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Characterization of a NIR absorbing thienyl curcumin contrast agent for photoacoustic imaging

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Abstract

The synthesis and characterization of a bis(2-dimethylaminothien-5-yl)curcumin boron difluoride chromophore is presented. Photophysical, electrochemical and computational investigations establish the properties of its absorption in the Vis-NIR spectral range relative to established curcumin dyes. Application of this thienyl curcumin dye as a photoacoustic contrast agent is investigated against the dicarbocyanine Cy5 dye in the 675 – 735 nm excitation range.

Graphical abstract

Curcumin is a naturally occurring compound that is found in rhizomes of the *curcuma longa* plant with a diverse range of therapeutic applications. The unique photophysical properties of curcumin has recently aroused interest in its potential as a diagnostic probe for imaging modalities such as photoacoustic tomography (PAT) - a rapidly emerging technique used for cancer diagnosis with the capability of high spatial resolution and high contrast in deep tissue.^{1–6} Recently it has been determined that compounds with a high fluorescence quantum yield are capable of exhibiting an amplified PA contrast via an excited-state absorption mechanism.⁷ In fact, the boron difluoride derivative of the naturally occurring curcumin, curcuminBF₂ (**1**) is also capable of such a nonlinear PA emission having exhibited an amplified PA contrast relative to the linear absorber PA standard crystal violet.

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^{8, 9} For in vivo applications it is preferred to employ chromophores with a strong absorption in the biological window (680 nm – 980 nm). While progress has been made with BODIPY-based NIR absorbing PA contrast agents^{10–14}, to the best of our knowledge, even with significant interest in curcuminoid dyes for biological imaging applications and many efforts to structurally tune their photophysical properties, a NIR absorbing curcuminoid dye has yet to be reported.^{9, 15–18} We hypothesized that substitution of the 4-hydroxy-3-methoxyphenyl feruloyl group, found in the naturally occurring curcumin chromophore at the terminal position of its β -diketonate backbone with an electron-rich thiophene substituent may destabilize the HOMO level enough to decrease the band gap,¹⁹ thus shifting the absorbance maximum toward the NIR biological imaging window.²⁰ Indeed, thiophene is often utilized as an ideal π -linker for promoting electronic communication across donor- π -acceptor organic frameworks as its electron rich aromatic nature contributes to it many interesting properties including electron and hole conduction as well as charge stabilization, which has long made it of interest in organic electronics materials such as in polymers²¹, molecular wires^{22–24}, LEDs^{25–27} and even polymeric PA probes.^{28–30} The bis(4-dimethylaminophenyl)curcuminBF₂ dye (**2**) inspired us to investigate its bis(2-dimethylaminothien-5-yl)curcumin boron difluoride analogue (**3**). The 4-dimethylaminophenyl substituents in curcuminoid **2** have already been shown to induce a significant red-shift in the $S_0 \rightarrow S_1$ absorption relative to **1**, in addition to a significant increase in molar extinction coefficient. Thus, the target bis(2-dimethylaminothien-5-yl)curcuminBF₂ dye (**3**) was prepared using a previously established synthetic protocol. The structures of all three curcuminoids (**1** – **3**) are included in Figure 1.

The UV-Vis-NIR electronic absorption and fluorescence emission spectra of curcuminoids **1** – **3** were recorded in acetonitrile at room temperature and are presented in Figure 2.

