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# Carbazole substituted BODIPY dyes: Synthesis, photophysical properties and antitumor activity

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ABSTRACT

In this study, two different BODIPYs containing carbazole groups at the meso position were designed and synthesized. All compounds were fully characterized by elemental analysis, FT-IR, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, $^{1}H$ and $^{13}C$ NMR spectroscopy. The photophysical properties of the new compounds were investigated by means of absorption and fluorescence spectroscopies in dilute dichloromethane solutions. We were also interested in the biological activity of these two novel carbazole-linked BODIPYs, particularly concerning their ability to inhibit human colon cancer HT29 cell lines. The photophysical studies revealed strong donor–acceptor interaction between carbazole and BODIPY and follow the order compound 5 > compound 4. Also, preliminary assay showed that compound 5 possessed higher cytotoxic activity than compound 4, with IC50 values of 8.3 ng/mL and 21.7 ng/mL respectively.



## 1. Introduction

4,4-Difluoro-4-borato-3a,4a-diaza-*s*-indacene (BODIPY) is one of the important families amongst the highly fluorescent dyes [1]. Since the first synthesis of BODIPY in 1968 by Treibs and co-worker [2], the chemistry of BODIPY has been the subject of intense study and major efforts have been devoted to obtain the well-designed BODIPY structures [3]. BODIPYs are highly colored compounds that often show intense florescence. They display highly desirable properties, such as high fluorescence quantum yields, intense absorption in the 450–750 nm regions, tunability of absorption range, and good solubility in most organic solvents [4,5]. Due to the photostable nature and insensitivity towards the environmental pH they gained attention as fluorescent labels (proteins and DNA) in the cells [6,7]. The study of the photophysical properties of these compounds is particularly interesting and highly dependent on the functional dye systems [8–11].

Carbazole and its derivatives are important class of aromatic heterocyclic compounds, having long attracted attention from researchers due to their valuable properties, such as facile functionalization, stability, low redox potential, hole transport properties, and strong electron donating ability [12–14]. Carbazole-based compounds are attractive as photoelectrical materials and dyes, as well as for supramolecular recognition and medicinal chemistry [12]. Such considerations have led to the preparation and characterization of carbazole conjugated BODIPY chromophores selected for their specific photophysical properties. Recently, Tour et al. have demonstrated the preparation of highly fluorescent BODIPY based nano-cars incorporating carbazole and *p*-carborane groups [15]. Long-wavelength fluorescent BODIPYs with mono- and di-carbazole groups attached to BODIPYs α-pyrrole position exhibited a reasonable two-photon absorption cross-section [16–18]. Donor-acceptor carbazole substituted BODIPY dyes were reported for DSSC (dye sensitized solar cells) application [13,19–21] and as dye, possessing solid-state red fluorescence and green metallic luster properties [22]. Also, carbazole-BODIPY derivatives have been explored for OLED green dopants [3]. As part of an ongoing investigation into the chemistry of carbazole-linked BODIPY, we now report the synthesis and characterization of compounds **4** and **5** (Scheme 1) as well as their absorption and emission spectroscopy studies in order to investigate the energy transfer from carbazole unit into the BODIPY core. Moreover, we were also interested in the biological activities of these two novel carbazole-linked BODIPYs, particularly concerning their ability to inhibit human colon cancer HT29 cell lines.

