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Synthesis and fluorescence study of 6,7-diaminocoumarin and its imidazolo derivatives

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ABSTRACT

Novel 6,7-diamino-4-methylcoumarin and derived imidazolocoumarins were synthesized and their absorption and fluorescence properties were recorded in solvents of varying polarity. 6,7-Diamino-4-methylcoumarin is highly fluorescent, with a quantum yield of 0.78 in dioxane. The fluorescence intensity of 6,7-diamino-4-methylcoumarin, when mixed with different carbonyl group containing compounds, decreased. Based on the interaction of this diaminocoumarin with the carbonyl compound a fluorescent sensor with high selectivity towards electron rich benzaldehydes and cinnamaldehyde was developed.

1. Introduction

Coumarins comprise a group of natural compounds found in a variety of plant sources. Coumarins are attractive fluorescent molecules due to their extended spectral range, high emission quantum yields, photostability and good solubility in common solvents. As a consequence of these features coumarins are widely used as laser dyes [1–3]. Typical features of these derivatives can be readily modified by introduction of substituents in the chromene ring as well as in the annulated benzene ring, imparting flexibility for various applications including as laser dyes [4], fluorescence markers [5], fluoride ion sensor [6], cyanide ion sensor [7,8] and metal ion sensors [9–12]. 2,3-Diaminonaphthalenes were employed as nitric oxide sensors [13,14]. There is increasing evidence that unsaturated aldehydes generated endogenously during the degradation process of biological molecules, such as lipid peroxidation, glycation and amino acid oxidation, are involved in the onset and progression of much pathology such as cardiovascular atherosclerosis, long-term complications of diabetes and neurodegenerative diseases [15,16]. Owing to the immense importance of coumarin dyes in the sensor field and the endogenous production of aldehydes, we have developed 6,7-diamino-4-methylcoumarin (**VI**) as a sensor for the detection of selected benzaldehydes. Therefore, in this paper we report the synthesis and photophysical properties of hitherto unreported **VI** and four of its imidazolo derivatives. The multistep linear synthesis is initiated from 3-aminophenol. The absorption and fluorescence properties of these compounds were studied in ten solvents of different polarity and the relative quantum yields were evaluated employing 9,10-diphenylanthracene as the standard. The photophysical properties of **VI** are drastically altered in presence of small amounts of a few select aromatic aldehydes having electron donating substituents at either the *ortho*- or *para* position and the α,β-unsaturated aldehyde, cinnamaldehyde.

2. Experimental

2.1. Materials

Spectroscopic grade organic solvents were obtained from Finar Chemicals. Starting and other chemicals and reagents were purchased from Sigma–Aldrich unless otherwise stated and were used without further purification. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminum plates (Merck).

2.2. Instrumentation

The melting points reported were uncorrected and determined in Polmon instrument (model no. MP-96). The IR spectra were

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recorded on Bruker Infrared model Tensor-27. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 300 MHz Ultrashield spectrometer. The EI mass spectra were recorded on a VG micro mass 7070-H. The LCMS spectra were recorded on LCMS 2010, Shimadzu, Japan. LC conditions were used C18 column, mobile phase methanol: water (90:10 v/v) and at UV–vis wavelength 254 nm. In the mass spectrum ESI technique is used. UV–vis spectra were recorded on Elico SL 159 UV–vis spectrophotometer. Steady state fluorescence was investigated on Shimadzu RF-5301PC spectrofluorophotometer with 5 nm excitation and emission slit widths at 25 °C employing 1 cm path length quartz cell. Elemental composition was determined by elemental analyzer, Elementar, Vario EL model. The pH measurements were carried out on a Global digital pH meter.

2.3. Synthesis of imidazolocoumarins

2.3.1. Synthesis of 3-carbethoxyaminophenol (**II**)

Ethylchloroformate (0.9 mL, 9.17 mmol) was added in one portion to a stirred suspension of m-aminophenol (1 g, 9.17 mmol) in ethyl acetate (15 mL). A white precipitate formed immediately. The reaction mixture was stirred for 2 h at room temperature. The amine hydrochloride precipitate was removed by filtration. Ethyl acetate was removed under reduced pressure to obtain colorless crystals of 3-carbethoxyaminophenol (1.4 g, 84.6%). m.p. 94–96 °C [17].

2.3.2. Synthesis of 7-carbethoxyamino-4-methylcoumarin (**III**)

3-Carbethoxyaminophenol (2 g, 11.04 mmol) and ethylacetoacetate (1.70 mL, 13.25 mmol) were suspended in 70% sulfuric acid (30 mL) and were stirred at room temperature for 4 h. The solution was poured in to an ice–water mixture (100 mL) resulting in the formation of a precipitate which was collected and crystallized from ethanol to give 7-carbethoxyamino-4-methylcoumarin (2.26 g, 83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.78 (s, 1H, –NH), 6.79 (d, 1H, J_{meta} = 7.4 Hz), 6.66 (d,, 1H, J_{meta} = 2.2 Hz), 6.54 (dd,, 1H, J_{ortho} = 7.4 Hz, J_{meta} = 2.2 Hz), 5.58 (s, 1H), 3.86 (q, 2H, $-OCH_2$), 2.33 (s, 3H, $-CH_3$), 1.40 (t, 3H, $-CH_3$). mp 185–187 °C [17].

