
53 7.41 (m, 3H), 7.36 (dd, *J* = 5.3, 1.9 Hz, 3H), 7.28 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 4.9 Hz,  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 2H), 7.08 (s, 1H), 2.42 (s, 3H), 2.37 (s, 3H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  161.89, 157.13,  
5  
6  
7 146.04, 145.06, 144.75, 143.17, 142.07, 139.73, 131.91, 131.01, 130.23, 129.90, 129.28,  
8  
9  
10 129.25, 128.96, 128.65, 128.41, 128.14, 128.01, 127.99, 127.47, 127.21, 119.29, 80.37,  
11  
12  
13  
14 21.08. HRMS (DART)  $m/z$ : 651.1259  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{34}\text{H}_{26}\text{BF}_2\text{IN}_3$  651.1263.

15  
16  
17 *BF<sub>2</sub> Chelate of [4-iodo-5-(5-methylthiophen-2-yl)-3-phenyl-1H-pyrrol-2-yl][5-(5-*  
18  
19  
20  
21 *methylthiophen-2-yl)-3-phenylpyrrol-2-ylidene]amine (11)*, coppery solid, yield 83.1%.  $^1\text{H}$   
22  
23  
24 NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.22 (d,  $J$  = 4.0 Hz, 1H), 8.04 – 7.98 (m, 2H), 7.78 (dt,  $J$  = 6.1,  
25  
26  
27 1.5 Hz, 2H), 7.73 (d,  $J$  = 3.7 Hz, 1H), 7.53 – 7.42 (m, 3H), 7.37 (dt,  $J$  = 5.6, 3.3 Hz, 3H),  
28  
29  
30  
31 7.18 (s, 1H), 6.97 – 6.91 (m, 2H), 2.62 (s, 3H), 2.60 (s, 3H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  
32  
33  
34  $\delta$  153.42, 151.56, 147.90, 147.15, 145.71, 144.75, 144.05, 143.51, 136.58, 133.88,  
35  
36  
37  
38 132.91, 131.29, 131.12, 130.96, 129.90, 129.81, 129.57, 129.14, 128.70, 128.59, 127.70,  
39  
40  
41  
42 126.17, 119.92, 16.15, 15.66. HRMS (DART)  $m/z$ : 663.0384  $[\text{M} + \text{H}]^+$ , calcd for  
43  
44  
45  $\text{C}_{30}\text{H}_{22}\text{BF}_2\text{IN}_3\text{S}_2$  663.0392.

46  
47  
48  
49 *BF<sub>2</sub> Chelate of [4-bromo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-7-*  
50  
51  
52 *methoxy-3-phenylethylbenzo[g]indole-2-ylidene]amine (12)*, coppery solid, yield 87.4%.  
53  
54  
55  
56  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (d,  $J$  = 9.1 Hz, 1H), 7.87 (dt,  $J$  = 6.4, 1.4 Hz, 2H), 7.81  
57  
58  
59  
60

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2  
3  
4 – 7.72 (m, 2H), 7.72 – 7.64 (m, 2H), 7.49 – 7.34 (m, 6H), 7.07 – 7.00 (m, 2H), 6.90 (dd,  $J$   
5  
6 = 9.0, 2.7 Hz, 1H), 6.81 (d,  $J$  = 2.6 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 2.94 (m, 4H).  $^{13}\text{C}$   
7  
8  
9  
10 NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  163.17, 159.95, 158.06, 149.07, 147.34, 145.41, 140.95,  
11  
12  
13  
14 139.21, 136.42, 133.16, 132.65, 131.75, 131.20, 130.58, 130.06, 129.67, 128.30, 127.72,  
15  
16  
17 127.58, 127.14, 122.60, 118.56, 114.02, 112.97, 112.71, 105.93, 54.97, 54.61, 29.73,  
18  
19  
20  
21 21.37. HRMS (DART)  $m/z$ : 661.1451  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{36}\text{H}_{28}\text{BBrF}_2\text{N}_3\text{O}_2$  661.1457.

