Synthesis of Aza-BODIPY Boron Difluoride PDT Agents to Promote Apoptosis in HeLa Cells

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Abstract: BF_2 Chelated azadipyrromethene dyes fluoresce in the near infrared and have potential applications in photodynamic therapy. When irradiated above 600nm these aza-BODIPY compounds react with triplet O_2 in the body to form a reactive singlet oxygen species which leads to cell death. A small library has been synthesized of these potential PDT agents *via* a four step process, with varying substituent's on the aromatic ring of the starting benzaldehyde and acetophenone. *In vitro* studies on HeLa cells have revealed an effective photosensitive compound with low dark cytotoxicity and promotion of apoptotic cell death when exposed to light.

Keywords: Azadipyrromethene, chalcone, HeLa cells, Michael's addition, photodynamic therapy.

INTRODUCTION

Photodynamic therapy (PDT) is a very attractive technique for the treatment of cancer as it is a non-invasive procedure. Porfimer sodium, also known as Photofrin, was the first PDT agent approved by the FDA in 1995. This drug has been used in the treatment of cervical, stomach, esophageal, bladder, endobroncheal, skin, and lung cancers [1]. However, Photofrin exists as a mixture of dimeric and oligomeric compounds. Other porphyrin based PDT that are commercially available are Visudyne [2], Foscan [3], Purlytin [4], Tookad [5], and Motexafin Lutetium [6]. In addition, Metvix [7] and 5-aminolevulinic acid [8] have also been used as PDT agents as they behave as pro-drugs, and are metabolized to porphyrins within the body. To date, very few non-porphyrin based PDT agents have been examined. Studies have been performed on methylene blue [9] as well as nile blue [10], however, both of these show dark toxicity (i.e. demonstrated cytotoxic ability in the absence of a light) which would impair their application as a PDT agent. Work has been done using a boron core in a non-porphyrin based PDT agent with promising results [11]. We wish to further examine this approach and evaluate whether these agents are active in PDT, show minimal dark toxicity, and can lead the cell to death *via* apoptosis.

The general theory of PDT is based upon the principle of *in vivo* synthesis of singlet oxygen (Fig. **1**). When an electron of the PDT agent is excited with visible or near-visible light, the compound may liberate this energy in two manners; either directly *via* fluorescence, which would bring the agent back down to its ground state, or *via* an intersystem crossing (ISC). If this latter approach occurs, the energy can be transferred to the natural occurring triplet state of oxygen which is thus excited to singlet oxygen [12]. The singlet oxygen can cause oxidative cellular damage, ultimately leading to cell death [13]. The original PDT agent can be re-excited as long as light is introduced. The short half-life (0.6×10^{-6} sec) [14] of singlet oxidation allows this technique to be very selective to the area irradiated.

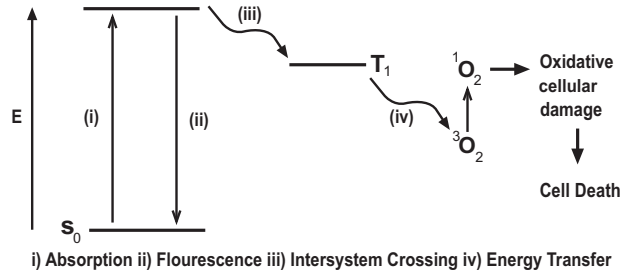

Fig. (1). Proposed Mode of Action of PDT agents.

In order to have a truly effective PDT agent, three factors must be optimized: 1) The ability of the agent to go through an ISC so that it can cause singlet oxygen to be generated, 2) have minimal-to-no dark toxicity, and 3) preferably induce cell death apoptotically versus necrotically. We have synthesized a small library of BF_2 chelated azadipyrromethene PDT agents and evaluated their activity on HeLa cancer cells.