2.3.3. Synthesis of 6-nitro-7-carbethoxyamino-4-methylcoumarin (**IV**)

Aluminum nitrate nonahydrate (1.29 g, 1.5 mmol) was added portionwise to 7-carbethoxyamino-4-methylcoumarin (1 g, 4.04 mmol) in acetic anhydride (25 mL) over a 10 min period and stirred at room temperature for overnight. After 12 h the reaction mixture was poured in to ice cold water (100 mL). The resulting yellow precipitate was filtered and washed with water. The solid was further purified by column chromatography employing hexane:ethyl acetate (9:1 v/v). Yield (0.54 g, 45.7%), m.p. 162–164 °C. Yellow color, ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.08 (s, 1H, –NH), 8.59 (s, 1H, aromatic), 8.48 (s, 1H, aromatic), 6.28 (s, 1H), 4.31 (q, 2H, $-OCH_2$), 2.47 (s, 3H, $-CH_3$), 1.38 (t, 3H, $-CH_3$). IR (KBr): ν_{max}/cm^{-1} 3318 (νNH) 1729 (νCO), 1716 (νCO). Mass spectral data: m/z = (M + 1) 293.

2.3.4. Synthesis of 6-nitro-7-amino-4-methyl-2H-chromen-2-one (**V**)

Deprotection of **IV** was carried out by heating 6-nitro-7-carbethoxyamino-4-methylcoumarin (1 g, 3.42 mmol) under reflux for 4 h in a mixture of ca. H_2SO_4 (3.05 g) and acetic acid (2.87 g). After cooling, the mixture was poured in to water (50 mL) and let stand overnight. The resulting suspension was made slightly basic with 50% NaOH with cooling by addition of ice chips. The yellow precipitate was filtered and washed with ice water (2 × 30 mL). Yield (0.65 g, 86.5%), m.p. >300 °C. Dark yellow color, ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.74 (s, 2H, NH_2, D_2O exchanged), 8.31 (s, 1H, aromatic), 6.80 (s, 1H, aromatic), 6.15 (s, 1H), 2.36 (s, 3H, $-CH_3$). IR (KBr): ν_{max}/cm^{-1} 3350 (νNH), 1726 (νCO). Mass spectral data: m/z = 221(M + 1). Elemental analysis (found C 54.38, H 3.37, N 12.61% $C_{10}H_8N_2O_4$ required C 54.55, H 3.66, N 12.72%).

2.3.5. Synthesis of 6,7-diamino-4-methyl-2H-chromen-2-one (**VI**)

6-Nitro-7-amino-4-methyl-coumarin (1 g, 4.54 m mol) was heated under reflux for 4 h with (0.89 g, 6.81 m mol) of tin in conc. hydrochloric acid (15 mL). After cooling, the mixture was poured in to water (50 mL) and let stand overnight. The resulting suspension was made slightly basic with 50% NaOH with cooling by addition of ice chips and extracted with ethyl acetate (3 × 100 mL). All the organic layers were combined and dried over sodium sulphate. Ethyl acetate was removed under vacuum to get yellow solid. Yield (0.2 g, 23.5%), m.p. 237–241 °C. Yellow color, ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.72 (s, 2H, NH_2, D_2O exchanged), 6.76 (s, 1H, aromatic), 6.48 (s, 1H, aromatic), 5.80 (s, 1H), 4.88(brs, 2H, NH_2, D_2O exchanged), 2.23 (s, 3H, $-CH_3$). ^{13}C (75 MHz, DMSO-d_6): δ = 161.9, 154.1, 148.5, 141.7, 132.1, 109.7, 107.9, 99.5, 18.52. IR (KBr): ν_{max}/cm^{-1} 3390 (νNH), 1677 (νCO). Mass spectral data: m/z = 191(M + 1). Elemental analysis found (C 62.94, H 5.22, N 14.45% C_6H_6 N_2O_2 required C 63.15, H 5.30, N 14.73%).

2.3.6. Synthesis of 8-methylchromeno[7,6-d]imidazol-6(3H)-one (**VIIa**)

6,7-Diamino-4-methyl-coumarin (0.1 g, 0.526 mmol) was heated under reflux for 4 h in formic acid (10 mL). After cooling, the mixture was poured in to water (30 mL) and the resulting suspension was made slightly basic with the addition of 5% $NaHCO_3$ with cooling by addition of ice chips. The TLC pure brown precipitate was filtered and washed with ice water. Yield (76 mg, 73%), m.p. >300 °C. Yellow color, ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.19 (s, 1H), 7.99 (s, br, 1H, –NH), 7.65 (s, 1H, aromatic), 7.48 (s, 1H, aromatic), 6.18 (s, 1H), 2.56 (s, 3H, $-CH_3$). ^{13}C (75 MHz, DMSO-d_6): δ = 160.2, 155.0, 153.4, 149.2, 140.9, 136.5, 114.4, 111.6, 110, 100.8, 18.7. IR (KBr): ν_{max}/cm^{-1} 3367 (νNH), 1720 (νCO). Mass spectral data: m/z = 201(M + 1). Elemental analysis (found C 65.82, H 4.10, N 13.73% $C_{11}H_8$ N_2O_2 required C 66.00, H 4.03, N 13.99%).

2.3.7. Synthesis of 2,8-dimethylchromeno[7,6-d]imidazol-6(3H)-one (**VIIb**)

Following the above method, compound **VIIb** was prepared by heating under reflux in acetic acid in place of formic acid. Yield (77 mg, 69%), m.p.>300 °C. Yellow color, ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.92 (s, br, 1H, –NH), 7.71 (s, 1H, aromatic), 7.32 (s, 1H, aromatic), 6.13 (s, 1H), 2.52 (s, 3H, $-CH_3$), 2.44 (s, 3H, $-CH_3$). ^{13}C (75 MHz, DMSO-d_6): δ = 160.5, 155.4, 153.6, 149, 140.8, 136.2, 114.6, 111.9, 110, 100.5, 18.7, 14.7. IR (KBr): ν_{max}/cm^{-1} 3362 (νNH), 1726 (νCO). Mass spectral data: m/z = (M + 1) 215. Elemental analysis (found C 67.13, H 4.47, N 12.92% $C_{12}H_{10}$ N_2O_2 required C 67.28, H 4.71, N 13.08%).

