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Synthesis of two-photon active cinnamoyl coumarins for high-contrast imaging of cancer cells and their photophysical characterization

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ABSTRACT

A series of two-photon (TP) active 4-dimethylaminocinnamoyl coumarins were synthesized. These compounds exhibit red shift in absorption and considerable Stokes shift in emission spectra in comparison to the parent coumarin. Large TP absorption cross-sections were measured for all the coumarins in dilute solutions. A correlation between the chemical structure and TP characteristics was established. TP confocal microscopy revealed that these coumarin derivatives can be internalized by cancer cells rendering them a potential candidate as a label in TP confocal imaging.

1. Introduction

Among all of the fluorescence microscopy techniques developed and optimized during the last few decades [1], the two-photon fluorescence (TPF) microscopy is of specific interest because of its potential applications in clinical imaging [2], immunology [3] and bio-engineering [4]. In TPF, a fluorescent molecule absorbs two near IR (NIR) photons simultaneously and emits a photon in the visible spectral region. Unlike conventional one-photon confocal microscopy, the TPF emission is highly localized reducing the photo damage and improving the spatial contrast in imaging. The widespread applications of the TPF microscopy have led to several design strategies and synthesis of organic molecules with large TPA cross-sections (δ) [5,6]. The δ value for organic molecules is usually small, thus it is desirable to search for newer organic molecules with high absorption cross sections. We have explored the possibility of using novel coumarin derivatives as molecular probes for confocal microscopy based bio-imaging.

Coumarins, a member of benzopyrone family, are a group of plant-derived polyphenolics and are well-known for their antibacterial, anticoagulant, antioxidant, anticancer, and enzyme inhibition properties [7–10]. These compounds are relatively non-toxic as no adverse effects of coumarin have been reported [11]. Apart from being biologically active, coumarins also possess considerable optical properties e.g. molar extinction coefficient, quantum efficiency of fluorescence, and emission in the visible spectral region [12]. These features render coumarin and its derivatives potential candidates for high contrast in vivo imaging of tumor cells. However, the application of coumarins for biological imaging has not been well-established. This could be attributed to the fact that the coumarins typically show small Stokes shift of the order of 10–30 nm [13–15]. However, Stokes shifts of 80 nm and larger are desirable to minimize the background from the exciting radiation and to reduce autofluorescence in cells [16]. In addition, molecules with high quantum efficiency of fluorescence are considered as prospective candidates for high contrast in vivo imaging. Therefore, fluorophores with a higher Stokes shift and high quantum yield (Φ) are promising candidates for NIR fluorescence bioassays, which can enhance the detection sensitivity to a large extent. With this

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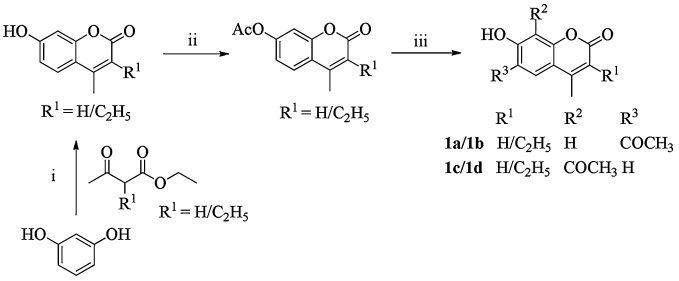


Scheme 1. Reagents and conditions: (i) $CH_3COCHR^1COOC_2H_5$, conc. H_2SO_4, 30 °C; (ii) $(CH_3CO)_2O$, DMAP, THF, 30 °C; (iii) $AlCl_3$, 125–170 °C.

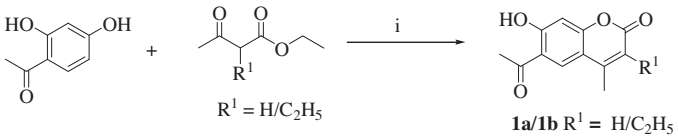
perspective we have designed and developed a series of fluorescent coumarin derivatives for bio-imaging applications.

Wu et al. reported that 1,3-diarylprop-2-enones (chalcones) exhibit promising nonlinear optical properties [17]. Therefore, it is expected that the incorporation of a 3-aryl-2-propenoyl (cinnamoyl) moiety on the coumarin skeleton might lead to new materials that are fluorescent and also possess nonlinear optical properties. A few such derivatives are reported in the literature; however, in almost all the cases the cinnamoyl moiety was kept confined at the C-3 or C-4 position of the coumarin skeleton (lactone ring) [18]. In addition, their capabilities for fluorescence imaging have not been fully explored [18]. Herein, we have synthesized C-6 and C-8 substituted 4-dimethylaminocinnamoyl coumarins and studied their photophysical and TP bio-imaging properties.