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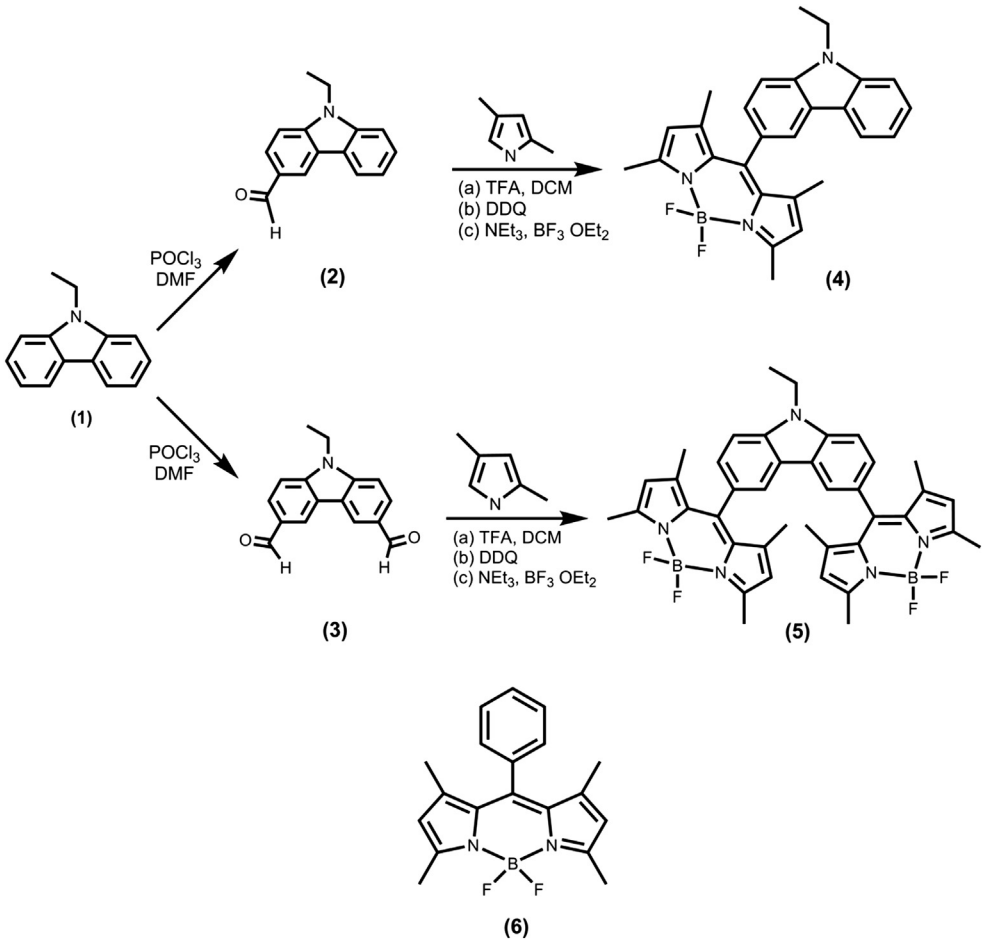


**Scheme 1.** Chemical structure and synthetic pathway of 2–5 and molecular structures of standards 1 and 6.

## 2. Experimental

### 2.1. Materials

The deuterated solvent ($CDCl_3$) for NMR spectroscopy was obtained from Merck. Following chemicals were obtained from Sigma Aldrich; 9-ethylcarbazole, 2,4-dimethylpyrrole, trifluoroacetic acid, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, trimethylamine, Boron trifluoride diethyl etherate. Silica gel 60 and dichloromethane were obtained from Merck Chemicals. 1,8,9-Anthracenetriol for the MALDI matrix was obtained from Fluka.

### 2.2. Equipment

Electronic absorption spectra were recorded with a Shimadzu 2101 UV spectrophotometer in the UV–visible region. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1 cm pathlength cuvettes at room temperature. The fluorescence lifetimes were obtained using Horiba-Jobin-Yvon-SPEX Fluorolog 3-2iHR instrument with Fluoro Hub-B Single Photon Counting Controller at an excitation wavelength of 470 nm. Signal acquisition was performed using a TCSPC module. Mass spectra were acquired in linear modes with average of 50 shots on a Bruker Daltonics Microflex mass spectrometer (Bremen, Germany) equipped with a nitrogen UV-Laser operating at 337 nm. FT-IR spectra were recorded on a Bruker Alpha-P in ATR in the range of 4000 $cm^{-1}$–650 $cm^{-1}$. Many different MALDI matrices were tried to find an intense molecular ion peak and low fragmentation under the MALDI-MS conditions for these compounds. 1,8,9-Anthracenetriol MALDI matrix yielded the best MALDI-MS spectra. 1,8,9-Anthracenetriol (2 mg/mL in tetrahydrofuran) matrix for compounds **2**–**6** were prepared. MALDI samples were prepared by mixing compounds **2**–**6** (2 mg/mL in tetrahydrofuran) with the matrix solution (1:10 v/v) in a 0.5 mL eppendorf micro tube. Finally 1 μL of this mixture was deposited on the sample plate, dried at room temperature and then analyzed. $^1H$ and $^{13}C$ NMR spectra were recorded in $CDCl_3$ solutions on a Varian 500 MHz spectrometer. Analytical thin layer chromatography (TLC) was performed on silica gel plates (Merck, Kieselgel 60, 0.25 mm thickness) with F254 indicator. Column chromatography was performed on silica gel (Merck, Kieselgel 60, 70–200 or 230–400 mesh). Suction column chromatography was performed on silica gel (Merck, Kieselgel 60A, 70–230 mesh).