2.3.8. Synthesis of 8-methyl-2-phenylchromeno[7,6-d]imidazol-6(3H)-one (**VIIc**)

6,7-Diamino-4-methyl-coumarin (0.02 g, 0.105 m mol) was taken in DMF (5 mL) solvent, to this benzaldehyde (0.012 g, 0.105 m mol) was added and was heated under reflux for 4 h. After cooling, the DMF was removed under reduced pressure. The resulting residue was washed with hexane (20 mL) to obtain yellow colored product. Yield (18 mg, 65%), m.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.14–8.15 (m, 2H), 7.53–7.60 (m, 5H), 6.29 (s, 1H), 2.54 (s, 3H, -CH_3). IR (KBr): ν_{max}/cm^{-1} 3360 (νNH), 1726 (νCO). Mass spectral data: m/z = 277.28 (M + 1). Elemental analysis

(found C 73.64, H 4.17, N 10.03% $C_{17}H_{12}N_2O_2$ required C 73.90, H 4.38, N 10.14%).

*2.3.9. Synthesis of 8-methyl-2-styrylchromeno[7,6-d]imidazol-6(3H)-one (**VIId**)*

6, 7-Diamino-4-methyl-coumarin (0.02 g, 0.105 m mol) was taken in DMF (5 mL) solvent, to this cinnamaldehyde (0.013 g, 0.105 m mol) was added and was heated under reflux for 4 h. After cooling, the DMF was removed under reduced pressure. The resulting residue was washed with hexane (20 mL) to obtain dark yellow colored product. Yield (21 mg, 68%), m.p. >300 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.94 (s, 1H, NH), 7.75–7.68 (m, 2H), 7.46–7.24 (m, 7H), 6.29 (s, 1H), 2.52 (s, 3H, $-CH_3$). IR (KBr): ν_{max}/cm^{-1} 3358 (νNH), 1724 (νCO). Mass spectral data: m/z = 303.28 (M + 1). Elemental analysis (found C 75.19, H 4.42, N 9.05% $C_{19}H_{14}N_2O_2$ required C 75.48, H 4.67, N 9.27%).

3. Results and discussion

7-Aminocoumarin is a bright fluorescent molecule. However on annulation with a pyrrole ring at 7,8-positions its fluorescence was enhanced significantly [18]. As the **VI** is a diaminocoumarin, it is expected to be fluorescent and a potential reagent for the determination of carbonylic compounds [19].

*3.1. Synthesis of **VI** and imidazolocoumarin*

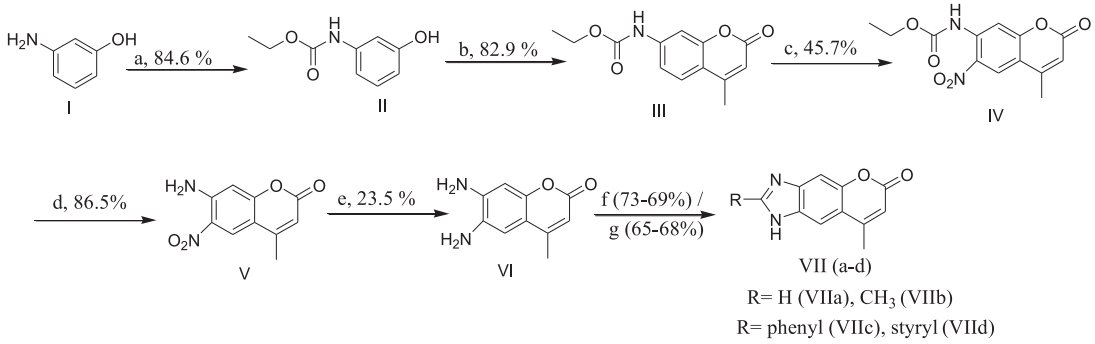
A variety of synthetic methodologies are known for the synthesis of benzimidazoles, e.g. the condensation of ortho phenylenediamine (OPDA) with either benzaldehydes or benzoic acids [20,21]. In the present study **VI** is considered as an analogue of OPDA and its synthesis was carried out following Scheme 1. Subsequently, the **VI** was condensed with acetic acid, formic acid, benzaldehyde and cinnamaldehyde to yield the respective imidazolocoumarins in good yields.

3-Aminophenol was treated with ethylchloroformate to give the protected aminophenol, ethyl-3-hydroxyphenylcarbamate, **II**. Its melting point of 94–96 °C coincided with the literature value [17]. Compound **II** was further treated with ethylacetoacetate to give the protected amino coumarin **III**. Compound **III** was characterized by melting point, proton NMR and mass spectra. In the ^{1}H NMR spectrum of **III**, the coumarin characteristic C3-H proton was observed as a singlet signal at 5.58 ppm. The singlet signal at 2.33 ppm was assigned to the methyl protons attached to C-4. The coumarin **III** was nitrated with aluminum nitrate nonahydrate in the presence of acetic anhydride to give 6-nitro-7-aminoprotected-4-methylcoumarin, **IV**. Its structure was confirmed by melting point, proton NMR and mass spectra. In the proton NMR spectrum disappearance of C-6 proton and appearance of singlet signals at 8.60 and 8.49 ppm confirms formation nitro derivative. The signal at 8.60 ppm was assigned to the proton *ortho* to the nitro group.

6-Nitro-7-protectedaimno-4-methylcoumarin, **IV** was heated under reflux in acetic acid and conc. sulfuric acid mixture to deprotect the amino group. The deprotection was confirmed by its proton NMR spectrum. In the proton NMR spectrum, the amine protons were observed at 7.80 ppm, C-5 and C-8 protons gave singlet signals at 8.37 and 6.85 ppm, respectively. Disappearance of the ethyl group proton signals in the aliphatic region indicated the formation of 6-nitro-7-amino-4-methyl-2H-chromen-2-one, **V**.