22  
23  
24 *BF<sub>2</sub> Chelate of [4-bromo-5-(4-tolyl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-7-methoxy-3-*  
25  
26  
27  
28 *phenylethylbenzo[g]indole-2-ylidene]amine (13)*, coppery solid, yield 93.6%.  $^1\text{H}$  NMR  
29  
30  
31 (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.61 (d,  $J$  = 9.1 Hz, 1H), 7.87 (dt,  $J$  = 6.6, 1.4 Hz, 2H), 7.71 – 7.64  
32  
33  
34 (m, 4H), 7.47 – 7.35 (m, 6H), 7.32 (d,  $J$  = 7.9 Hz, 2H), 6.89 (dd,  $J$  = 9.0, 2.7 Hz, 1H), 6.81  
35  
36  
37 (d,  $J$  = 2.6 Hz, 1H), 3.87 (s, 3H), 2.94 (dt,  $J$  = 7.4, 3.2 Hz, 4H), 2.45 (s, 3H).  $^{13}\text{C}$  NMR (151  
38  
39  
40  
41 MHz,  $\text{CDCl}_3$ )  $\delta$  163.27, 158.49, 149.03, 147.49, 145.51, 140.81, 139.39, 138.91, 136.17,  
42  
43  
44  
45 133.32, 132.90, 132.83, 132.75, 131.22, 130.53, 130.04, 129.67, 128.34, 127.98, 127.69,  
46  
47  
48 127.59, 127.45, 127.14, 118.48, 114.03, 113.01, 105.70, 54.97, 29.71, 21.39, 21.06.  
49  
50  
51  
52 HRMS (DART)  $m/z$ : 645.1501  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{36}\text{H}_{28}\text{BBrF}_2\text{N}_3\text{O}$  645.1508.  
53  
54  
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59  
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4 *BF<sub>2</sub> Chelate of [4-bromo-5-(5-methylthiophen-2-yl)-3-phenyl-1H-pyrrol-2-yl][4,5-*  
5  
6  
7 *Dihydro-7-methoxy-3-phenylethylbenzo[g]indole-2-ylidene]amine (14)*, coppery solid,  
8  
9  
10  
11 yield 89.4%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.74 (d, *J* = 9.2 Hz, 1H), 7.85 – 7.78 (m, 3H),  
12  
13  
14 7.71 – 7.65 (m, 2H), 7.48 – 7.35 (m, 6H), 6.98 (dd, *J* = 9.2, 2.7 Hz, 1H), 6.92 (d, *J* = 3.7  
15  
16  
17 Hz, 1H), 6.84 (d, *J* = 2.6 Hz, 1H), 3.91 (q, *J* = 2.1, 1.4 Hz, 3H), 2.96 (s, 4H), 2.61 (s, 3H).  
18  
19  
20  
21 <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 163.74, 158.29, 148.11, 146.05, 145.30, 142.75, 141.90,  
22  
23  
24 139.46, 137.69, 133.83, 133.34, 133.20, 131.65, 131.18, 130.78, 130.26, 128.89, 128.41,  
25  
26  
27 128.20, 127.72, 126.18, 119.31, 114.66, 113.67, 55.63, 30.39, 22.01, 15.61. HRMS  
28  
29  
30  
31 (DART) *m/z*. 651.1063 [M + H]<sup>+</sup>, calcd for C<sub>34</sub>H<sub>26</sub>BBrF<sub>2</sub>N<sub>3</sub>OS 651.1072.  
32  
33

34  
35 *BF<sub>2</sub> Chelate of [4-iodo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-7-*  
36  
37  
38 *methoxy-3-phenylethylbenzo[g]indole-2-ylidene]amine (15)*, coppery solid, yield 85.4%.  
39  
40  
41  
42 <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.61 (d, *J* = 9.0 Hz, 1H), 7.86 – 7.78 (m, 2H), 7.75 – 7.63  
43  
44  
45 (m, 4H), 7.53 – 7.34 (m, 6H), 7.09 – 7.01 (m, 2H), 6.91 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.82 (d,  
46  
47  
48 *J* = 2.6 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 2.94 (q, *J* = 4.4, 3.8 Hz, 4H). <sup>13</sup>C NMR (151  
49  
50  
51 MHz, CDCl<sub>3</sub>) δ 163.18, 159.89, 158.06, 152.32, 147.20, 145.41, 141.87, 141.02, 139.27,  
52  
53  
54  
55 133.29, 132.68, 132.38, 131.85, 130.57, 130.13, 129.65, 128.28, 127.71, 127.57, 127.03,  
56  
57  
58  
59  
60