### 2.3. The parameters for fluorescence quantum yields

The fluorescence quantum yield values of the samples (**4** and **5**) were determined in dichloromethane by comparing with the fluorescence of Rhodamine 6G as a standard. Fluorescence quantum yields ($\Phi_F$) were calculated by the comparative method (Eq. (1)) [23].

$$\Phi_F = \Phi_F(\mathrm{Std})\frac{F \cdot A_{Std} \cdot n^2}{F_{Std} \cdot A \cdot n_{Std}^2} \quad (1)$$

where $\Phi_F$(Std) is the fluorescence quantum yield of standard. Rhodamine 6G was employed as the standard ($\Phi_F = 0.76$ in water) [24]. F and $F_{Std}$ are the areas under the fluorescence emission curves of samples (**4** and **5**) and the standard, respectively. A and $A_{Std}$ are the respective absorbance of the samples and standard at the excitation wavelengths. $\eta^2$ and $\eta_{Std}^2$ are the refractive indices of solvents used for the sample and standard, respectively. The concentration of the solutions at the excitation wavelength fixed at $5 \times 10^{-7}$ mol dm$^{-3}$. According to the Eq. (2) [25,26], where ΦENT is the energy transfer quantum yield, ΦF (dyad) and ΦF (donor) are the fluorescence quantum yields of the dyad (the donor part) and the donor without connecting to the acceptor, respectively.

$$\Phi\mathrm{ENT} = 1 - \Phi\mathrm{F(dyad)}/\Phi\mathrm{F(donor)} \quad (2)$$

### 2.4. Cell culture

HT-29 cells were obtained from SAP Institute (Ankara, TURKEY). The cells were grown in phenol red free Dulbecco's modified Eagle's medium (DMEM) (high glucose) supplemented with 10% fetal calf serum, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C in 5% $CO_2$ in humudified incubator in T25 culture flasks. All culture media were obtained from Biochrom (Berlin, GERMANY). Before cell viability assays cells in T25 flasks were grown upon reaching 70% in vitro confluency, in the meantime culture media were changed every other day. Before quantification assay cells were first washed with ice-cold Phosphate Buffered Saline (PBS) solution twice and then subjected to trypsin enzyme for 5 min at 37 °C and activity of trypsin was stopped by the addition of serum containing DMEM (7 mL medium per ml of trypsin solution), 10 μL of medium was taken for cell counting by using hemocytometer and cell concentration was adjusted to $1 \times 10^4$ cells/100 μL culture medium.

### 2.5. Cell viability assay

Cell viability was performed by using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) [27]. HT-29 cells are plated in 96-well microplates at $1 \times 10^4$ cells/well, and incubated overnight for proper attachment in conditions described elsewhere. Treatments were applied in seven groups in triplicates along with vehicle control (DMSO only). Cells were treated with increasing concentrations of mono- and bis-BODIPY carbazoles 4–5 (0, 5, 10, 25, 50, 100, 250 ng/mL). Data were gathered over 24 by removing cell culture media from the cells, then washing with PBS and adding 100 μL of new media which contains 10 μL of 12 mM MTT reagent (Molecular Probes). Cells were further incubated 4 h and 100 μL of 10% SDS solution prepared from 0.01 M HCl was added. Absorbances of plates were read spectrophotometrically at 570 nm with automatic micro-plate reader (Bio-RAD). Average results were obtained and cell viability was calculated according to the formula below:

$$\%\ \text{Cell viability} = [(\text{Absorbance of treated cells})/ \times (\text{Absorbace of controls untreated cells})] \times 100$$

## 3. Synthesis

Compounds **2** and **3** were prepared according to procedures described previously [28]. The synthesis procedure of compound **6** was as follows based on literature with a minor revision [29] (Scheme 1).

### 3.1. Synthesis of compound **4**

A 500 mL round bottomed flask was charged with dichloromethane (150 mL), and purged with Ar for 20 min. To the flask were added Compound **1** (0.263 g, 1.18 mmol) and 2,4-dimethylpyrrole (0.246 g, 2.60 mmol) were dissolved in dichloromethane and stirred 20 min and few drops of trifluoroacetic acid was added to the reaction mixture and stirred for 2–3 h. 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) (0.16 g, 0.71 mmol) was dissolved in 80 mL of dichloromethane and added to the reaction mixture. The reaction mixture was stirred for 2 h at room temperature and triethyl amine (5 mL, 57 mmol) was added to the mixture drop by drop and stirred for 30 min. $BF_3$. $OEt_2$ (5 mL, 66 mmol) was added to the mixture drop by drop. The reaction mixture was stirred at room temperature 2–3 h and filtered from G4 sintered filter. Solvent was removed under reduced pressure. Compound **4** has been isolated from column chromatography with silica gel (4:1; Hexane: Ethylacetate) Yield: 72 mg (14%).