RESULTS AND DISCUSSION

The synthesis (Scheme **1**) of our azadipyrromethene boron difluoride PDT agents began with the formation of a diaryl α,β-unsaturated ketone (**1A**) [15]. The chalcone was synthesized from benzaldehyde and acetophenone derivatives which allows for the formation of a diverse

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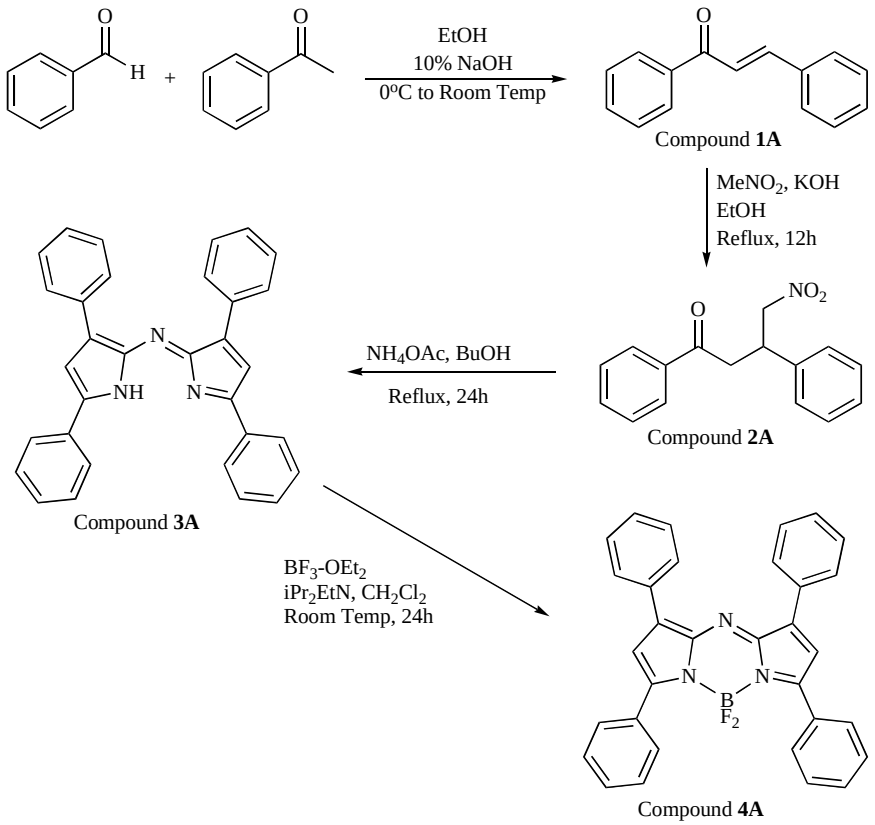


Scheme 1. Synthetic scheme for the aza-BODIPY dye compounds.

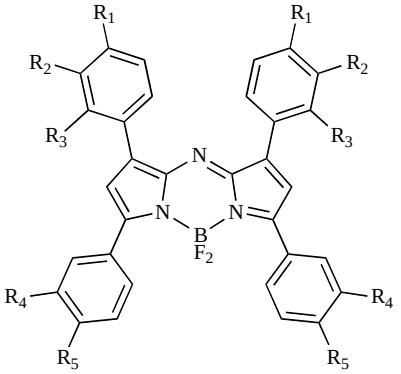
library of final products with variations on the tetraphenyl system. Michael adduct (**2A**) was then formed at nearly quantitative yields, followed by ring closure/dimerization with ammonium acetate (**3A**). Good yields were obtained with the final chelation of the azadipyrromethenes with BF_2 (**4A**) [11] affording the aza-borodipyrromethene (aza-BODIPY).

A total of 7 different aza-BODIPY boron difluorides were synthesized using this scheme (Table **1**). The compounds vary in electron withdrawing, electron donating, non-polar and polar groups off the ortho, meta, and para positions of the tetraphenyl system. This was to examine the effects of different substituents on the molecules fluorescent and cellular toxicity properties.

HeLa cervical cancer cells were grown in the presence of different aza-BODIPY compounds and exposed to either light treatment for photoactivation or dark treatment to determine toxicity affects. Apoptotic cells which detach from the flask [13], and necrotic cells which remain attached, were isolated and their DNA was extracted. Agarose gel electrophoresis and gel imaging were used to characterize and quantitate apoptotic and necrotic effects of the compounds relative to their photodynamic activation or toxicity.