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3  
4 124.14, 118.54, 114.02, 112.97, 112.59, 78.08, 54.96, 54.60, 29.72, 21.41. HRMS  
5  
6  
7 (DART)  $m/z$ . 709.1309 [M + H]<sup>+</sup>, calcd for C<sub>36</sub>H<sub>28</sub>BF<sub>2</sub>IN<sub>3</sub>O<sub>2</sub> 709.1318.  
8  
9

10 *BF<sub>2</sub> Chelate of [4-iodo-5-(4-tolyl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-7-methoxy-3-*  
11 *phenylethylbenzo[g]indole-2-ylidene]amine (16)*, coppery solid, yield 89.0%. <sup>1</sup>H NMR  
12  
13  
14  
15  
16  
17 (400 MHz, CDCl<sub>3</sub>) δ 8.59 (d,  $J$  = 9.1 Hz, 1H), 7.84 – 7.78 (m, 2H), 7.68 – 7.63 (m, 2H),  
18  
19  
20  
21 7.61 (d,  $J$  = 7.9 Hz, 2H), 7.47 – 7.35 (m, 6H), 7.32 (d,  $J$  = 7.9 Hz, 2H), 6.88 (dd,  $J$  = 9.0,  
22  
23  
24 2.7 Hz, 1H), 6.80 (d,  $J$  = 2.6 Hz, 1H), 3.86 (s, 3H), 2.94 (td,  $J$  = 7.4, 4.7 Hz, 4H), 2.46 (s,  
25  
26  
27 3H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>) δ 163.27, 158.42, 152.35, 147.33, 145.49, 141.73,  
28  
29  
30  
31 140.75, 139.41, 138.82, 133.42, 132.84, 132.76, 132.38, 130.53, 130.13, 129.66, 129.00,  
32  
33  
34  
35 128.32, 127.89, 127.68, 127.58, 127.03, 118.48, 114.02, 113.01, 77.75, 54.97, 29.71,  
36  
37  
38 28.70, 21.42, 21.08. HRMS (DART)  $m/z$ . 693.1356 [M + H]<sup>+</sup>, calcd for C<sub>36</sub>H<sub>28</sub>BF<sub>2</sub>IN<sub>3</sub>O  
39  
40  
41 693.1369.  
42  
43  
44

45 *BF<sub>2</sub> Chelate of [4-iodo-5-(5-methylthiophen-2-yl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-*  
46 *7-methoxy-3-phenylethylbenzo[g]indole-2-ylidene]amine (17)*, coppery solid, yield  
47  
48  
49 87.2%. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.71 (d,  $J$  = 9.1 Hz, 1H), 7.79 – 7.73 (m, 2H), 7.68 –  
50  
51  
52 7.63 (m, 2H), 7.59 (d,  $J$  = 3.7 Hz, 1H), 7.47 – 7.36 (m, 6H), 6.96 (dd,  $J$  = 9.0, 2.7 Hz, 1H),  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 6.90 (dd,  $J = 3.7, 1.1$  Hz, 1H), 6.83 (d,  $J = 2.6$  Hz, 1H), 3.90 (s, 3H), 2.97 – 2.93 (m, 4H),

5  
6  
7 2.61 (d,  $J = 1.1$  Hz, 3H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )  $\delta$  163.92, 158.80, 148.17, 146.18,

8  
9  
10 145.85, 144.54, 142.63, 142.16, 139.75, 134.11, 133.48, 133.05, 131.14, 130.85, 130.27,

11  
12  
13 130.07, 129.89, 128.93, 128.35, 128.20, 127.62, 125.88, 55.63, 30.37, 22.07, 15.61.

14  
15  
16  
17 HRMS (DART)  $m/z$ : 699.0923  $[\text{M} + \text{H}]^+$ , calcd for  $\text{C}_{34}\text{H}_{26}\text{BF}_2\text{IN}_3\text{OS}$  699.0933.