6-Nitro-7-amino-4-methylcoumarin, **V** was treated with Sn, HCl at 100 °C to give 6,7-diamino-4-methylcoumarin, **VI**. The structure of 6,7-diamino-4-methylcoumarin was confirmed by NMR and IR spectroscopy and mass spectrometry and microanalysis. In the proton NMR spectrum broad singlet signals at 7.81 ppm and 4.93 ppm are due to two amino group protons attached to C6 and C7. The C-8 and C-5 carbon attached protons gave singlet signals at 6.57 ppm and 6.84 ppm, respectively. The C-3 attached proton gave a singlet signal at 5.80 ppm and C-4 carbon attached methyl protons gave a singlet signal at 2.23 ppm. In the ^{13}C NMR spectrum, the lactone carbonyl carbon gave a signal at 161.9 ppm, C-3 carbon at 154.1, the coumarin oxygen adjacent carbon gave a signal at 148.5. The amino group ipso carbon atoms gave signals at 141.7 and 132.1 ppm. In the mass spectrum, the molecular ion peak observed at 191 [M + 1] indicates the formation of 6,7-diamino-4-methylcoumarin.

6,7-Diamino-4-methylcoumarin, **VI** was treated with formic acid, acetic acid, benzaldehyde and cinnamaldehyde separately to yield respective imidazole derivatives, **VII(a–d)**. In the ^{1}H NMR spectrum of **VIIa**, the imidazole ring carbon attached proton was observed as a singlet signal at 8.19 ppm, imidazole ring nitrogen attached proton was observed at 7.99 as a broad singlet. The C-9, C-4 and C-7 carbon attached protons appeared as singlets at 7.65, 7.48 and 6.18 ppm, respectively. The methyl protons attached to coumarin gave a singlet at 2.56 ppm. In the ^{13}C NMR spectrum of **VIIa**, the lactone carbonyl carbon gave a signal at 160.2 ppm, C-2 carbon at 155.0, C-8 carbon at 153.4 and coumarin oxygen adjacent carbon gave a signal at 149.2. The amino attached ipso carbons gave signals at 140.9, 136.5 ppm. In the mass spectrum of **VIIa**, a molecular ion peak observed at 201 [M + 1] and indicates the formation of imidazolocoumarin **VIIa**.



a. Ethyl chloroformate, EtOAC, rt, 4 h, b. Ethyl acetoacetate, 70% H_2SO_4, rt, 4 h, c. Al $(NO_3)_3.9H_2O$, acetic anhydride, rt, 12 h, d. H_2SO_4, AcOH, 110°C, 4 h, e. Sn, HCl, 100°C, 2 h, f. Formic acid / acetic acid, reflux, 6 h, g. Benzaldehyde / cinnamaldehyde, DMF, 90°C, 4 h

Scheme 1. Synthesis of 6,7-diamino-4-methylcoumarin and its imidazole derivatives.

In the ^{1}H NMR spectrum of **VIIb**, the imidazole ring methyl protons were observed as a singlet at 2.52 ppm and in its mass spectrum, a molecular ion peak at *m*/*z* 215 [M + 1] indicates the formation of imidazolocoumarin, **VIIb**.

In the ^{1}H NMR spectrum of **VIIc**, the imidazole substituted phenyl protons were observed as a mutiplet at 7.53–7.60 ppm and in its mass spectrum, a molecular ion peak at *m*/*z* 277 [M + 1] indicates the formation of imidazolocoumarin, **VIIc**. Similarly when everything being equal in the ^{1}H NMR spectrum of VIId, the imidazole substituted phenyl protons resonated as a multiplet at 7.46–7.24 ppm and the two ethylinic protons signal was merged in the aromatic multiplet signal at 7.46–7.24 ppm. Further the structure of VIId was established by its molecular ion peak at *m*/*z* 303 [M + 1].