Spectral data of **4**: (Found: C 73.45, H 5.96, N 9.54%, $C_{27}H_{26}BF_2N_3$ (441) requires C 73.48, H 5.94, N 9.52%). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 7.7 Hz, 1H), 8.00 (s, 1H), 7.55–7.51 (m, 2H), 7.48 (d, J = 8.1 Hz, 1H), 7.34 (dd, J = 8.3, 1.6 Hz, 1H), 7.29–7.26 (m, 1H), 5.98 (s, 2H), 4.43 (t, J = 7.2 Hz, 2H), 2.59 (s, 6H), 1.51 (t, J = 7.2 Hz, 3H), 1.32 (s, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 155.21, 143.51, 143.34, 140.50, 140.13, 132.44, 126.88, 126.43, 125.46, 125.28, 123.54, 122.78, 121.17, 121.16, 120.74, 120.22, 119.44, 109.19, 108.97, 47.63, 37.97, 29.85, 14.79, 14.74, 14.72, 13.96. IR (ATR) $\upsilon_{max}$ (cm$^{-1}$): 2955 (aromatic CH), 2920 (aliphatic CH), 2856 (aliphatic CH), 1601 (aromatic C=C), 1546 (aromatic C=C), 1505 (B–F), 1463 (aliphatic CH), 1370, (aliphatic CH), 1305 (C–N), 1255, 1190, 1155, 1122, 975, 807, 765, 726. MS (MALDI-TOF) m/z (%): 442 $[M + H]^+$, 423 $[M-F]^+$.

### 3.2. Synthesis of compound **5**

A 500 mL round bottomed flask was charged with dichloromethane (250 mL), and purged with Ar for 20 min. To the flask were added Compound **3** (1.25 g, 5 mmol) and 2,4-dimethylpyrrole (2.1 g, 22 mmol) were dissolved in dichloromethane and stirred 20 min and few drops of trifluoroacetic acid was added to the reaction mixture and stirred for 2–3 h. 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) (2.26 g, 6 mmol) was dissolved in 150 mL of dichloromethane and added to the reaction mixture. The reaction mixture was stirred for 2 h at room temperature and triethyl amine (5 mL, 57 mmol) was added to the mixture drop by drop and stirred for 30 min. $BF_3$. $OEt_2$ (5 mL, 66 mmol) was added to the mixture drop by drop. The reaction mixture was stirred at room temperature 2–3 h and filtered from G4 sintered filter. Solvent was removed under reduced pressure. Compound **5** has been isolated from column chromatography with silica gel (4:1; Hexane: Ethylacetate) yield: 350 mg (10%).

Spectral data of **5**: (Found: C 69.84, H 5.74, N 10.21%, $C_{40}H_{39}B_2F_4N_5$ (687) requires C 69.89, H 5.72, N 10.19%). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.94 (s, 2H), 7.58 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 8.3 Hz, 2H), 5.97 (s, 4H), 4.48 (q, J = 7.0 Hz, 2H), 2.57 (s, 12H), 1.59 (d, J = 7.1 Hz, 3H), 1.32 (s, 12H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 155.49, 155.25, 143.49, 143.07, 142.77, 142.61, 142.14, 140.40, 126.10, 125.76, 125.74, 121.11, 120.28, 109.38, 47.49, 29.68, 14.78, 14.58. FT-IR (ATR) $\upsilon_{max}$ (cm$^{-1}$): 2958 (aromatic CH), 2923 (aliphatic CH), 2853 (aliphatic CH), 1541 (aromatic C=C), 1505 (B–F), 1469 (aliphatic CH), 1409, (aliphatic CH), 1305 (C–N), 1190, 1155, 973, 807, 765, 726. MS (MALDI-TOF) *m/z* (%): 688 $[M + H]^+$, 669 $[M-F]^+$.