18  
19  
20  
21 *BF<sub>2</sub> Chelate of [4-iodo-5-(thiophen-2-yl)-3-phenyl-1H-pyrrol-2-yl][4,5-Dihydro-7-*  
22  
23  
24 *methoxy-3-phenylethylbenzo[g]indole-2-ylidene]amine (18)*, coppery solid, yield 83.4%.

25  
26  
27  
28  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.70 (d,  $J = 9.1$  Hz, 1H), 7.78 (dt,  $J = 6.4, 1.4$  Hz, 2H), 7.71

29  
30  
31 (dd,  $J = 3.8, 1.2$  Hz, 1H), 7.66 (dd,  $J = 7.6, 2.1$  Hz, 2H), 7.61 (dd,  $J = 5.1, 1.2$  Hz, 1H),

32  
33  
34 7.48 – 7.36 (m, 6H), 7.23 (dd,  $J = 5.1, 3.7$  Hz, 1H), 6.95 (dd,  $J = 9.0, 2.7$  Hz, 1H), 6.83 (d,

35  
36  
37  $J = 2.6$  Hz, 1H), 3.90 (s, 3H), 2.96 (td,  $J = 6.8, 4.5$  Hz, 4H).  $^{13}\text{C}$  NMR (151 MHz,  $\text{CDCl}_3$ )

38  
39  
40  $\delta$  164.21, 159.72, 148.52, 146.49, 144.95, 142.36, 141.43, 140.12, 134.50, 133.74,

41  
42  
43 133.00, 132.54, 132.38, 131.00, 130.82, 130.28, 129.05, 128.99, 128.33, 128.23, 127.64,

44  
45  
46  
47 127.05, 119.02, 114.73, 113.79, 55.66, 30.33, 22.09. HRMS (DART)  $m/z$ : 685.0771  $[\text{M} +$

48  
49  
50  
51  
52  $\text{H}]^+$ , calcd for  $\text{C}_{33}\text{H}_{24}\text{BF}_2\text{IN}_3\text{OS}$  685.0777.

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3  
4 *Synthetic procedure for mixed halogenated aza-BODIPY photosensitizers BF<sub>2</sub> Chelate*  
5  
6  
7 *of [4-bromo-5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][4-iodo-5-(4-methoxyphenyl)-*  
8  
9  
10 *3-phenylpyrrol-2-ylidene]amine (5):*

11  
12  
13  
14 **4** (0.036 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (6 ml), acetic acid (2 ml) was added, followed  
15  
16  
17 by the addition of NBS (0.036 mmol) and allowed to react at room temperature for 30 min.

18  
19  
20  
21 The mixture were washed with sodium sulfate aqueous and CH<sub>2</sub>Cl<sub>2</sub> for three times, and  
22  
23  
24 the organic phases were combined and separated by column chromatography with

25  
26  
27  
28 CH<sub>2</sub>Cl<sub>2</sub> as eluent. The product was recrystallized from CH<sub>2</sub>Cl<sub>2</sub>/petroleum ether (1/1) to  
29  
30  
31 give the final product (coppery solid, yield 88.7%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.89 –

32  
33  
34  
35 7.67 (m, 8H), 7.47 – 7.41 (m, 6H), 7.02 – 6.97 (m, 4H), 3.89 – 3.85 (m, 6H). <sup>13</sup>C NMR (151  
36  
37  
38 MHz, CDCl<sub>3</sub>) δ 161.72, 161.55, 160.31, 157.62, 147.71, 145.08, 144.07, 142.66, 132.43,

39  
40  
41  
42 131.94, 130.74, 129.46, 129.41, 127.96, 127.87, 123.26, 121.76, 113.59, 113.44, 55.32,  
43  
44  
45 55.30, 53.43. HRMS (DART) *m/z*. 761.0268 [M + H]<sup>+</sup>, calcd for C<sub>34</sub>H<sub>25</sub>BBrF<sub>2</sub>IN<sub>3</sub>O<sub>2</sub>

46  
47  
48  
49 761.0267.