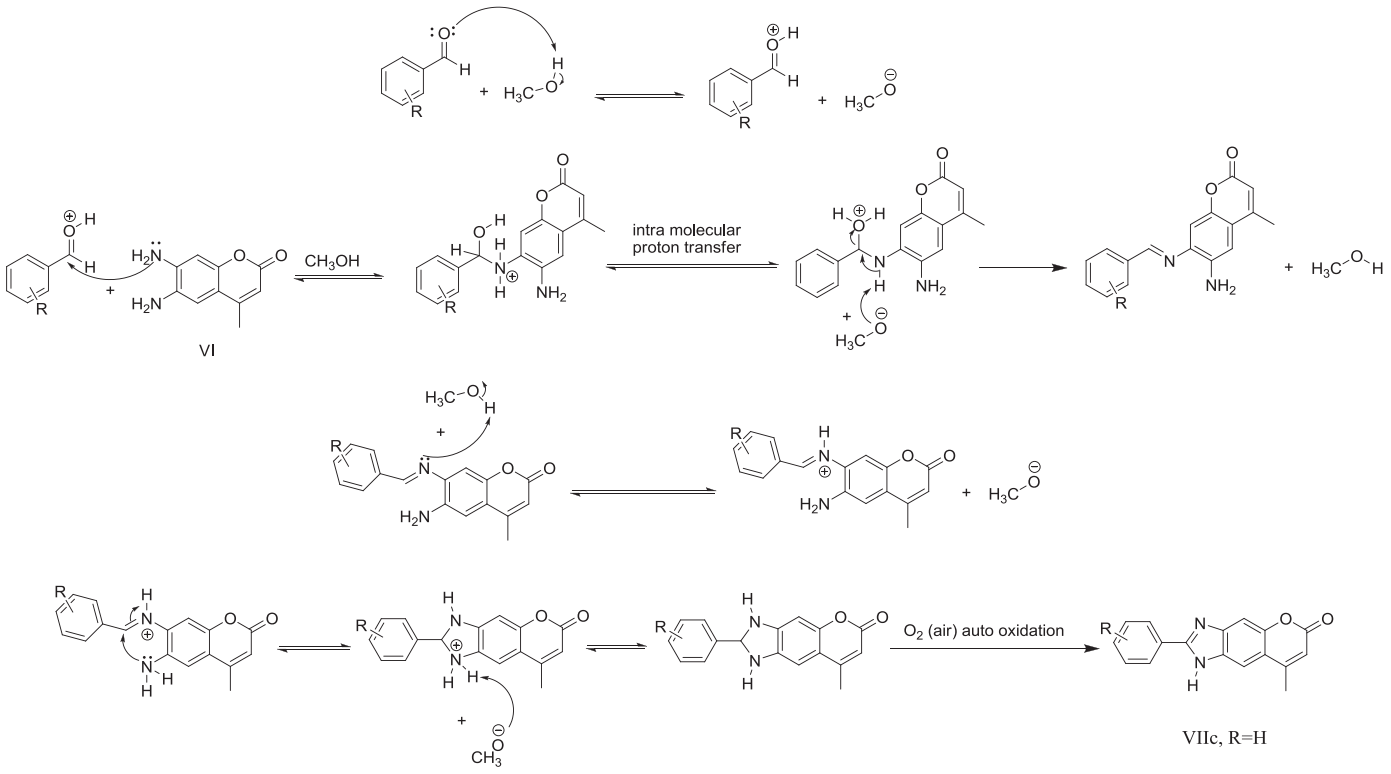
The mass spectra of **VII** clearly indicates that the final product is imidazolocoumarin and not the dihydroimidazolocoumarin or imine. A possible mechanism for the formation of imidazolocoumarin is given in Scheme 2. Hidenori et al. reported that when OPDA was reacted with benzaldehyde the intermediate dihydroimidazole that was obtained unstable and it was readily converted to benzimidazole by aerial oxidation [22–24]. It is further confirmed by the LC-MS studies that when the LC-MS of the mixture of **VI** and benzaldehyde and **VI** and cinnamaldehyde was taken the former gave a peak at *m*/*z* 277 [M + 1] and the later gave a peak at *m*/*z* 303 [M + 1], respectively indicating that the corresponding dihydroimidazole was not present.

3.2. Electronic absorption spectral study

In Fig. 1 the electronic absorption spectra of **VI** in six different solvents are shown. From Fig. 1 it can be noticed that the longer wavelength absorption maximum of **VI** in chloroform solvent is at 326 nm and in dimethylsulfoxide it is red shifted to 389 nm exhibiting solvatochromism. The spectral data obtained in the electronic absorption studies are given in Table 1.The longer wavelength absorption maxima of **VI**, **VIIa**, **VIIb**, **VIIc** and **VIId** are varying in the range of 326–389 nm, 307–329 nm, 321–327 nm, 340–346 nm and 360–374 nm, respectively. It can be noticed that the electronic absorption maxima of imidazolocoumarins, **VII**, depend on the substituents present in the imidazole part of the molecule. This may be due to the change in the basic absorbing chromophore unit. The conversion of **VI** to imidazolocoumarin brings a change in the electronic transition from coumarin chromophore to benzimidazole chromophore. Compound **VIId** has absorption maxima in between 360 nm and 374 nm in various solvents. The absorption maximum at longer wavelength in **VIId** may be due to the extended styryl chromophore moiety while there is no such extended chromophore is present in VIIa and its absorption maximum observed was between 307 nm and 329 nm in different solvents. Compound **V** is an immediate precursor of **VI** and has a nitro group in a complimentary position which resulted in the absorption maximum shifting to a longer wavelength than the latter. The solvent polarity and the hydrogen bond between the solvent and coumarin make the absorption wavelength occurring at longer wavelength while the hydrogen bond between the solvent and the amino nitrogen atom of imidazole ring has an opposite effect on the position of the long wavelength absorption maximum.

3.3. Fluorescence study

7-Aminocoumarins are the most important subset of coumarins with wide spread applications as fluorescent probes [25,26]. Coumarin fluorescent probes exhibit significant solvatochromism and has been an area of intense investigation for almost 40 years. The main aim of the present study is to investigate the photophysical and photochemical properties of **VI**, **VI** and subsequently its utility in the detection of carbonyl containing compounds. **VI** is a structural analogue of ortho phenylenediamine (OPDA) and can



Scheme 2. Possible mechanism for the formation of benzimidazole ring.

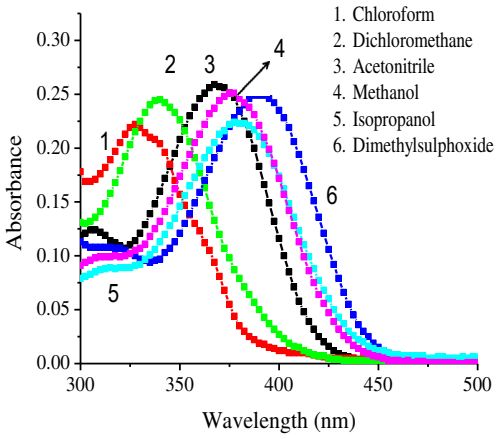


Fig. 1. The UV–visible absorption spectra of **VI** [2.75×10^{-5} M] in different solvents at 25 °C.