## 4. Results and discussion

### 4.1. Synthesis and structural characterization

9-Ethylcarbazole **1** was chosen for this study as a linker in order to generate new carbazole-based BODIPY systems. According to literature, the carbazole ring systems are easily functionalized and covalently linked to other molecules [30]. In particular, carbazole undergo Vilsmeier-Haack formylation to obtain an appropriate carbazole carbaldehyde using dimethyl formamide and phosphoryl chloride [29]. This reaction therefore provides a ready entry to suitable aldehyde precursors for the BODIPY synthesis. The first step was the formylation of 9-ethylcarbazole **1** by Vilsmeier-Haack conditions using phosphoryl chloride and dimethyl formamide to get mono- and diformyl carbazoles **2** and **3** [29]. With the 9-ehtyl-9H-carbazole-3-carbaldehyde (**2**) and 9-ehtyl-9H-carbazole-3,6-dicarbaldehyde (**3**) in hand, it was of interest to use 9-ehtyl-carbazole-3-carbaldehyde (**2**) as a monomeric model for the preparation of mono-BODIPY carbazole before extending the chemistry to bis-BODIPY carbazole. The synthetic strategy for the preparation mono-BODIPY carbazole involved the treatment of carbazole-3-carbaldehyde (**2**) with 2,4-dimethylpyrrole in the presence of trifluoroacetic acid followed by the oxidation of dipyrromethane derivatives to dipyrromethenes using DDQ and finally dipyrromethene precursors were transformed to compound **4** by boron trifluoride etherate in the presence of trimethylamine (Scheme 1). Identification of the compound **4** was performed through FT-IR, $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry and elemental analysis and the results were consistent with the predicted structures, as shown in the experimental section. The FT-IR, elemental analysis results and the mass spectral data for the newly synthesized carbazole substituted BODIPY **4** were consistent with the assigned formulations. The mass spectra of compounds **4**, was obtained by MALDI-TOF MS using 1,8,9-anthracenetriol as the MALDI matrix, and the spectrum revealed the peak groups representing the protonated molecular ions at 442 Da and molecular ion rupture fluor at 423 Da (Fig. 6). Well-resolved $^{1}H$ NMR spectra of **4** showed sets of signals for *meso*-carbazole protons ~ 7–8 ppm region. The pyrrole rings –CH protons appeared as sharp singlets ~5.9 ppm and $-CH_3$ protons ~2.5 ppm and ~1.3 ppm. The $-CH_3$ and $-CH_2$ protons on the carbazole were observed ~1.4 ppm as triplet and ~4.4 ppm as quarted respectively.

With the successful execution of monomeric model in hand, preparation of related bis-BODIPY carbazole system was examined. The BODIPY synthetic methodology was again employed for the preparation of compound **5** (Scheme 1). The targeted compound bis-BODIPY carbazole **5** was purified by column chromatography and/or preparative TLC techniques. The structure of the compound **5** was supported by FT-IR, $^{1}H$ and $^{13}C$ NMR spectroscopic data. The $^{1}H$ NMR spectrum in $CDCl_3$ showed the loss of two aldehyde CHO protons ~10.1 ppm as a result of BODIPY formation and displayed characteristic pyrrole -rings $CH_3$ protons ~1.3 and ~2.5 ppm as singlets. Further structural verification was obtained via mass spectrometry, with MALDI-TOF MS using 1,8,9-anthracenetriol as the MALDI matrix. The spectrum showed the peak groups representing the protonated molecular ions at 688 Da and molecular ion rupture fluor at 669 Da, consistent for the bis-BODIPY carbazole **5** (Fig. 6).

### 4.2. Photo-physical properties

#### 4.2.1. Electronic absorption spectra

The absorption spectra of **4** and **5** were recorded in dichloromethane and are shown in Fig. 1. Compounds **4** and **5** showed two major characteristic absorption bands at 330–350 and 503 nm. The spectra of the compounds **4** and **5** exhibited similar features to other carbazole compounds with a maximum absorption wavelength at 330 and 347 nm. The longer wavelength band corresponding to the $\pi-\pi^*$ transition of the BODIPY moiety appeared at ca. 503 nm [6].

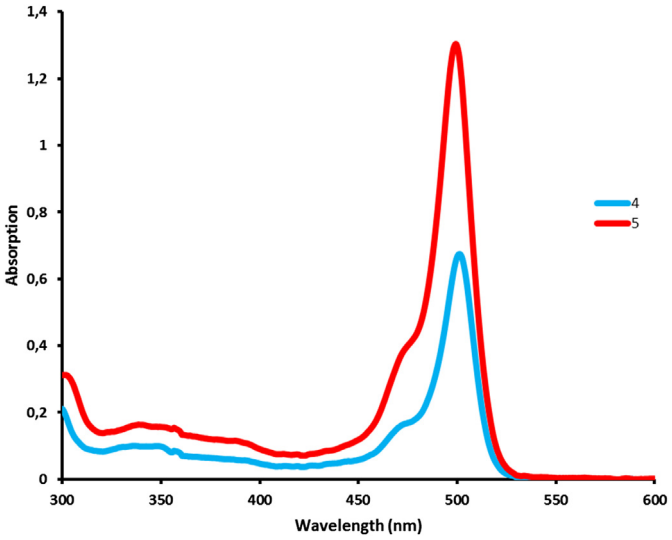


**Fig. 1.** Electronic absorption spectra of compounds **4** and **5** in dichloromethane.

#### 4.2.2. Fluorescence spectra

The fluorescence properties of compounds **4** and **5** were investigated in dichloromethane by both steady state and time resolved fluorescence techniques. The fluorescence emission spectra of compounds **4** and **5** were investigated with an excitation wavelength of 470 nm in dichloromethane at room temperature (Fig. 2). Fluorescence emission peaks were observed at around 512 nm for all the compounds in dichloromethane. The fluorescence spectra of **4** and **5** exhibit characteristic emission of BODIPYs such as narrow bandwidth, small stokes shift and high quantum yield. When comparing to these two carbazole-linked BODIPY dyes, the bis-BODIPY carbazole dye (**5**) showed higher fluorescence