#### 50 51 52 **4.2 Absorption and emission spectra.**

53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 The absorption and emission spectra of **4** in various reagent were recorded on a 759S  
4  
5  
6  
7 UV-visible spectrophotometer (Lengguang Tech, China) and F98 fluorescence  
8  
9  
10 spectrophotometer (Lengguang Tech, China). All the measurements were carried out at  
11  
12  
13  
14 room temperature.  
15  
16

### 17 4.3. Fluorescence quantum yield.

18  
19  
20  
21 Fluorescence quantum yields ( $\Phi_f$ ) were measured as reported and calculated by the  
22  
23  
24 equation:  $\Phi_{f(s)} = (F_s/F_{ref}) \cdot (n_s^2/n_{ref}^2) \cdot (A_{ref}/A_s) \cdot \Phi_{f(ref)}$ ,<sup>52</sup> wherein F, A and n represent the  
25  
26  
27 area under the emission peak of measured fluorescence, the absorbance at the excitation  
28  
29  
30 position (670 nm), and the refractive index of the solvent, respectively. Compound **A** was  
31  
32  
33 used as the reference in  $\text{CHCl}_3$  ( $\Phi_f = 0.36$ ). The emission spectra were obtained in very  
34  
35  
36 dilute solutions ( $\text{Abs} \leq 0.010$ ) to minimize reabsorption effect of radiation.  
37  
38  
39  
40

### 41 4.4 Singlet oxygen quantum yield.

42  
43  
44  
45 The singlet oxygen quantum yields ( $\Phi_\Delta$ ) were determined as reported by using DPBF  
46  
47  
48 as the singlet oxygen scavenger and ZnPc as the reference ( $\Phi_\Delta = 0.56$  in DMF).<sup>66</sup> The  
49  
50  
51 light source is composed of a 75 W halogen lamp, a 590 nm cutoff optical filter (Sipeida,  
52  
53  
54  
55 China) and a water tank for cooling. A solution of DPBF (50  $\mu\text{M}$ ) containing the  
56  
57  
58  
59  
60

1  
2  
3 photosensitizer (1  $\mu\text{M}$ ) in DMF was prepared in the dark and then irradiated with filtered  
4  
5  
6  
7 red light ( $\lambda > 590 \text{ nm}$ ). The maximum absorption values (411 nm) of DPBF was monitored  
8  
9  
10 along with irradiated time. The  $\Phi_{\Delta}$  values were determined according to the equation  $\Phi_{\Delta(s)}$   
11  
12  
13  $= (K_s/K_{\text{ref}}) \cdot (A_{\text{ref}}/A_s) \cdot \Phi_{\Delta(\text{ref})}$ , where  $\Phi_{\Delta(\text{ref})}$  is the singlet oxygen quantum yield of reference  
14  
15  
16  
17 ZnPc in DMF,  $K_s$  and  $K_{\text{ref}}$  are the photobleaching rates of DPBF in the presence of the  
18  
19  
20 samples and ZnPc,  $A_s$  and  $A_{\text{ref}}$  represent the absorption areas of Q band (590–750 nm)  
21  
22  
23  
24 of the samples and ZnPc respectively. As for the relative rate of DPBF degradation,  
25  
26  
27  
28 methylene blue (1  $\mu\text{M}$ ) was used as reference in  $\text{tPrOH}$  and emulsified PBS system.  
29  
30

#### 31 **4.5. Cell lines and culture conditions.**

32  
33  
34  
35 Human cervical cancer cell line HeLa, human breast cancer cell line MCF-7 and human  
36  
37  
38 colon cell line SW480 were obtained from American Type Culture Collection (ATCC). All  
39  
40  
41  
42 cells were cultured in Dulbecco's modified Eagle's medium (GE Healthcare Life Sciences,  
43  
44  
45 USA), supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (GE  
46  
47  
48  
49 Healthcare Life Sciences, USA) in a humidified incubator at 37 °C with an atmosphere of  
50  
51  
52 5%  $\text{CO}_2$ .  
53  
54  
55

