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SYNTHESIS OF COUMARIN DERIVATIVES BASED FLUORESCENT DYE TOWARDS THE DETECTION OF CANCER CELL

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Abstract

Fluorescent readout is a powerful technique for the detection of cell cancer. The fluorescent biomarkers (or dyes) should exhibit the ability of the light emission when excited, and the biocompatibility. This work reports on the synthesis of coumarin derivatives based fluorescent dyes. The characterization results, obtained by NMR, HR-MS, absorption and emission spectroscopic method, revealed that these compounds are promising candidates for fluorescent biomarker applications.

Keywords. Coumarin derivatives, fluorescence, biomarker, cancer cell, Palladium-catalyzed direct arylation.

1. INTRODUCTION

Cancer, term used to describe collections of the uncontrolled growth and spread of abnormal cells that can arise from almost any type of tissue cells [1], is a leading cause of death worldwide [2-3]. In fact, one is more likely to survive if the cancer is found in early stage [4]. Fluorescent based assays, using fluorescent biomarkers (or fluorescent dyes), is a powerful technique for the detection of cancer cells. The photophysical-photochemical properties, biocompatibility, stability, solubility and relative easy synthesis of fluorescent dyes play an important role in the high sensitive cancer cell detection [5].

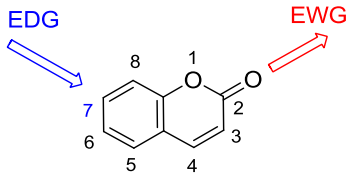


Figure 1: Coumarin's "push-pull" structural feature

Coumarin (*2H*-chromen-2-one) and its derivatives, constitute a very large and important family of heterocyclic compounds, that have received considerable attention in recent years because of their application as components of sensors, switches [6], tunable dye lasers [7], therapeutic agents [8, 9] and fluorescence probes [11]. Despite its small size, the extended π-conjugation of benzopyrone system provides desirable spectrophotometric properties for coumarins. Furthermore, coumarin core substituted at 7-position with an electron-donating group (EDG) has been reported to exhibit strong fluorescence [11]. It is well known that an extended π-conjugation system connecting the electron-withdrawing carbonyl moiety with the electron-donating substituent of 7-position can generate a "push-pull" structural feature (figure 1) [11].

Electron donors such as amino, hydroxyl and methoxy groups at the 7-position were demonstrated to enhance the fluorescence of coumarins [11]. Indeed, 7-aminocoumarins, especially 7-diethyl-aminocoumarins with high quantum yield, excellent stability, large Stokes' shift have been used as optical brighteners and fluorescent probes in physicochemical and biochemical studies. Recent studies have focused on increasing the π-conjugation system by attaching various aromatic substituents to coumarin core at the 3-position via both theoretical and experimental studies [12-14].

Polycyclic aromatic sulfur heterocycles have been widely known in the studies about organic semiconductor materials. They are organic compounds with intrinsic fluorescence whose properties can be modified by changing the number of aromatic rings and the nature of substituents. Furthermore, these heterocycles can be easily derivatized with active groups, which react with biomolecules to form bioconjugation in fluorescent probes [11]. Thus, these organic materials are also

fluorescent markers for biological application [15].

To improve fluorescent properties and create new fluorescent dyes based on the coumarin skeleton, we have performed synthetic protocols to substitute the 3-position of 7-diethylaminocoumarin scaffold by a C-C cross-coupling reaction of Palladium-catalyzed direct arylation [16]. Using this transformation, the attachment of thieno[3,2-*b*]thiophene, benzo[1,2-*b*:5,4-*b'*]dithiophene moieties were expected to alter significantly the fluorescence properties of 7-diethylaminocoumarin.

2. EXPERIMENTAL

2.1 General information

The Palladium-catalyzed reactions were performed in Schlenk tubes under argon in anhydrous *N*,*N*-dimethylacetamide (DMA, 99.8 %, Sigma-Aldrich) and potassium acetate (KOAc, > 99 %, Sigma-Aldrich). Column chromatography was performed with silica gel (230–400 mesh). ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were acquired with samples in $CDCl_3$ solution at 298 K. Chemical shifts are reported in ppm and were referenced to $CDCl_3$ (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.0 ppm).