readily yield benzimidazole system on treatment with a carboxyl compound. The synthesis of 2-substituted benzimidazoles involves the treatment of 1,2-phenylenediamines either with carboxylic acids [27] or their derivatives (nitriles, imidates, or orthoesters) [28], under acidic conditions and sometimes combined with very high temperatures (i.e., polyphosphoric acid, 180 °C) or the use of microwave irradiation [29]. The benzimidazoles were also often generated from the condensation of phenylenediamines with aldehydes [30] under oxidative conditions [31] using various oxidative and catalytic reagents, such as nitrobenzene (high-boiling point oxidant/solvent) [32], $PhI(OAc)_2$ [33], 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) [34], $NaHSO_3$ [35], H_2O_2/HCl [36], and $Na_2S_2O_5$ [37], air [24], sulfamic acid [38], $FeCl_3 \cdot 6H_2O$ [39], $Sc(OTf)_3$ [40], $KHSO_4$ [41], TsOH/graphite and N,N-dimethylaniline/graphite [42].

Table 1
Spectral properties of **VI** and imidazolocoumarins.

Compound number	Solvent	λ_{abs} (nm)	λ_{ex}(nm)	λ_{em} (nm)	Stokes shift $\Delta\nu$ (cm^{-1})	Φ_f
VI	1,4-Dioxane	366	370	468	5660	0.783
	Chloroform	326	339	413	5285	0.526
	Ethyl acetate	366	369	474	6003	0.550
	Dichloromethane	345	340	419	5545	0.697
	Acetone	373	370	483	6324	0.535
	Acetonitrile	367	370	485	6409	0.463
	Methanol	375	378	502	6675	0.265
	Ethanol	378	378	498	6375	0.383
	Isopropanol	378	379	497	6265	0.338
	DMSO	389	387	498	5759	0.488
	Water (pH7.4)	368	368	516	7800	0.140
VIIc	1,4-Dioxane	344	348	404	3984	0.097
	Chloroform	345	345	388	3213	0.260
	Ethyl acetate	343	349	417	4672	0.090
	Dichloromethane	346	346	389	3195	0.193
	Acetone	340	349	417	4672	0.101
	Acetonitrile	341	349	417	4672	0.104
	Methanol	343	348	421	4900	0.261
	Ethanol	343	348	421	4900	0.283
	Isopropanol	342	346	419	5036	0.276
	DMSO	346	353	421	4576	0.165
	Water (pH7.4)	345	345	424	5401	0.182
VIId	1,4-Dioxane	360	363	418	3625	0.156
	Chloroform	369	360	429	4460	0.015
	Ethyl acetate	365	367	423	3607	0.262
	Dichloromethane	367	370	431	3825	0.018
	Acetone	368	368	422	3477	0.284
	Acetonitrile	367	369	423	3607	0.244
	Methanol	366	366	422	3425	0.225
	Ethanol	366	365	422	3700	0.247
	Isopropanol	364	364	420	3663	0.231
	DMSO	374	373	430	3554	0.492
	Water (pH7.4)	364	364	430	4429	0.205

The effect of solvent polarity on the photophysical properties of **VI** and **VII** was investigated in different organic solvents of varying polarity. Table 1 (supplementary information tables, Tables S1, S2) summarizes the spectral characteristics, i.e., absorption maxima (λ_A), excitation maxima ($\lambda_{ex.max}$), fluorescence maxima ($\lambda_{flu.max}$), Stokes shift ($\Delta\nu$ (cm^{-1})) and fluorescence quantum yield (Φ) of **VI**, **VIIa**, **VIIb**, **VIIc** and **VIId** in various solvents.

The relative fluorescence quantum yield, Φ is evaluated by employing Eq. (1) [43]:

$$\Phi_{unk} = \Phi_{std}\left(\frac{I_{unk}}{I_{std}}\right)\left(\frac{A_{unk}}{A_{std}}\right)\left(\frac{\eta_{unk}}{\eta_{std}}\right)^2 \quad (1)$$

where Φ, Φ_{std}, I_{unk}, I_{std}, A_{std}, A_{unk}, η_{unk} and η_{std} are the fluorescence quantum yields, the integral of the emission intensities, the absorbance at the excitation wavelength and the refractive indexes of the corresponding solvents of the unknown samples and the standard, respectively. 9,10-Diphenylanthracene was used as standard ($\Phi = 0.9$) [43].

Compound **VI** is highly fluorescent while its precursors are less fluorescent. The fluorescence spectra of **VI** in different solvents are shown in Fig. 2. From Fig. 2, it can be observed that the **VI** exhibited a single emission maximum in all solvents except in dichloromethane. The single emission maximum in chloroform is at 413 nm and occurs at shorter wavelength when compared to the remaining solvents. In dichloromethane, two emission maxima were obtained. Among these two emission maxima, one is characteristic to that obtained in chloroform at 413 nm and the other is characteristic to that obtained in the remaining solvents at longer wavelength, at about 500 nm. Similar to the 7-aminocoumarin, **VI** with two amino groups experienced a strong red-shift in polar solvents and correlate well with the hydrogen bonding. The H-bond acceptor nucleophilic solvents also caused a strong red-shift in the emission maximum of **VI**. This can be attributed to the preferential solvation in the excited state [44]. From Table 1 it can be observed that the Stokes shift vary in between 5285 cm^{-1} in chloroform and 7800 cm^{-1} in water (pH 7.4). In polar protic solvents, the Stokes shift was maximum. This is largely due to the occurrence of fluorescence emission maxima at longer wavelengths. In polar aprotic solvents like DMSO, acetone and acetonitrile the emission maxima