#### 56 **4.6. Cytotoxicity study.**

1  
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3  
4 The phototoxicity of photosensitizers were determined in human cervical cancer cells  
5  
6  
7 following the procedures as reported. Briefly, the photosensitizer ( $6.0 \times 10^{-6}$  mol) was  
8  
9  
10 dissolved in THF (2.0 ml) and a mixture of Cremophor EL (CrEL)/1,2-propanediol (10:3,  
11  
12  
13 v:v) (0.1 ml) was added, then the solution was placed in a sonic bath for 10 min. THF was  
14  
15  
16 removed under reduced pressure, then the mixture was dissolved in phosphate-buffered  
17  
18  
19 saline (PBS) (7 ml). The solution was then passed through a filter membrane (13 mm  $\times$   
20  
21  
22 0.22  $\mu$ m, Tansoole, China). The exact concentration of the photosensitizer was confirmed  
23  
24  
25 by UV – Visible spectral when used for assaying.  $3 \times 10^3$  cells/well were seeded on a 96-  
26  
27  
28 well culture plates (Corning Inc., USA) and allowed to adhere overnight. The cells were  
29  
30  
31 incubated with various photosensitizers of different concentrations for 3 h and then  
32  
33  
34 irradiated with a light dose of 54 J/cm<sup>2</sup> ( $\lambda > 590$  nm), followed by incubation for 24 h in a  
35  
36  
37 humidified incubator at 37 °C. After treatment, the above loading media was removed and  
38  
39  
40 then 100  $\mu$ L of MTT solution (0.5 mg/mL in PBS) was added to each well, which was then  
41  
42  
43 incubated for 4 h at 37 °C in a humidified incubator. Subsequently, the MTT-containing  
44  
45  
46 medium was removed and DMSO was added (100  $\mu$ L per well) to dissolve the formazan  
47  
48  
49 crystals. The absorbance at 490 nm was measured using a SpectraMax M5 microplate  
50  
51  
52  
53  
54  
55  
56  
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60

1  
2  
3 reader (Molecular Devices, USA). For dark toxicity, no light dose was provided and cell  
4  
5  
6  
7 viability was measured as described above.  
8  
9

#### 10 **4.7 Apoptosis assay.**

11  
12 Apoptosis assay were conducted using annexin V-FITC/PI (Beyotime Biotechnology,  
13  
14 China) double staining following the manufacturer's protocol. Briefly, HeLa cells were  
15  
16  
17 seeded in six-well plate and incubated with **4** for 3 h and then irradiated with a light dose  
18  
19  
20  
21 of 54 J/cm<sup>2</sup> ( $\lambda > 590$  nm), followed by incubation for 24 h in a humidified incubator at 37  
22  
23  
24  
25 °C. After treatment, the above loading media was removed and cells were harvested  
26  
27  
28  
29 respectively, then washed with PBS and stained with a mixture of annexin V(2  $\mu$ L) and PI  
30  
31  
32 (2  $\mu$ L) followed by incubation for 20 min at room temperature. Apoptosis was then  
33  
34  
35  
36  
37  
38 determined immediately using flow cytometry (Beckman, USA).  
39  
40

#### 41 **4.8 Subcellular localization.**

42  
43 HeLa cells were seeded on 35 mm diameter glass-bottom culture dish (NEST,  
44  
45  
46 Cat.No.801001) and allowed to adhere overnight in a humidified incubator at 37 °C under  
47  
48  
49  
50  
51  
52 5% CO<sub>2</sub> atmosphere. The medium was replaced with fresh medium containing 5  $\mu$ M  
53  
54  
55  
56 photosensitizer **4** and incubated in dark for 2 h. The medium was then removed, washed  
57  
58  
59  
60

1  
2  
3 twice with PBS, followed by incubation with Mito-Tracker Green FM (250 nM, 30 min,  
4  
5  
6  
7 Molecular Probes, Yeasen, China), ER-Tracker Green (2  $\mu$ M, 45 min, Molecular Probes,  
8  
9  
10 Yeasen, China) and Lyso-Tracker Green DND-26 (160 nM, 30 min, Molecular Probes,  
11  
12  
13 Life Technologies, USA) in a humidified incubator at 37 °C with an atmosphere of 5%  
14  
15  
16  
17 CO<sub>2</sub>. Then the trackers-containing medium were removed and washed twice with PBS,  
18  
19  
20  
21 the cells were re-fed with fresh DMEM and imaged on a confocal scanning microscope  
22  
23  
24 (Carl Zeiss LSM 710) equipped with a 488 nm argon laser and a 633 nm laser. The  
25  
26  
27  
28 excitation wavelength of all Trackers was 488 nm and their fluorescence was collected at  
29  
30  
31 500–560 nm, while **4** was excited at 633 nm and its fluorescence was monitored at  
32  
33  
34  
35 650–740 nm.