HRMS: The mass spectra were recorded at the Materials Chemistry Laboratory, Faculty of Chemistry of Hanoi University of Science, Vietnam National University with a Thermo Scientific LQT Orbitrap XL instrument with an ESI, [M+H] source.

Emission Spectroscopy: The emission spectra were recorded at the Nano Optoelectronic Laboratory, Advance Institute for Science and Technology (AIST), Hanoi University of Science and Technology with a Fluorescence Spectroscopy Systems of Horiba.

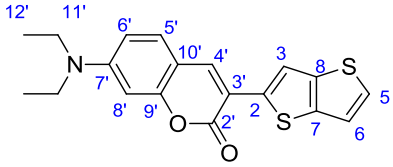
2.2 Preparation of Intermediates

3-Bromo-7-(diethylamino)-*2H*-chromen-2-one (**Cou-Brom**) was prepared via two steps from 4-(diethylamino)-2-hydroxybenzaldehyde [20-21], thieno[3,2-*b*]thiophene (**TT**) was prepared as Fuller's process [22] and benzo[1,2-*b*:4,5-*b'*] dithiophene was obtained like the reference [23].

2.3. Procedure for the Synthesis

The reaction of **Cou-Brom** (1 equiv.), **TT** or **BDT** (2-3 equiv.), $PdCl(C_3H_5)$ dppb [dppb = bis-(diphenylphosphino)-butane] (3 mol-%) and KOAc (5 equiv.) in DMA (10 mL) under argon in a Schlenk tube at 140-150 °C for 24-36 h. The arylated products were purified by silica gel column chromatography.

7-(diethylamino)-3-(thieno[3,2-*b*] thiophen-2-yl)-*2H*-chromen-2-one (Cou-TT)



From **Cou-Brom** (592 mg, 2 mmol) and thieno[3,2-b] thiophene **TT** (840 mg, 6 mmol), **Cou-TT** was obtained in 55% yield as an orange solid (335 mg).

^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.95 (s, 1H, H-3), 7.82 (s, 1H, H-4'), 7.35 (d, 1H, ^{3}J = 5.0 Hz, H-5), 7.30 (d, 1H, ^{3}J = 9.0 Hz, H-5'), 7.21 (d, 1H, ^{3}J = 5.5 Hz, H-6), 6.59 (q, 1H, J^{3} = 9.0 Hz, ^{4}J = 2.5 Hz, H-6'), 6.50 (d, 1H, J^{4} = 2.5 Hz, H-8'), 3.40 (q, 4H, *J* = 7.0 Hz, H-11'), 1.22 (t, 6 H, *J* = 7.0 Hz, H-12').

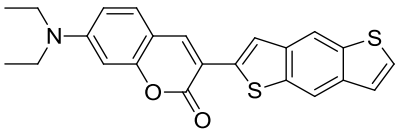
^{13}C NMR (125 MHz, $CDCl_3$): δ = 160.8 (C-2'), 155.5 (C-10'), 150.6 (C-9'), 140.0 and 139.8 (C-2 / C-8), 138.7 (C-4'), 128.9 (C-5'), 127.2 (C-5), 119.4 (C-6), 117.8 (C-3), 114.8 (C-7), 109.3 (C-6'), 108.7 (C-7'), 97.0 (C-8'), 44.8 (C-11'), 12.4 (C-12').

HSQC ($^{1}J_{CH}$): H-3 with C-3, H-4' with C-4', H-5 with C-5, H-5' with C-5', H-6 with C-6, H-6' with C-6', H-8' with C-8', H-11' with C-11', H-12' with C-12'.

HMBC ($^{2}J_{CH}$ and $^{3}J_{CH}$): H-3 with C-7, C-3'; H-4' with C-5', C-3', C10', C-2'; H-5 with C-6, C-8; H-5' with C-4', C-9', C-10'; H-6 with C-5, C-8; H-6' with C-8', C-7'; H-8' with C-7', C-10'.

HR-MS (ESI–[M+H]): calculated for $C_{19}H_{18}NO_2S_2$ 356.0778; found 356.0789.

3-(benzo[1,2-*b*:4,5-*b'*] dithiophen-2-yl)-7-(dietylamino)-*2H*-chromen-2-one (Cou-BDT)



From **Cou-Brom** (592 mg, 2 mmol) and **BDT** (760 mg, 4 mmol), **Cou-BDT** was obtained in 60% yield as an orange solid (487 mg).