Indeed, **3** exhibits a strong and broad $S_0 \rightarrow S_1$ based absorption band which stretches out to 750 nm, therefore possessing the most red-shifted absorbance of any curcumin dye reported to date. The benchmark curcuminBF₂ dye (**1**) exhibits a λ_{max} at 498 nm (fwhm = 3,207 cm⁻¹) in acetonitrile with a molar extinction coefficient of $\epsilon = 9.44 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Introduction of the electron-donating 4-dimethylaminophenyl group at the terminal ends of the π -conjugated bis-vinyl boron difluoride diketonate backbone in **2** results in a strong bathochromic shift of the absorption maxima to 596 nm (fwhm = 3,013 cm⁻¹) with a strong enhancement in absorptivity with $\epsilon = 1.39 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. Finally, switching the phenyl linker to the 2,5-substituted thiophene ring further shifts the absorption maximum to 675 nm (fwhm = 2,065 cm⁻¹) albeit with a slight decrease in absorptivity with $\epsilon = 8.35 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, comparable in intensity to **1**. Thienyl-bridged donor-acceptor dyes have been reported to have an increased transition dipole moment (μ_{ge}), relative to their phenyl-bridged counterparts, therefore this reduction in ϵ is most likely due to a reduced Franck-Condon factor, which may be attributed to the differing aromatic nature of the two ring structures.³¹ This observation is consistent with a similar trends of ϵ previously observed in related BODIPY molecules when heterocycles are substituted for phenyl.^{32–34} The emission profiles of **1** – **3** trends similar to their absorption spectra with maxima observed at 588 nm, 684 nm and 705 nm for **1**, **2** and **3** respectively. It is also noteworthy that the Stokes shifts reduce across the series from 3,074 cm⁻¹ for **1**, to 2,595 cm⁻¹ for **2** and to just 598 cm⁻¹ for

3. A narrowing Stoke's shift would be inconsistent with increasing donor strength for a series of dipolar chromophores, however, this is not uncommon for symmetric quadrupolar donor- π -acceptor- π -donor systems. The fluorescence quantum yield of **1** recorded in acetonitrile is modest at $\Phi_{\text{fl}} = 0.05$ ($^1\tau = 1.80$ ns) while **2** exhibits the strongest emission of the three dyes with $\Phi_{\text{fl}} = 0.22$ ($^1\tau = 0.74$ ns). This apparent increase in fluorescence quantum yield is likely due to the elimination of H-bonding upon substitution of the feruloyl group in **2**. In contrast, dye **3** exhibits a weaker fluorescence with $\Phi_{\text{fl}} = 0.03$ ($^1\tau = 0.90$ ns). This reduced fluorescence efficiency is likely due to a combination of its narrow Stoke's shift but also a probable enhanced intersystem crossing to its T_1 triplet state as a consequence of the heavier S-atoms in both of its thienyl rings.

To gain more insight into the electronic influence of the thiophene ring on the photophysical properties of **3** and the origin of electronic transitions in the electronic absorption spectra of dyes **1** – **3**, single-point energy time-dependent density functional theory (TDDFT) calculations were carried out following prior DFT optimization and frequency analysis. A quantitative comparison of frontier molecular orbital energies for each dye is presented in Fig. 3, which also includes a qualitative comparison of their HOMO and LUMO surfaces. A full description of the theoretical parameters employed is provided in the supporting information.

TDDFT analysis confirms that the HOMO–LUMO electronic transition is the sole contributor to the lowest energy $S_0 \rightarrow S_1$ absorption band of each dye. Consistent with experimental electronic absorption data, the DFT calculated HOMO–LUMO band-gaps narrow from **1** (2.14 eV) to **2** (1.78 eV) to **3** (1.68 eV). This was also confirmed by cyclic voltammetry electrochemical analysis where each dye exhibits irreversible oxidation and redox waves (Fig. 4). The reduction potentials show a slight cathodic shift at -1.18 V, -1.32 V and -1.40 V vs the ferricenium/ferrocene ($\text{Fc}^{+/0}$) redox couple for **1**, **2** and **3**, respectively, consistent with increasing electron donating character of their terminal substituents. A much stronger cathodic shift is observed across all three oxidation potentials at $+0.88$ V, $+0.45$ V and -0.01 vs. $\text{Fc}^{+/0}$ for **1**, **2** and **3**, respectively, confirming our hypothesis that a greater destabilization of the HOMO level, relative to the LUMO level, is inherent from the 2-dimethylaminothien-5-yl functional group and is overall responsible for the bathochromic shift observed in their electronic absorption spectra.