Results for all aza-BODIPY dyes tested showed similar cell death by necrosis as the parent compound **4A** with the exception of compound **4B** [16] (Fig. **2**). Compound **4B** (R_1 = $(CH_3)_2N$) demonstrated apoptosis, as shown by the characteristic laddering pattern rather than a smear after gel electrophoresis of the detached cell DNA. The effect was increased by approximately 30 percent in the presence of light (Fig. **3**). No effect was observed in the viable attached

Table 1. Synthesized aza-BODIPY dyes (4A-G)



4	R_1	R_2	R_3	R_4	R_5
A	H	H	H	H	H
B	$N(CH_3)_2$	H	H	H	H
C	H	H	H	OCH_3	H
D	H	Br	H	H	H
E	Br	H	H	H	H
F	H	H	H	CH_3	CH_3
G	H	H	H	CH_3	H

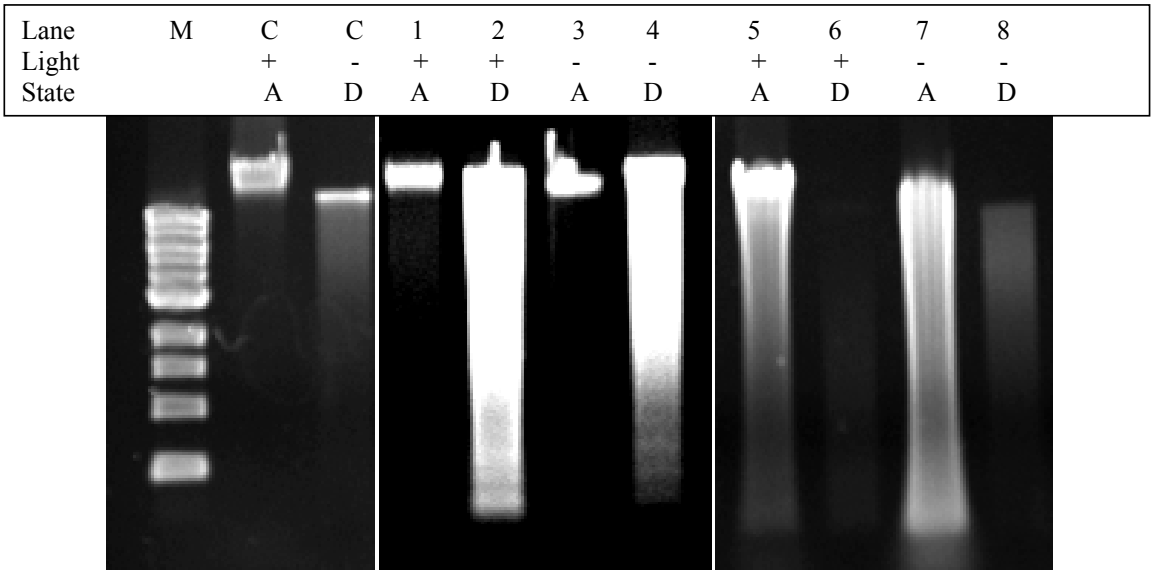


Fig. (2). Aza-BODIPY mediated necrosis and apoptosis in HeLa cells in culture. Lanes: M, Lambda marker DNA; C, control attached (A) and detached (D) DNA; lanes 1-4, Compound **4B** treated DNA isolation, attached (A) and detached (D) in the presence (+) and absence (-) of light; lanes 5-8, Compound **4A** treated DNA isolation, attached (A) and detached (D) in the presence (+) and absence (-) of light.

cell fraction for **4B**; in contrast with compound **4A**, where cells were completely necrotic independent of light and with no detectable detached cell fraction present. Compound **4B** has been previously synthesized, and although not examined for its ability as a PDT agent, it was studied for its absorbance and fluorescence profile [17]. It was revealed there was a distictive red shift in absorbance at high solution pH values. In addition, this scaffold has been covalently linked to a solid support and examined as a fluorescent sensor [18].

CONCLUSION

In summary, seven PDT agents have been synthesized and were tested to look at their cytotoxicity and ability to induce apoptosis on HeLa cells. All of these aza-BODIPY compounds showed a classic necrotic cell death profile, with the exception of **4B**. Although the synthesis of **4B** has been recently reported [17], it has not been examined as a possible PDT agent. Its ability to induce apoptosis opposed to necrosis and a >30% selectivity in light versus dark conditions has encouraged us to further examine this particular amino moiety for future synthesis.