^{1}H NMR (125 MHz, $CDCl_3$): δ = 8.23 (s, 1H), 8.21 (s, 1H), 8.15 (s, 1H), 7.91 (s, 1H), 7.45 (d, 1H, *J* = 5.5 Hz), 7.35 (m, 2H), 6.62 (q, 1H, ^{3}J = 8.5 Hz, ^{4}J = 2.5 Hz), 6.53 (d, 1H, ^{4}J = 4.0 Hz), 3.45 (q, 4 H, 7.0 Hz), 1.23 (t, 6 H, 7.0 Hz).

^{13}C NMR (500 MHz, $CDCl_3$): 160.0, 155.8, 150.9, 138.8, 138.6, 138.3, 137.6, 137.3, 136.2, 129.2, 126.9, 123.0, 122.0, 117.0, 116.1, 109.4,

108.7, 97.076, 44.9, 12.5.

HR-MS: (ESI–[M+H]): calculated for $C_{23}H_{20}NO_2S_2$ 406.0935; found 406.0947.

3. RESULTS AND DISCUSSION

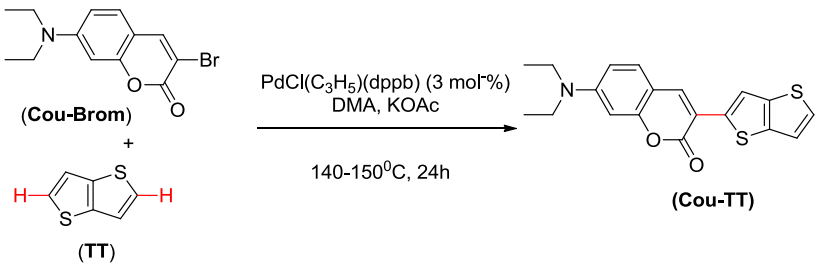
3.1. Synthesis of Coumarin derivatives

Two new coumarin derivatives presented in this report contain 7-(diethylamino)-3-(thieno[3,2-*b*] thiophen-2-yl)-*2H*-chromen-2-one (**Cou-TT**) and 3-(benzo[1,2-*b*:4,5-*b*']dithiophen-2-yl)-7-(diethylamino)-*2H*-chromen-2-one (**Cou-BDT**). The method for the synthesis of these compounds is the palladium-catalyzed direct arylation, green and straightforward strategy in comparison to the traditional methods reported by Negish [17], Suzuki [17], Heck [17] or Stille [18]. In this work, the advantage of the developing method is that it does not require the preliminary synthesis of one or two organometallic derivatives [16]. By using this arylation, the 3-bromine atom of 3-bromo-7-diethylaminocoumarin (**Cou-Brom**) was replaced by thieno[3,2-*b*] thiophene-2-yl or benzo[1,2-*b*:4,5-*b'*] dithiophene-2-yl moiety to give **Cou-TT** or **Cou-BDT**, respectively.

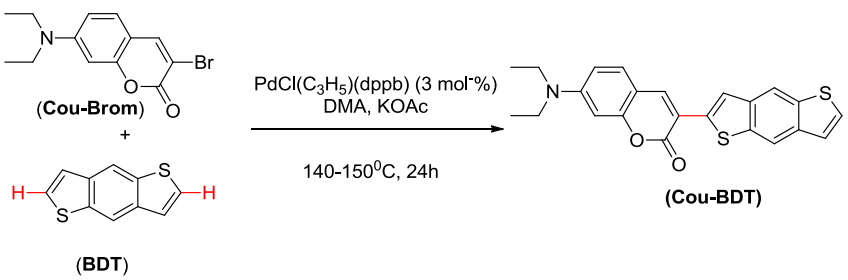
In this transformation, a number of parameters, including reaction temperature, transition metal as catalyst and the ratio of the reactants, were investigated for optimal reaction effectiveness, table 1. Two catalysts were used for the direct arylation: (1) palladium(II)acetate ($Pd(OAc)_2$) and (2) $PdCl(C_3H_5)$ (dppb). However, our attempts to prepare **Cou-TT** and **Cou-BDT** in the presence of $Pd(OAc)_2$ was not successful. Only 7-diethylaminocoumarin was obtained from the reduction of 3-brom-7-diethyl-aminocoumarin (**Cou-Brom)** (the formation of this by-product was demonstrated by the ^{1}H-NMR spectrum). Therefore, the reaction was catalyzed by the catalyst (2) with other conditions in table 1, both **Cou-TT** and **Cou-BDT** were formed in good yields.