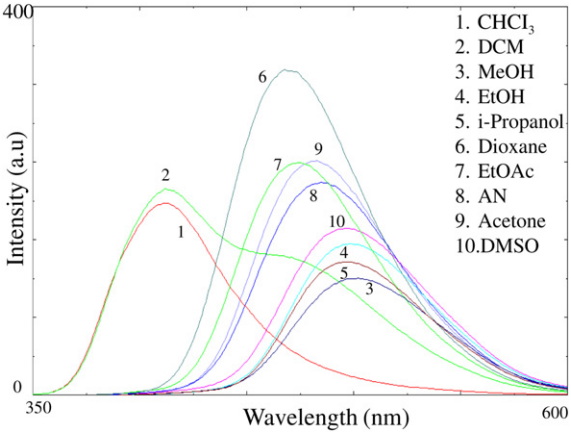


Fig. 2. The fluorescence spectra of **VI** [2.75×10^{-5} M] in different solvents at 25 °C.

also occurred at longer wavelength. But in the case of chlorinated solvents, chloroform and dichloromethane, the emission maxima were blue shifted. The fluorescence quantum yield of **VI** varied between 0.14 in water (pH 7.4) and 0.78 in dioxane solvent. The fluorescence quantum yield of **VI** in polar protic solvents like water (pH 7.4), methanol, ethanol and isopropanol is diminished and appears at 0.14, 0.265, 0.383 and 0.338, respectively. This can be attributed to an increased nonradiative decay rate in protic solvents [45]. The order of fluorescence quantum yield in the solvents investigated is 1,4-dioxane > DCM > acetone > ACOEt > $CHCl_3$>DMSO > AN > EtOH > isopropanol > methanol > water (pH 7.4). A similar observation was made in the case of 7-aminocoumarin.

The interactions between the solvent and solute affect the energy difference between the ground and excited states. Lippert–Mataga equation provides the relationship between the spectral shift and the solvent polarity [46] [Eq. (2)].

$$\nu_A - \nu_F = \frac{2}{hc}\left(\frac{\varepsilon-1}{2\varepsilon-1} - \frac{\eta^2-1}{2\eta^2+1}\right)\frac{(\mu_E-\mu_G)^2}{a^3} + \text{Const} \qquad (2)$$

where h is Planck's constant, C is the speed of light, and a is the radius of the cavity in which the fluorophore resides. ν_A and ν_F are the absorption and emission maxima in cm^{-1}, η is the refractive index and ε is the dielectric constant, respectively. The first parentheses in Eq. (2), is difference of two terms $((\varepsilon-1)/(2\varepsilon+1)$ and $(\eta^2-1)/(2\eta^2+1))$, the difference of these terms accounts for the spectral shifts due to reorientation of the solvent molecules and hence called the orientation polarizability (Δf) Eq. (3).

$$\Delta f = \left(\frac{\varepsilon-1}{2\varepsilon-1} - \frac{\eta^2-1}{2\eta^2+1}\right) \qquad (3)$$

In order to determine the solvatochromic behavior of **VI**, a plot was drawn between $\Delta\nu$ ($\nu_A - \nu_F$) and polarity parameter Δf. The Lippert–Mataga plot is given in Fig. 3. From Fig. 3 it can be observed that the relationship between the Stokes shift and the polarity parameter is non-linear. The non-linear relationship between the Stokes shift and the polarity implies that the spectral shifts are dependent on specific solvent solute interactions like H-bonding.

VI has functional groups which can act as H-acceptor and H-donor. Therefore, the Lippert–Mataga correlation followed two distinctive lines, one for protic and one for non-protic solvents. In protic solvents, there is an increased reorientation of solvent molecules around the dye through hydrogen bonding. On the other hand, in proton accepting polar aprotic solvents, **VI** in its exited and ground state is stabilized by hydrogen bonding interactions *via* its amino groups. The emission spectral shifts of **VI** are large when compared to its absorption spectral shifts with change in polarity of the solvent. The smaller shift in the absorption spectra and the larger shifts in the emission clearly indicate that the dipole moment of the exited state is higher compared to that of ground state leading to the preferential solvation of exited state over the ground state [44].