EXPERIMENTAL

All commercial reagents were obtained from Sigma-Aldrich Chemical Company. Thin-layer chromatography (SiliCycle Inc.) was performed on 0.25-mm silica-gel plates with aluminum backing, visualized using short-wave UV

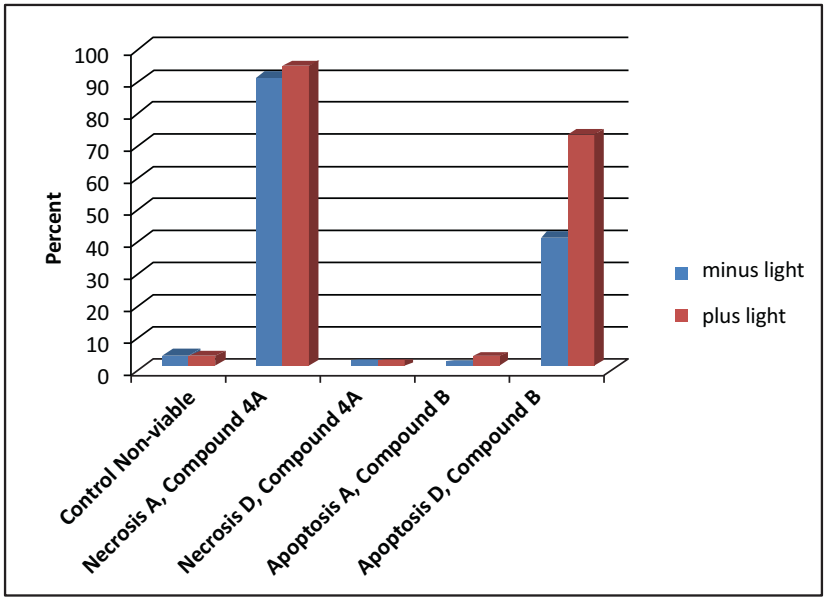


Fig. (3). Apoptosis and necrosis cell fraction percent distributions after treatment with Compound **4A** and **4B** and light.

light. ^{1}H and ^{13}C-NMR were recorded on a Varian 400-MHz instrument, and all spectra of synthesized compounds were compared to previously reported spectra [11, 15, 17].

General Procedure for the Synthesis of Chalcone 1A-G

A solution of an acetophenone (1 eq) in absolute ethanol (10 mL) was added to an aqueous solution of 10% NaOH (30 mL) at 0°C. After stirring for 15 min, a benzaldehyde (1 eq) was added to the solution and stirred for an additional 15 min. The reaction mixture was then stirred at room temperature for 24 hours. The product precipitated from the solution over the course of the reaction. The solid was collected by filtration and washed with water to yield the desired chalcone. Some compounds did not form solids, but an oil. This oil was concentrated, washed with ethyl acetate, filtered, and evaporated to yield an oil. The product was used without purification for the next step.

1,3-diphenylprop-2-en-1-one (1A)

δ_H (400MHz, $CDCl_3$): 8.3 (d, 2H, *J*=7.6Hz), 7.8 (d, 1H, *J*=15.6Hz), 7.65 (d, 2H, *J*=3.2Hz), 7.59 (t, 2H, *J*=8.0Hz), 7.56 (d, 1H, *J*=15.6Hz), 7.51 (t, 2H, *J*=8.0Hz), 7.42 (d, 2H, *J*=4.0Hz).

3-(4-dimethylaminophenyl)-1-phenylprop-2-en-1-one (1B)

δ_H (400MHz, $CDCl_3$): 8.01 (d, 2H, *J*=6.0Hz), 7.79 (d, 1H, *J*=15.6), 7.55 (m, 3H), 7.49 (t, 2H, *J*=7.2), 7.34 (d, 1H, *J*=15.6Hz), 6.70 (m, 2H), 3.05 (s, 6H).

1-(4-methoxyphenyl)-3-phenylprop-2-en-1-one (1C)

δ_H (400MHz, $CDCl_3$): 8.16 (d, 2H, *J*=8.4Hz), 7.92 (d, 1H, *J*=15.6Hz), 7.76 (m, 2H), 7.67 (d, 1H, *J*=15.6), 7.52 (m, 3H), 7.09 (d, 2H, *J*=8.0Hz), 4.00 (s, 3H).