Having established the fundamental photophysical and electronic properties of **3**, optophotoacoustic z-scan (OPAZ) studies were conducted over a range of excitation wavelength (675 – 735 nm, Figs ESI-1 & ESI-2). Due to the negligible/weak absorption of both **1** and **2** at these wavelengths the 1'-diethyl-2,2'-dicarbocyanine iodide dye (Cy5, $\lambda_{\text{max}} \sim 707$ nm) was here used as a suitable reference based upon the popularity of cyanine dyes for PAI and the likeness of their absorption properties (Table ESI-1). As both dyes **3** and Cy5 exhibit similar absorption cross sections at ~ 688 nm in acetonitrile this was chosen as the wavelength of choice for comparison (o.d. = 0.3). Both **3** and Cy5 exhibit saturable absorber⁸ behaviour at 688 nm excitation over a range of laser fluences (5 – 35 mJ cm⁻²; Fig. ESI-3) allowing their PA response to be described by the simple linear relationship $\text{PA} = \epsilon C I \Phi_{\text{nr}} F$, where ϵ is the $S_0 \rightarrow S_1$ molar extinction coefficient, C is the sample concentration, I is the laser fluence, Φ_{nr} is the nonradiative decay quantum yield and F is the Gruneissen

coefficient; a parameter that quantifies a medium's ability to conduct sound.³⁵ The observed saturable absorber behaviour of both dyes is consistent with their weak fluorescence emission in acetonitrile under similar conditions (Table ESI-1). The slight depletion of optical absorption for **3** at increasing laser fluence is likely due to its slightly longer lifetime ($^1\tau = 0.90$ ns) relative to Cy5 ($^1\tau = 0.03$ ns). Nonetheless **3** still displays a slightly improved PA emission response relative to Cy5.

Subsequently both dyes were investigated by PAT also at 688 nm excitation. As illustrated in Fig. 6 the bis(2-dimethylaminothien-5-yl) curcuminoid dye **3** exhibits a very strong PA response, comparable to that of the Cy5 dye, with an excellent contrast against the blank solvent background.

Conclusions

The electronic properties of the bis(2-dimethylaminothien-5-yl) curcuminoid dye **3** have been probed using UV-Vis-NIR electronic absorption and fluorescence emission spectroscopies, in addition to electrochemical and density functional theory methods, to establish the nature of its low-energy electronic transition. OPAZ-scan and PAT studies have established a strong PA emission and contrast for **3** relative to the dicarbocyanine Cy5 reference dye indicating great promise for this class of naturally inspired organic chromophore for biomedical PAI applications. Future work will now be focussed towards the biocompatibility of **3** and related curcuminoid dyes with further tuning of their absorption properties into the extended NIR region of the spectrum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of the difluoroboron curcuminoid dyes **1** – **3**.

Figure 2. Electronic absorption (top) and fluorescence emission (bottom) spectra of dyes **1**, **2** and **3** recorded in acetonitrile at room temperature.

Figure 3.

Frontier molecular orbital energy levels (eV) and HOMO-LUMO surfaces (isofactor = 0.04), and oscillator strengths (f) as calculated by DFT and TDDFT analysis. Electron occupancy is removed for clarity thus HOMO and LUMO levels are highlighted in blue and red, respectively. DFT and TDDFT analysis of compounds **1–3** were performed with the B3LYP/6-311g(d,p)/acetonitrile-CPCM functional/basis set/solvent-model combination. The cis(d) keyword was used to account for configuration interaction in TDDFT calculations to determine oscillator strengths (f).

Figure 4.

Cyclic voltammograms of **1**, **2** and **3** recorded at 1 mM concentration under 1 atm of argon in 0.1 M Bu_4NPF_6 supporting electrolyte at a glassy carbon disc electrode (3 mm diameter) at a scan rate of $\nu = 0.10 \text{ V s}^{-1}$.

Figure 5. Photoacoustic emission of **3** and Cy5 recorded in acetonitrile at room temperature and plotted as a function of laser fluence at 688 nm pulsed ($\tau = 3$ ns) excitation.

Figure 6. Photoacoustic tomography (PAT) imaging of **3** and Cy5 recorded in acetonitrile at room temperature in 1 mm internal diameter borosilicate glass tubes.