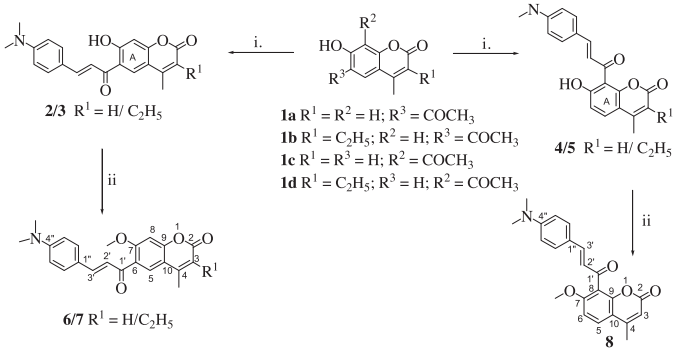
2. Results and discussion

2.1. Chemistry

The reaction of β-resorcinol with corresponding ethylacetoacetate under Pechmann condensation yielded 7-hydroxy-3-alkyl-4-methyl-2*H*-benzopyran-2-one [19]. Acetylation of these compounds with acetic anhydride under catalytic amount of dimethylaminopyridine (DMAP) in THF resulted the corresponding 7-acetoxy derivatives. The C-6 acetyl (**1a**, **1b**) and C-8 acetyl (**1c**, **1d**) coumarin derivatives (Scheme 1) were then obtained from 7-acetoxy-3-alkyl-4-methylcoumarin via Fries migration in 1:9 ratio, respectively [20,21]. As the yield of the 6-acetyl-7-hydroxy-4-methyl-2*H*-chromen-2-one is low via Fries migration, we have synthesized it by following another literature procedure (Scheme 2) in about 60% yield [22]. The structural assignment of **1a** and **1c** was unambiguously carried on the basis of ^{1}H NMR. For compound **1a** the protons H-5 and H-8 were observed as singlets at δ 7.94 and 6.82 respectively, however for compound **1c** the two



Scheme 2. Reagents and conditions: (i) $HClO_4{\cdot}SiO_2$, 130 °C, 30–90 min, solvent free.



Scheme 3. Reagents and conditions: (i) DMB (4-dimethylaminobenzaldehyde), ethanol, reflux; (ii) CH_3I, dimethylformamide, K_2CO_3, 30 °C.

ortho-coupled protons H-5 and H-6 were observed at δ 7.60 and 6.82 (J = 8.8 Hz) respectively. The isomeric compounds **1b** and **1d** were also characterized on a similar basis.

The C-6 and C-8 substituted 4-dimethylaminocinnamoyl coumarins (**2**–**5**) were synthesized by piperidine catalyzed Claisen–Schmidt condensation of 4-dimethylaminobenzaldehyde (DMB) with 6/8-acetyl-7-hydroxy-3-alkyl-4-methylcoumarin. Compounds **6**, **7**, and **8** in turn were synthesized by carrying *O*-methylation of compounds **2**, **3**, and **4** respectively using methyl iodide (Scheme 3). The use of a weak base piperidine was preferred because chalcones having hydroxyl group at *ortho* to carbonyl group in ring A may get cyclized to the corresponding flavanones under stronger alkaline conditions. The compounds reported here are novel except **2** [23] and **4** [24] and fully characterized.

2.2. Photochemistry

The optical properties of all of the six 4-dimethylaminocinnamoyl coumarins (**2**, **4**–**8**) were studied. For spectroscopic measurement, the compounds were dissolved in DMSO (1 mg/mL) and diluted further to get the required concentration for optical studies. A quartz cuvette of 1 cm path-length was used to record both absorption and one-photon fluorescence spectra. All of the compounds exhibited strong absorption maxima

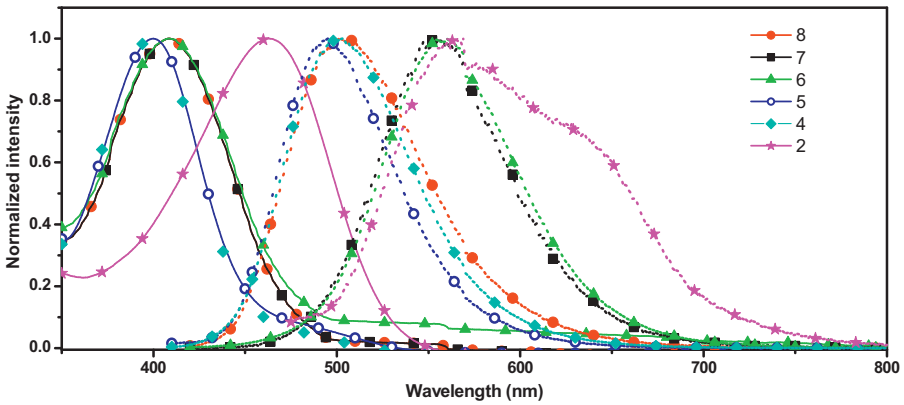


Fig. 1. Normalized one-photon absorption (solid lines) and emission (dotted lines) spectra of cinnamoyl coumarins.