3-(3-bromophenyl)-1-phenylprop-2-en-1-one (1D)

δ_H (400MHz, $CDCl_3$): 8.03 (d, 2H, *J*=7.9Hz), 7.81 (s, 1H), 7.73 (d, 1H, *J*=15.6), 7.61 (t, 1H, *J*=7.2Hz), 7.50-7.57 (m, 5H), 7.31 (t, 1H, *J*=7.6Hz).

3-(4-bromophenyl)-1-phenylprop-2-en-1-one (1E)

δ_H (400MHz, $CDCl_3$): 8.01 (d, 2H, *J*=7.2Hz), 7.75 (d, 1H, *J*=16.0Hz), 7.50-7.60 (m, 8H).

1-(3,4-dimethylphenyl)-3-phenylprop-2-en-1-one (1F)

δ_H (400MHz, $CDCl_3$): 8.00 (d, 2H, *J*=7.2Hz), 7.75 (d, 1H, J=15.8Hz), 7.51-7.64 (m, 6H), 7.46 (d, 1H, *J*=15.8Hz), 2.41 (s, 3H), 2.36 (s, 3H).

1-(4-methylphenyl)-3-phenylprop-2-en-1-one (1G)

δ_H (400MHz, $CDCl_3$): 7.94 (d, 2H, *J*=8.0Hz), 7.80 (d, 1H, J=15.6Hz), 7.64-7.75 (m, 7H), 7.54 (d, 1H, *J*=15.6Hz) 2.38 (s, 3H).

General Procedure for the Synthesis of Michael's Adduct 2A-G

A solution of the chalcone (**1A-G**) (1 eq), nitromethane (20 eq), and NaOH (0.2 eq) in absolute ethanol (1 mL) was heated at 60 °C for 12 hours. After cooling to room temperature, the solution was concentrated and the oily residue obtained was dissolved in ethyl acetate and washed with water (3 x 50 mL). The organic layers were washed with brine, dried over magnesium sulfate, filtered, and evaporated to yield the desired oily residue.

4-nitro-1,3-phenylbutan-1-one (2A)

δ_H (400MHz, $CDCl_3$): 7.92 (d, 2H, *J*=7.2Hz), 7.58 (t, 2H, *J*=7.6Hz), 7.46 (t, 2H, *J*=7.6Hz), 7.33 (d, 2H, J=7.6Hz), 7.29 (m, 2H), 4.84 (dd, 1H, *J*=6.0, 12.8Hz), 4.69 (dd, 1H, *J*=4.8, 12.8Hz), 4.23 (p, 1H, *J*=7.6Hz), 3.46 (dd, 1H, *J*=6.4Hz, 16.8Hz), 3.38 (dd, 1H, *J*=7.6Hz, 16.8Hz).

3-(4-dimethylaminophenyl)-4-nitro-1-phenylbutan-1-one (2B)

δ_H (400MHz, $CDCl_3$): 7.91 (d, 2H, *J*=8.0Hz), 7.57 (t, 1H, *J*=7.2Hz), 7.45 (t, 2H, *J*=7.6Hz), 7.12 (d, 2H, *J*=8.4Hz), 6.66 (d, 2H, *J*=7.2Hz), 4.77 (dd, 1H, *J*=5.2, 12.0Hz), 4.63 (dd, 1H, *J*=7.8, 12.0Hz), 4.12 (m, 1H), 3.41 (dd, 1H, *J*=6.8, 16.8Hz), 3.35 (dd, 1H, *J*=7.6, 16.8Hz), 2.95 (s, 6H).

1-(4-methoxyphenyl)-4-nitro-3-phenylbutan-1-one (2C)

δ_H (400MHz, $CDCl_3$): 7.90 (d, 2H, *J*=8.8Hz), 7.24-7.35 (m, 5H), 6.92 (d, 2H, *J*=8.8Hz), 4.84 (dd, 1H, *J*=6.4, 12.4Hz), 4.67 (dd, 1H, *J*=4.4, 12.4Hz), 4.21 (m, 1H), 3.40 (dd, 1H, *J*=6.0, 17.0Hz), 3.32 (dd, 1H, *J*=7.2, 17.0Hz), 3.87 (s, 3H).