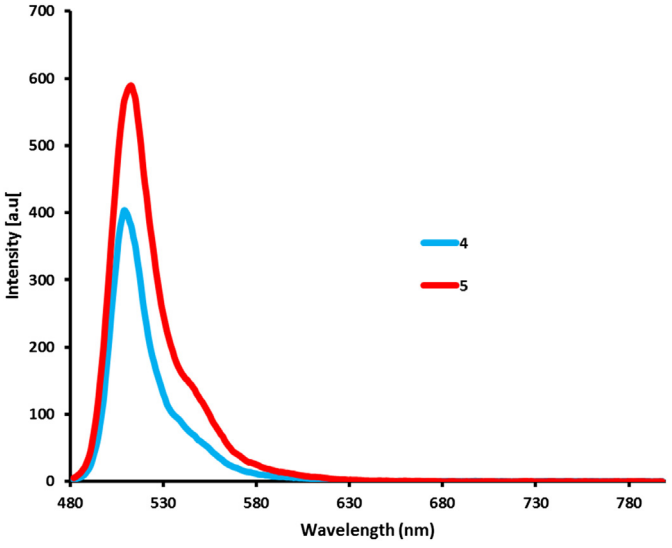


**Fig. 2.** Emission spectra of **4** and **5** in dichloromethane. Excitation wavelength = 470 nm.

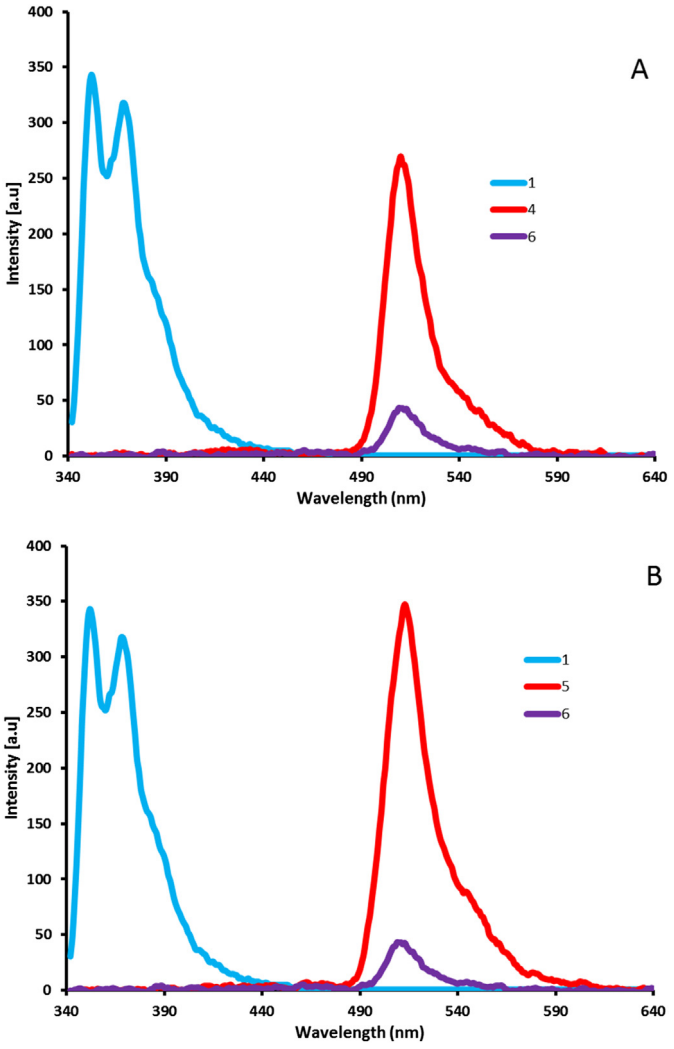


**Fig. 3.** Emission spectra of A) **1**, **4**, **6** and B) **1**, **5**, **6** in dichloromethane. Excitation wavelength = 330 nm.