3.2. Spectra

The chemical structures of the products were elucidated by ^{1}H NMR, ^{13}C NMR and HR-MS (extra HMBC and HSQC were also recorded for **Cou-TT** compound). All the ^{1}H and ^{13}C chemical shifts and other characteristics of the spectra are given in the experimental section. The resonance signals in ^{1}H NMR and ^{13}C NMR spectrums have confirmed the appearance of a thieno[3,2-*b*]thiophen-2-yl moiety in **Cou-TT**, a benzo[1,2-*b*:4,5-*b'*]dithiophen-2-yl in **Cou-BDT** and the 7-(diethylamino)-*2H*-chromen-2-one-3-yl moiety (or 7-diethylaminocoumarin-3-yl) in both of the compounds. The assignments of the hydrogen and carbon atoms based on HSQC and HMBC spectra have demonstrated more clearly the structure of **Cou-TT**. The exact mass of **Cou-TT** and **Cou-BDT** recorded by high resolution mass spectrometry (HR-MS) with ESI – [M+H] source are 356.0789 and 406.0947, respectively. By using single crystal X-ray diffraction for analogous derivatives, we have investigated the selectivity for the arylation of thieno[3,2-*b*]thiophene (**TT**) and benzo[1,2-b:4,5-*b'*]dithiophene (**BDT**) structures (the investigation is now on progress and will be reported in due course).



Scheme 1: Pd-catalyzed arylation of thieno[3,2-*b*] thiophene



Scheme 2: Pd-catalyzed arylation of benzo[1,2-*b*:4,5-*b'*] dithiophene

Table 1: Reaction conditions of the Palladium-catalyzed direct arylation

Compound	Catalyst	Ratio of the reactants	T (^oC)	Yield* (%)
Cou-TT	$PdCl(C_3H_5)$dppb (3 mol-%)	**Cou-Brom**:**TT** 1:3 (equiv.)	140-150	55
Cou-BDT	$PdCl(C_3H_5)$dppb (3 mol-%)	**Cou-Brom**:**BDT** 1:2 (equiv.)	140 -150	60

(*) Isolated yield.

3.3. Photophysical Properties

The UV-vis absorption spectra of the two synthetic 7-diethylaminocoumarin derivatives (**Cou-TT**, **Cou-BDT**) were recorded in chloroform solutions at room temperature with their normalized intensities (figure 2, table 2). We have also included the photophysical data of 3-bromo-7-diethylaminocoumarin (**Cou-Brom**) in ethanol solutions as a parent chromophore, (table 2) to compare the photophysical properties of the parent chromophore to those of our synthetic dyes.

All of these dyes have dual absorptions in the region from 240 to 600 nm, but their spectra were dominated by intense absorption bands in the visible light range. **Cou-Brom** exhibits strong absorption at the wavelength of λ = 400 nm, while the absorption bands of the two corresponding arylated products, **Cou-TT** and **Cou-BDT,** appear at longer wavelength, λ = 401 nm and 463 nm, respectively. Thus, the new synthetic coumarin dyes show bathochromic shift in comparison with the parent coumarin.

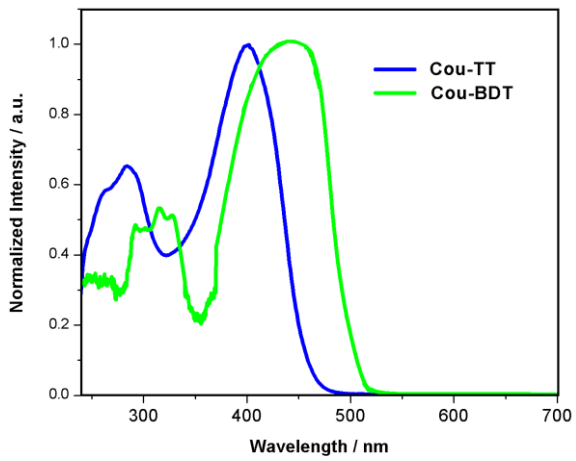


Figure 2: Normalized absorption spectra of **Cou-TT** and **Cou-BDT** in $CHCl_3$ (10^{-4} mol/lit) at 298 K