3-(3-bromophenyl)-4-nitro-1-phenylbutan-1-one (2D)

δ_H (400MHz, $CDCl_3$): 7.92 (d, 2H, *J*=7.2Hz), 7.59 (t, 1H, *J*=7.2Hz), 7.38-7.49 (m, 4H), 7.2 (t, 2H, *J*=7.6Hz), 4.82 (dd, 1H, *J*=6.0, 12.8Hz), 4.66 (dd, 1H, *J*=4.8, 12.8Hz), 4.21 (m, 1H), 3.44 (dd, 1H, *J*=5.8, 17.2Hz), 3.37 (dd, 1H, *J*=6.8, 17.2Hz).

3-(4-bromophenyl)-4-nitro-1-phenylbutan-1-one (2E)

δ_H (400MHz, $CDCl_3$): 7.91 (d, 2H, *J*=6.8Hz), 7.59 (t, 1H, *J*=7.2), 7.44-7.49 (m, 4H), 7.18 (d, 2H, *J*=8.4Hz), 4.81 (dd, 1H, *J*=6.4, 12.8Hz), 4.66 (dd, 1H, *J*=4.0, 12.4Hz), 4.21 (m, 1H), 3.43 (dd, 1H, J=4.0, 17.6Hz), 3.34 (dd, 1H, J=5.2, 17.6Hz).

1-(3,4-dimethylphenyl)-4-nitro-3-phenylbutan-1-one (2F)

δ_H (400MHz, $CDCl_3$): 7.89 (d, 2H, *J*=7.0Hz), 7.55 (t, 1H, *J*=6.8Hz), 7.32-7.46 (m, 5H), 4.80 (dd, 1H, *J*=5.8, 12.8Hz), 4.63 (dd, 1H, *J*=4.2, 12.8Hz), 4.18 (m, 1H), 3.48 (dd, 1H, *J*=5.2, 16.8Hz), 3.35 (dd, 1H, *J*=7.0, 16.8Hz), 2.45 (s, 3H), 2.41 (s, 3H).

1-(4-methylphenyl)-4-nitro-3-phenylbutan-1-one (2G)

δ_H (400MHz, $CDCl_3$): 7.82 (d, 2H, *J*=8.4Hz), 7.22-7.36 (m, 7H), 4.84 (dd, 1H, *J*=6.0, 12.4Hz), 4.68 (dd, 1H, *J*=4.4, 12.8Hz), 4.22 (m, 1H), 3.42 (m, 2H), 2.41 (s, 3H).

General Procedure for Synthesis of Azadipyrromethene 3A-G

The oily Michael adduct (**2A-G**) (1 eq), ammonium acetate (35 eq), and butanol (10 mL) were added to a round bottom flask and heated under reflux for 24 hours. After cooling to room temperature the solution was evaporated to approximately a quarter of its original volume, filtered, and

washed with absolute ethanol, to yield the desired blue-purple azapyrromethane solid.

[3,5-diphenyl-1H-pyrrol-2-yl][3,5-diphenylpyrrol-2-ylidene]amine (3A)

δ_H (400MHz, $CDCl_3$): 8.07 (d, 4H, *J*=8.0Hz), 7.97 (d, 4H, *J*=7.6Hz), 7.55 (t, 4H, *J*=7.6Hz), 7.48 (d, 2H, *J*=7.2Hz), 7.44 (t, 4H, *J*=8.4Hz), 7.37 (d, 2H, *J*=7.2Hz), 7.22 (s, 2H).

[3-(4-dimethylaminophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(4-dimethylaminephenyl)-5-phenylpyrrol-2-ylidene]amine (3B)

δ_H (400MHz, $CDCl_3$): 8.05 (d, 4H, *J*=8.8Hz), 7.95 (d, 4H, *J*=7.2Hz), 7.52 (t, 4H, *J*=7.2Hz), 7.43 (t, 2H, *J*=7.6Hz), 7.06 (s, 2H), 6.79 (d, 4H, *J*=8.8Hz), 3.05 (s, 12H).

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

δ_H (400MHz, $CDCl_3$): 8.07 (d, 4H, *J*=8.0Hz), 7.90 (d, 4H, *J*=8.0Hz), 7.43 (t, 4H, *J*=6.4Hz), 7.35 (t, 2H, *J*=6.0Hz), 7.15 (s, 2H), 7.06 (d, 4H, *J*=8.0Hz), 3.92 (s, 6H).