emission than the mono-BODIPY carbazole dye (**4**). In the cases where two different dyes were used, it may result in fluorescence resonance energy transfer (FRET) [31,32]. Therefore **4** and **5** constitute a potential donor–acceptor pair in energy transfer. Indeed, all the compounds **4** and **5** exhibited an energy transfer phenomenon from carbazole unit to the BODIPY core. In order to obtain more information about the energy transfer, the emission spectrum of compound **4** with the emission spectra of their reference compounds **1** and **6** were measured under the same conditions ($5 \times 10^{-7}$ mol dm$^{-3}$ in dichloromethane, exc 330 nm) and are shown in Fig. 5A. In the emission spectrum of compound **4** excited from carbazole at 330 nm, there is significant quenching of donor emission at around 350 and 370 nm and enhancement in acceptor emission at around 510 nm. In addition to that, compound **6** excited at 330 nm has very weak emission at around 510 nm (Fig. 3A). In the emission spectrum of compound **5** excited from pyrene at 330 nm, similar results were obtained. There is a significant quenching of donor emission at around 350 and 370 nm and enhancement in acceptor emission at around 510 nm (Fig. 3B). This shows that all increase in emission at 510 nm is coming from energy transfer from donor groups (Fig. 3A, B). The fluorescence quantum yields of compounds 4 and 5 in dichloromethane are 0.0022 and 0.0026, respectively. According to Eq. (2), the energy transfer quantum yield values of **4** and **5** were estimated. The ΦENT values were found 0.90 for compound **4** and 0.88 for compound **5**, showing that this is a very efficient energy transfer process in **4** and **5**. The lifetimes were also measured with the time correlated single photon counting (TCSPC) technique in dichloromethane, excited at 470 nm. Our values of the lifetimes were found to be 4.926 and 6.073 ns respectively (Fig. 4). These results indicate a strong electronic interaction between carbazole and the BODIPY.

### 4.3. Biological properties

Following synthesis and characterization, our targeted mono- and bis-BODIPY carbazoles **4** and **5** were subjected to biological assessment against human colon cancer HT29 cell lines. In vitro cytotoxic activity of the compounds 4 and 5 were evaluated on HT29 cells in different concentrations. IC50 values of the novel carbazole-linked BODIPY were obtained as 21.7 and 8.3 ng/mL over 24 h, respectively. Although the mechanism of **4** and **5** in terms of its toxic effect has not been studied for this cell type yet, there is

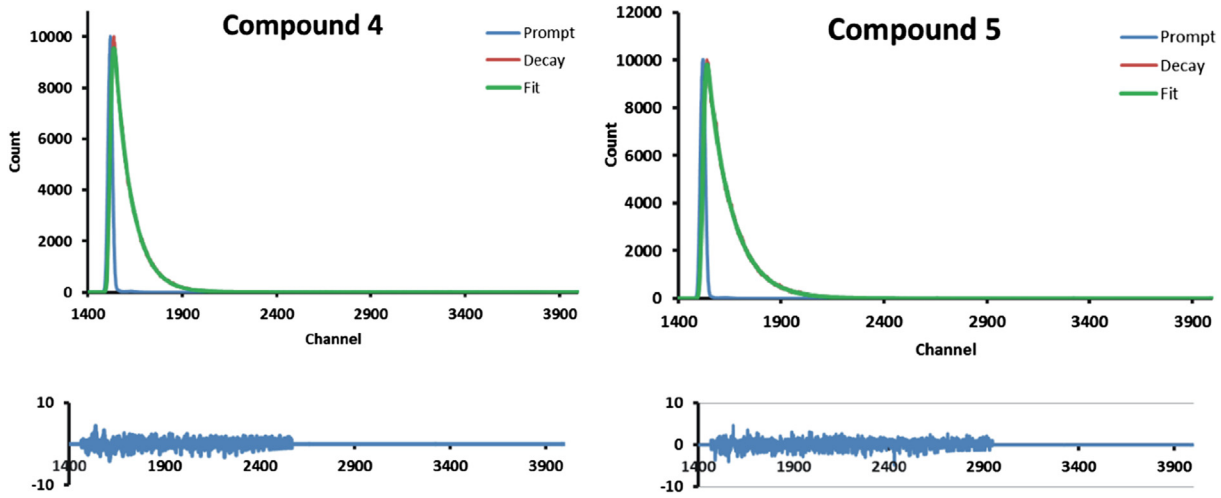


**Fig. 4.** The fluorescence decay profiles of compounds **4** and **5**. The Excitation wavelength used was 470 nm, collected at 512 nm.