#### 38 **4.9 Investigation of intracellular ROS level.**

39  
40  
41  
42 HeLa cells were incubated with solutions of **4** with variant concentrations (0.004, 0.02,  
43  
44  
45 0.10, 0.5, 2.5  $\mu$ M) for 3 h on 35 mm diameter glass-bottom culture dish (Nest, Cat. No.  
46  
47  
48 801001) or 96-well plates (Corning Inc., costar, 3603). The cells were then washed twice  
49  
50  
51  
52 with PBS and incubated with H<sub>2</sub>DCFDA (Molecular Probes, Life Technologies, D399) in  
53  
54  
55  
56 PBS (10  $\mu$ M, 100  $\mu$ L) for 20 min at 37 °C in the incubator. After being washed twice with  
57  
58  
59  
60

1  
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3  
4 PBS, the cells were re-fed with PBS and irradiated with a light dose of 54 J/cm<sup>2</sup> ( $\lambda > 590$   
5  
6  
7 nm). The cells were then imaged on a confocal scanning microscope (Carl Zeiss LSM  
8  
9  
10 710). Meanwhile, the fluorescence signal was measured by a SpectraMax M5 microplate  
11  
12  
13 reader (Molecular Devices, USA) using a 485 nm excitation filter and a 538 nm emission  
14  
15  
16  
17 filter.  
18

#### 21 **4.10. Animals.**

22  
23  
24 Female Balb C nu/nu mice were purchased from Shanghai Slac Laboratory Animal Co.  
25  
26  
27 Ltd. (Shanghai, China). HeLa tumor cells were injected subcutaneously into the right back  
28  
29  
30 of nude mice and the cell density was about  $5 \times 10^6$  per mouse. On the seventh day after  
31  
32  
33 inoculation, nude mice with a body weight of 18–22 g and a tumor volume of 80–100 mm<sup>3</sup>  
34  
35  
36 were subjected to PDT and started to count as day zero. The animal experimental  
37  
38  
39 procedures were carried out according to the Guidelines of the Animal Ethical Care and  
40  
41  
42 Use Committee, Fudan University.  
43  
44  
45

#### 49 **4.11 *In vivo* and *ex vivo* imaging.**

50  
51  
52 The mice were injected with **4** (2 mg/kg) via the tail vein and imaged using a VISQUE  
53  
54  
55 Invivo Smart imaging system (Vieworks Co., Ltd., Korean) with excitation of 680 nm and  
56  
57  
58

1  
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3 emission of 720 nm. The living mice were then anesthetized with isoflurane and imaged  
4  
5  
6  
7 at various time points after 15 min of tail vein injection. To investigate the organ  
8  
9  
10 biodistribution of **4**, the mice was then euthanized, major normal organs (heart, liver,  
11  
12  
13 spleen, lungs, kidneys) as well as tumors were extracted, which were placed on black  
14  
15  
16 paper and *ex vivo* fluorescence images were obtained. Images were analyzed using  
17  
18  
19 VISQUE Invivo Smart analysis system. Regions of interest (ROI) were drawn over the  
20  
21  
22 tumor and major organs to acquire the quantitative comparison. All results were obtained  
23  
24  
25 as the mean  $\pm$  standard deviation (SD) for a group of three mice. All fluorescence images  
26  
27  
28 were acquired using a 1 s exposure time (f/stop = 4), with animal remaining sedated to  
29  
30  
31 obtain multiple angles samples.  
32  
33  
34  
35  
36  
37