[3-(3-bromophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(3-bromophenyl)-5-phenyl pyrrol-2-ylidene]amine (3D)

δ_H (400MHz, $CDCl_3$): 8.11 (s, 2H), 8.01 (d, 2H, *J*=7.2Hz), 7.94 (d, 4H, *J*=7.2Hz), 7.49-7.55 (m, 6H), 7.37 (t, 4H, *J*=8.0Hz), 7.20 (s, 2H).

[3-(4-bromophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(4-bromophenyl)-5-phenyl pyrrol-2-ylidene]amine (3E)

δ_H (400MHz, $CDCl_3$): 7.91 (d, 4H, *J*=7.2Hz), 7.39-7.66 (m, 10H), 7.17 (t, 4H, *J*=8.0Hz), 6.78 (s, 2H).

[5-(3,4-dimethylphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(3,4-dimethylphenyl)-3-phenylpyrrol-2-ylidene]amine (3F)

δ_H (400MHz, $CDCl_3$): 8.02 (d, 4H, *J*=7.0H), 7.37-7.58 (m, 8H), 7.32 (t, 4H, *J*=7.6Hz), 7.18 (s, 2H), 2.46 (s, 3H), 2.39 (s, 3H).

[5-(4-methylphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(4-methylphenyl)-3-phenylpyrrol-2-ylidene]amine (3G)

δ_H (400MHz, $CDCl_3$): 8.06 (d, 4H, *J*=7.2H), 7.85 (d, 4H, *J*=8.0Hz), 7.43 (t, 4H, *J*=7.2Hz), 7.35 (t, 6H, *J*=8.0Hz), 7.18 (s, 2H), 2.46 (s, 6H).

General Procedure for Synthesis of Aza-BODIPY 4A-G

A flame dried flask was charged with the azapyrromethane (**3A-G**) (1eq), and flushed with argon. Dry dichloromethane (30mL) and dry diisopropylethylamine (11eq) were added, and the solution was stirred for 15 min, whereby BF_3•OEt_2 (15.6eq) was slowly added. After stirring for 24 h at room temperature, the mixture was washed with water, the organic layer dried over magnesium sulfate, and evaporated to give the target compound.

BF_2 chelated [3,5-diphenyl-1H-pyrrol-2-yl][3,5-diphenyl-pyrrol-2-ylidene]amine (4A)

δ_H (400MHz, $CDCl_3$): 8.03-8.08 (m, 8H), 7.43-7.5 (m, 12H), 7.04 (s, 2H).

BF_2 chelated [3-(4-dimethylaminophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(4-dimethylaminephenyl)-5-phenylpyrrol-2-ylidene]amine (4B)

δ_H (400MHz, $CDCl_3$): 8.09 (d, 4H, *J*=8.8Hz), 8.01 (d, 4H, *J*=7.2Hz), 7.34-7.51 (m, 6H), 6.82 (s, 2H), 6.78 (d, 4H, *J*=8.8Hz), 3.10 (s, 12H).

BF_2 chelated [5-(4-methoxyphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(4-methoxyphenyl)-3-phenyl pyrrol-2-ylidene]amine (4C)

δ_H (400MHz, $CDCl_3$): 8.04-8.09 (m, 8H), 7.41-7.47 (m, 6H), 6.98-7.03 (m, 6H), 3.87 (s, 6H).

BF_2 chelated [3-(3-bromophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(3-bromophenyl)-5-phenyl pyrrol-2-ylidene]amine (4D)

δ_H (400MHz, $CDCl_3$): 8.18 (s, 2H), 8.01-8.05 (m, 6H), 7.56 (d, 2H, *J*=7.6Hz), 7.49-7.51 (m, 6H), 7.42 (t, 2H, *J*=7.6Hz), 7.05 (s, 2H).

BF_2 chelated [3-(4-bromophenyl)-5-phenyl-1H-pyrrol-2-yl][3-(4-bromophenyl)-5-phenyl pyrrol-2-ylidene]amine (4E)

δ_H (400MHz, $CDCl_3$): 8.03 (dd, 4H, *J*=4.0, 7.6Hz), 7.91 (d, 4H, *J*=8.4Hz), 7.61 (d, 4H, *J*=8.8Hz), 7.42-7.59 (m, 6H), 7.03 (s, 2H).