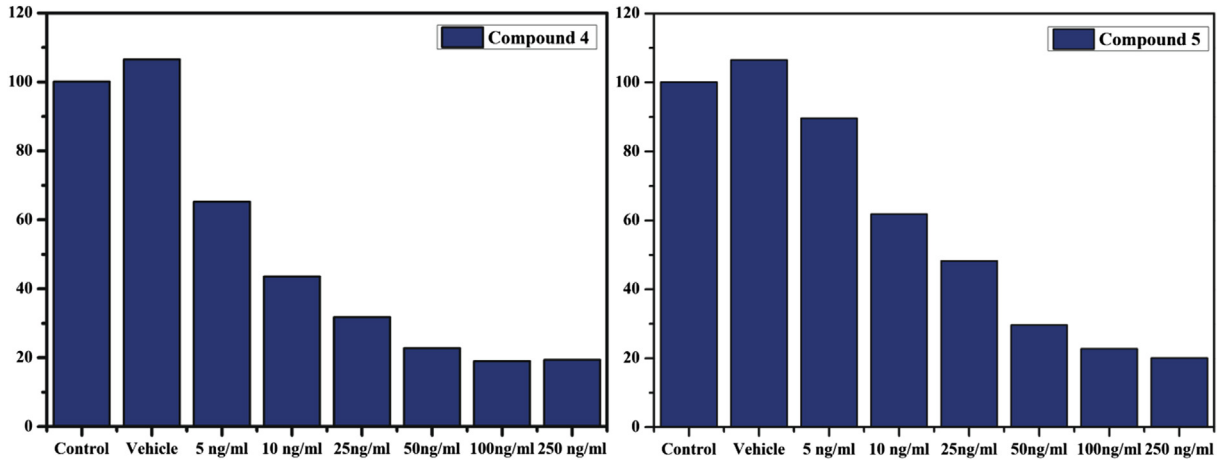


**Fig. 5.** Effects of the compounds 4 and 5 on viability of HT-29 Cells. Cell proliferation was determined by MTT assay HT-29 cells were treated with different concentrations of the compounds 4 and 5 (0, 5, 10, 25, 50, 100, 250 ng/mL) over 24 h.

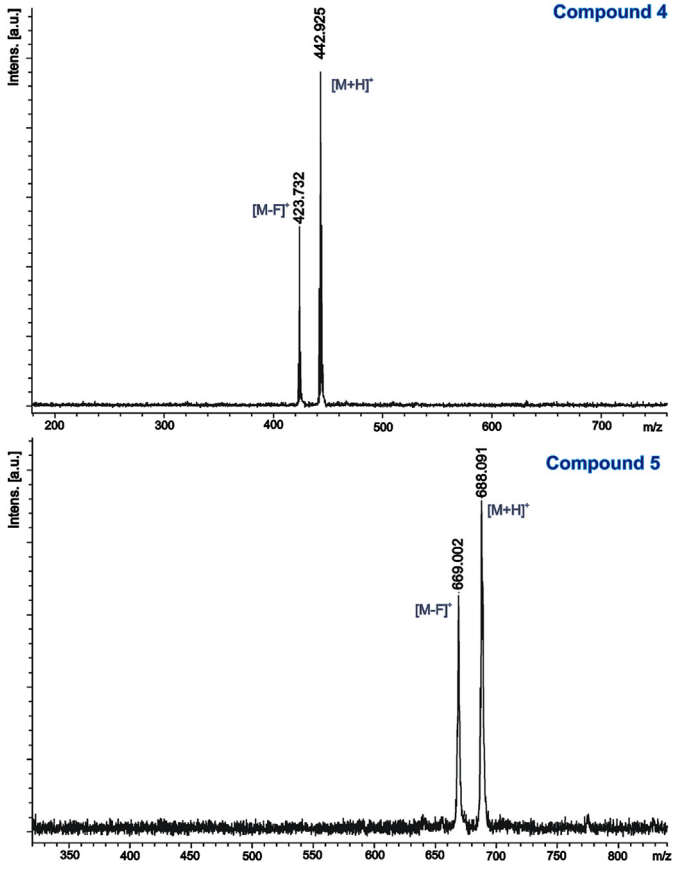


**Fig. 6.** Positive ion and linear mode MALDI TOF-MS spectra of **4** and **5** were obtained in 1,8,9-anthracenetriol (2 mg/mL THF) MALDI matrix using nitrogen laser accumulating 50 laser shots.

promising evidence displaying its potential in dose-dependent manner (Fig. 5).

## 5. Conclusion

In conclusion, the synthesis and characterization of carbazole linked boron-dipyrrins were successfully achieved. Absorption, emission, and time resolved fluorescence studies showed that energy transfer between the carbazole and BODIPY, as well as fluorescence behavior was better on compound **5** than compound **4**. A preliminary screening of the targeted BODIPY compounds developed throughout this body of work showed reasonable cytotoxic activity against HT29 cell lines.

## Acknowledgment

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