#### 38 **4.12 PDT effects on HeLa xenograft model.**

39  
40  
41 **ADPM06** and the commercially available photosensitizer **Ce6** were used as references  
42  
43  
44 to compare the anti-tumor effects of **4**. Nude mice were randomly divided into four groups,  
45  
46  
47 including control group, **ADPM06** group, **4** group and **Ce6** group, each group contained  
48  
49  
50 five mice. The control group was given the same amount of saline. The experimental  
51  
52  
53 group was injected with the photosensitizer (2 mg/kg) via tail vein injection. After 15 min  
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3 of injection, the tumor site was irradiated with light. The light source contains a 75 W  
4  
5  
6 halogen lamp, a color filter with cut-on at 590 nm (Sipeida, China) and a water tank for  
7  
8  
9  
10 cooling. The irradiation time of each nude mouse was 10 min and the light dose was 54  
11  
12  
13 J/cm<sup>2</sup>. After treatment, the long (L) and short diameter (W) of tumors were recorded using  
14  
15  
16  
17 a digital vernier caliper every other day, and the body weight was measured as well.  
18  
19  
20  
21 Tumor volume was calculated using the formula:  $V = 0.5 \times (L \times W^2)$ .  
22  
23

#### 24 **4.13 Statistical analysis.**

25  
26  
27 Comparisons among the groups were analyzed using t tests one-way ANOVA followed  
28  
29  
30  
31 by Tukey's multiple comparison tests. Results were considered statistically significant at  
32  
33  
34  
35 a value of  $P < 0.05$ .  
36  
37

## 38 **ASSOCIATED CONTENT**

### 39 **Supporting Information**

40  
41  
42 The Supporting Information is available free of charge at  
43  
44  
45  
46  
47  
48  
49 <https://pubs.acs.org/doi/XX.XXXX/acs.jmedchem.XXXXXXX>.  
50

51  
52 Additional spectroscopic data and absorption spectra, figures of photodegradation of DPBF,  
53  
54 light dose-dependent cell viability, thermal stability of PSs, imaging of ROS generation in cells  
55  
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57  
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2  
3 and organ distributions in mice, copies of  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra, HRMS spectra (DART)  
4  
5  
6  
7 and HPLC spectra (PDF).  
8  
9

10 Molecular formula strings and the associated data (CSV).  
11  
12

## 13 14 **AUTHOR INFORMATION**

### 15 16 17 **Corresponding Author**

18  
19  
20  
21 \*For W.Z.: E-mail, zhaoweili@fudan.edu.cn; phone, +86 21 51980111.  
22  
23

24  
25 \*For X.D.: E-mail, xcdong@fudan.edu.cn; phone, +86 21 51980123.  
26  
27  
28

### 29 30 **Author Contributions**

31  
32  
33 #These authors contributed equally to this work. The manuscript was written through  
34  
35  
36 contributions of all authors. All authors have approved the final version of the manuscript.  
37  
38  
39

### 40 41 **Notes**

42  
43 The authors declare no competing financial interest.  
44  
45  
46

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53  
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55  
56  
57

## ABBREVIATIONS LIST

PDT, photodynamic therapy; PS, photosensitizer; BODIPY, 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene; NIR, near infrared; NBS, *N*-bromosuccinimide; NIS, *N*-iodosuccinimide; DMF, *N,N*-dimethylformamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; TFA, trifluoroacetic acid; PBS, phosphate buffered saline; PI, propidium iodide; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle's Medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPBF, 1,3-diphenylisobenzofuran; ROS, reactive oxygen species; H<sub>2</sub>DCFDA, 2',7'-dichlorodihydrofluorescein diacetate;  $\Phi_{\Delta}$ , singlet oxygen quantum yields;  $\Phi_f$ , fluorescence quantum yields.

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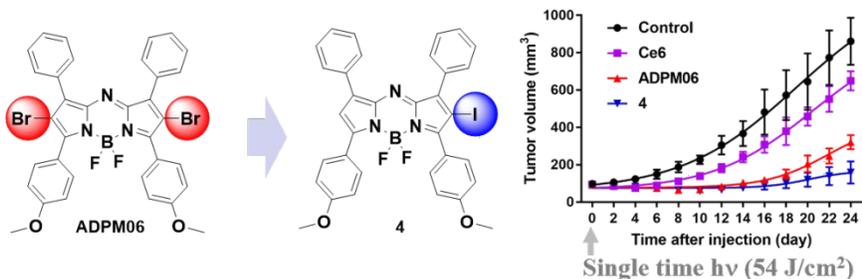
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### Table of Contents Graphic



**Intense NIR absorption / High photo-toxicity / Low dark-toxicity**  
**Good thermal-stability / High photo-stability / Excellent *in vivo* efficacy**