Both of the studied compounds, **Cou-TT** and **Cou-BDT,** have single emissions in $CHCl_3$ solution under excitation with 355 nm and 400 nm, respectively. The emission spectra of **Cou-TT** has the maximum emission energy at λ_{em} = 508 nm, while the emission spectra of **Cou-BDT** is narrower with λ_{em} = 518 nm (table 2, figure 2). Compared with the emission of **Cou-Brom**, both of the new dyes also show bathochromic shifts from 400 to more than 500 nm. These red-shifted emissions mirror the similar bathochromic trends observed in the absorption spectra. Thus, changes to the nature of the added group, which based on polycyclic sulfur aromatics, allow a significant modification of the emission wavelength of the coumarin core.

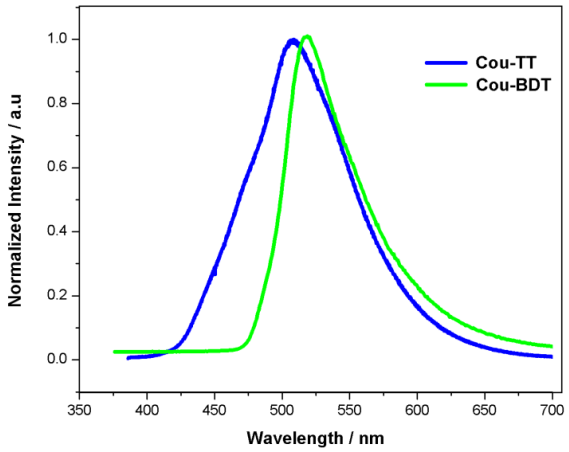


Figure 3: Normalized fluorescence spectra in $CHCl_3$ (10^{-4} mol/lit) at 298 K with **Cou-TT**: λ_{exc} = 355 nm; **Cou-BDT**: λ_{exc} = 400 nm

Our experimental results express that the introduction of the thieno[3,2-*b*]thiophene (**TT**), benzo[1,2-*b*:5,4-*b'*]dithiophene (**BDT**) moieties into the 7-diethylaminocoumarin scaffold at the 3-position causes a great change to its fluorescence property. Because of the small and planarity moieties, similar small steric hindrance of **TT** and **BDT** in the new dyes does not much affect the oscillator strength in the excited state, which is one of the most important causes of decreasing fluorescence. On the other hand, the extension of the π-conjugation leads to the increases of electron donating-accepting ability in the "push-pull" structural feature. As a result, the enhancement of intramolecular charge-transfer transition causes the

bathochromic shifts of absorption and emission spectra. This is also related to the difference in the photophysical properties of both compounds. Shorter extended π-conjugation system in **TT** moiety leads to the emission of dye **Cou-TT** at shorter wavelength.

Table 2: Photophysical data of 7-diethylaminocoumarin derivatives

	UV-vis	Fluorescence	Stokes' shift
Cou-Brom [19]	255; 324; 400 (ethanol)	482 (ethanol)	82
Cou-TT	284 (0.65); 401 (1.00)	508	107
Cou-BDT	327 (0.56); 463 (1.00)	518	55

We have also calculated the Stokes' shifts of the new dyes (**Cou-TT** and **Cou-BDT**) and compare with the Stokes' shift of the parent coumarin (**Cou-Brom**) (table 2). The **Cou-TT** dye exhibits the larger Stokes' shift than the **Cou-Brom** dye, while the other dye has the smallest Stokes' shift. Interestingly, a very large Stokes' shift of the **Cou-TT** dye (more than 100 nm) revealed that this compound is a promising candidate as a component of fluorescent probes.

4. CONCLUSION

In this report, two new coumarin derivatives were prepared straightforwardly by Palladium-catalyzed direct arylation. The combination of a coumarin skeleton containing 7-diethylamnion as an electron-donating group with fused polycyclic sulfur-containing aromatic rings creates new dyes, which show strongly fluorescence at room temperature. Especially, 7-(diethylamino)-3-(thieno[3,2-*b*]thiophen-2-yl)-*2H*-chromen-2-one (**Cou-TT**), with the emission wavelength at 508 nm (the visible green light) with a very large Stokes' shift (more than 100 nm), is a potential fluorescent dye for the development of fluorescence probes.

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