BF_2 chelated [5-(3,4-dimethylphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(3,4-dimethylphenyl)-3-phenylpyrrol-2-ylidene]amine (4F)

δ_H (400MHz, $CDCl_3$): 8.06 (d, 4H, *J*=8.0Hz), 7.98 (d, 4H, *J*=8.0Hz), 7.38-7.45 (m, 4H), 7.31 (d, 4H, *J*=8.4Hz), 7.04 (s, 2H), 2.57 (s, 6H), 2.43 (s, 6H).

BF_2 chelated [5-(4-methylphenyl)-3-phenyl-1H-pyrrol-2-yl][5-(4-methylphenyl)-3-phenylpyrrol-2-ylidene]amine (4G)

δ_H (400MHz, $CDCl_3$): 8.06 (d, 4H, *J*=8.0Hz), 7.97 (d, 4H, *J*=7.6Hz), 7.38-7.52 (m, 6H), 7.29 (d, 4H, *J*=8.0Hz), 7.04 (s, 2H), 2.42 (s, 6H).

Procedure for Determining Apoptosis and Necrosis of HeLa with Aza-BODIPY Compounds

HeLa cells were maintained at 37°C and 5% CO_2. HeLa cells were grown in 25cm^2 TPP tissue culture flasks containing 5 mL of RPMI + Glutamax and supplemented with 10% fetal bovine serum (FBS). Flask media was grown to 70% confluency prior to treatment at which time each flask media was changed with 5 mL of fresh media, and 40uM concentrations of the aza-BODIPY compounds were mixed directly into the media. Flasks were incubated for 24 hours at 37°C in the dark to allow the drug to accumulate intercellularly. After 24 hours, media from all flasks was replaced with fresh media. Dark toxicity flasks were wrapped with aluminum foil to eliminate light exposure and all flasks were placed under broad spectrum light for 60 minutes delivering 8 J/cm^2. Flasks were returned to the incubator for 24 hours before DNA isolation. All sample assays were carried out in triplicate and averages of the results were used for viability apoptosis and necrosis results.

Media plus detached cells were removed from all flasks and the cells were washed with 5 mL 1% phosphate buffered saline (PBS) on ice. The remaining attached cells were trypsonized and washed with 5 mL of 1% PBS on ice and all samples were centrifuged at 3000 rpms for 20 minutes at 5°C. After centrifugation, the supernatant was removed and cell pellets were resuspended in 500 µL of ice cold DNA Isolation Buffer (0.15 M NaCl, 0.015 M Na citrate, 10 mM EDTA and 1% Na lauryl sarkosinate), and homogenized by pipetting. Then 10 µL of Proteinase K was added to the lysate, incubated for 1 hr at 50°C and 1 mL of ice cold 95% ethanol was added to the tube. The samples were inverted several times and then placed in a -20°C freezer for 20 minutes and centrifuged at 16G for 20 minutes at 5°C to pellet the DNA. The supernatant was poured off and the pellet dried for 1 hour. Pellets were mixed with 50µL of ice cold 0.5% Tris/Borate/EDTA buffer by pipetting and then 5 µL of DNAse-free RNAse was added to the mixture. Samples were vortexed for 30 seconds and incubated at 37°C for 15 minutes then mixed with 5µL of DNA loading dye. Samples were loaded onto a 1 % agarose gel and run for 65 minutes at 65 Volts and DNA visualized and photographed in a Bio Rad Gel imager. Cell distribution in the apoptosis and necrosis samples was determined by counting using a Hausser Scientific Counting Chamber. Following trypsonization, a uniform suspension of cells was obtained by pipetting and a 1:2 dilution of cell suspension in trypan blue was prepared using 20 µL of cell suspension. Subsequently, 10 µL of the mixtures were loaded onto the hemocytometer and the total number of cells overlying each of the four 1mm^2 areas was counted for three replicates. These numbers were averaged, divided by the dilution and multiplied by the number of mL in the flask.

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[16] ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.10 (s, 12H), 6.78 (d, 4H, J=8.8Hz), 6.82 (s, 2H), 7.44 (m, 6H), 8.01 (d, 4H, J=7.2Hz), 8.09 (d, 4H, J=8.8Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 39.8, 109.4, 112.4, 123.5, 125.1, 129.0, 129.4, 131.4, 134.8, 141.3, 148.6, 151.8, 156.1. HR-MS (ESI: M+H$^+$) Exact mass calculated for $C_{36}H_{33}N_5BF_2$ 584.2792, found 584.27899.

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