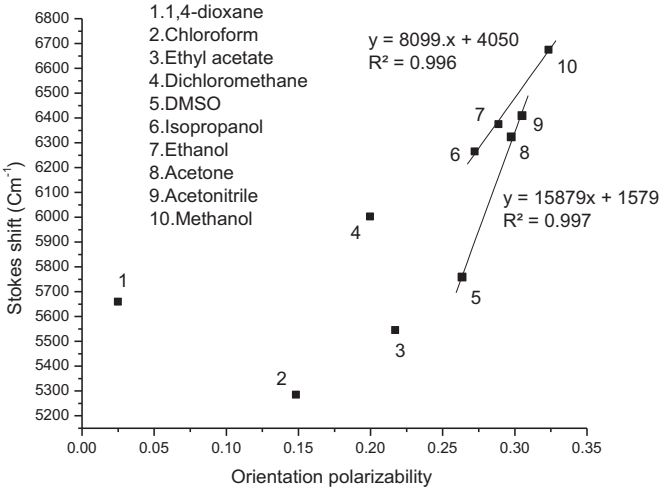


Fig. 3. The Lippert–Mataga plot for **VI.**

3.4. Detection of aldehydes

The effect of different aldehydes, ketones, esters and acids on the fluorescence of **VI** was studied in methanol at 25 °C without addition of catalyst and heating. The aldehydes used were benzaldehyde, benzaldehydes with electron donating and electron withdrawing groups at *ortho* and *para* positions, cinnamaldehyde and retinal. The ketones used were acetophenone and acetone. The ester employed was ethyl acetate and the acids used were acetic acid and formic acid. Based on the spectral results, the carbonyl containing compounds that interacted with **VI** were categorized into three types. The first categories of carbonyl containing compounds are those that can shift the emission maximum of **VI** to longer wavelength and quench its fluorescence. These include all the aliphatic aldehydes, acetophenone and retinal. They quench the fluorescence between 1.06 and 1.99 times as shown in Table 2. The quenching ratio was obtained by dividing the fluorescence quantum yield of **VI** with the fluorescence quantum yield of **VI** in presence of respective carbonyl containing compounds. The second category of carbonyl containing compounds are substituted benzaldehydes with an electron withdrawing group at either the *ortho* or *para* or *ortho* and *para* positions. These shifted the fluorescence emission maxima of **VI** from 502 nm to lower wavelength but not below 480 nm. From Table 2 it can be observed that these carbonyl containing compounds quenched the fluorescence quantum yield of **VI** by 1.90–7.80 times. The third categories of carbonyl containing compounds are benzaldehyde, benzaldehyde derivatives with electron donating substituents and cinnamaldehyde. These quenched the fluorescence quantum yield of **VI** by 3.27–24.09 times. Examination of Table 2 reveals that benzaldehyde and cinnamaldehyde have a remarkable effect on the fluorescence of **VI** and had prompted for further investigation.

From Fig. 4A it can be observed that addition of benzaldehyde (3.28×10^{-3} M) to the methanolic solution of **VI** (2.75×10^{-5} M), the emission and excitation wavelengths of the latter had shifted from 502 nm and 377 nm to 421 nm and 347 nm, respectively. The possible reason for the substantial quenching of fluorescence and blue shift of excitation and emission wavelengths of **VI** in the presence of a few selected benzaldehydes required detailed examination. In an ongoing effort to understand the mechanism of the profound blue shift and quenching of the fluorescence of **VI**, synthesis of imidazolocoumarins was undertaken as depicted in Scheme 1. Compounds phenylimidazolocoumarin, **VIIc** and styrylimidazolocoumarin, **VIId** are the imidazolocoumarins derived from **VI** on reaction with benzaldehyde and cinnamaldehyde. From Fig. 4A it is evident that, the wavelength of fluorescence emission maxima of **VIIc** and **VI** in presence of benzaldehyde [1.64×10^{-3} M] are coincident (Tables 1 and 2). Therefore it can be considered that **VI**, when mixed with benzaldehyde, readily upon irradiation at its absorption maximum is converted to an imidazolocoumarin which is less fluorescent than **VI**. The formation of phenylimidazolocoumarin, VIIc in the spectroscopic solution comprising

Table 2
Spectral property of **VI** on addition of aldehydes in methanol.

Entry	Compound **VI** + aldehyde	Solvent	λ_{ex} (nm)	λ_{em} (nm)	Stokes shift	Φ	Φ_f of diamine/Φ_f diamine + aldehydes
1	Nil	MeOH	378	502	6675	0.265	–
2	Benzaldehyde	MeOH	354	423	4900	0.011	24.09
3	2-Metyl Benzaldehyde	MeOH	365	434	4390	0.081	3.27
4	3-Metyl Benzaldehyde	MeOH	367	423	3600	0.072	3.68
5	4-Metyl Benzaldehyde	MeOH	360	419	3910	0.064	4.14
6	4-Methoxy Benzaldehyde	MeOH	374	438	3906	0.018	14.72
7	4-isopropyl benzaldehyde	MeOH	368	421	3421	0.071	3.73
8	Cinnmaldehyde	MeOH	386	424	3425	0.013	20.38
9	4-Choloro Benzaldehyde	MeOH	378	497	6334	0.04	6.62
10	2-Choloro Benzaldehyde	MeOH	408	485	3890	0.053	5.0
11	2,4-dichlorobenzaldehyde	MeOH	406	485	4012	0.139	1.90
12	4-Nitrobenzaldehyde	MeOH	400	502	5079	0.124	2.13
13	4-fluorobenzaldehyde	MeOH	374	483	6034	0.034	7.79
14	2-fluorobenzaldehyde	MeOH	409	487	3915	0.041	6.46
15	2-bromobenzaldehyde	MeOH	409	484	3787	0.046	5.76
16	Acetaldehyde	MeOH	376	502	6674	0.224	1.18
17	Propanal	MeOH	413	502	4292	0.218	1.21
18	Butanal	MeOH	412	504	4430	0.187	1.41
19	Pentanal	MeOH	412	502	4350	0.203	1.30
20	Hexanal	MeOH	412	502	4350	0.226	1.17
21	Heptanal	MeOH	414	504	4313	0.219	1.21
22	Octanal	MeOH	413	507	4489	0.186	1.42
23	Isoverlaldehyde	MeOH	412	505	4350	0.147	1.80
24	Cyclohexyl-1-al	MeOH	416	506	4275	0.133	1.99
25	Furfuraldehyde	MeOH	380	501	6355	0.057	4.64
26	Retinal	MeOH	378	502	6675	0.240	1.10
27	Acetophenone	MeOH	378	502	6675	0.248	1.06
28	Acetic Acid	MeOH	378	502	6675	0.248	1.06

VI and benzaldehyde was further supported by the LC-MS data. The LC-MS of the spectroscopic solution of **VI** and benzaldehyde is shown in Fig. 5. From Fig. 5 it can be noticed that the retention time 4.13 min (methanol: water (80:20 v/v)) correspond to *m*/*z*/277 (M + 1) in the mass spectrum which indicates the formation of **VIIc** in the spectroscopic solution. The LC-MS of the spectroscopic solution of **VI** and benzaldehyde and **VIIc** in other mobile phase (methanol:water (90:10 v/v)) the retention time was 33 s while **VIIc** has exhibited a retention time of 33.5 s which correspond to *m*/*z*/277 (M + 1) in the mass spectrum. Similar phenomena were observed with the other substituted benzaldehydes possessing electron donating substituents.

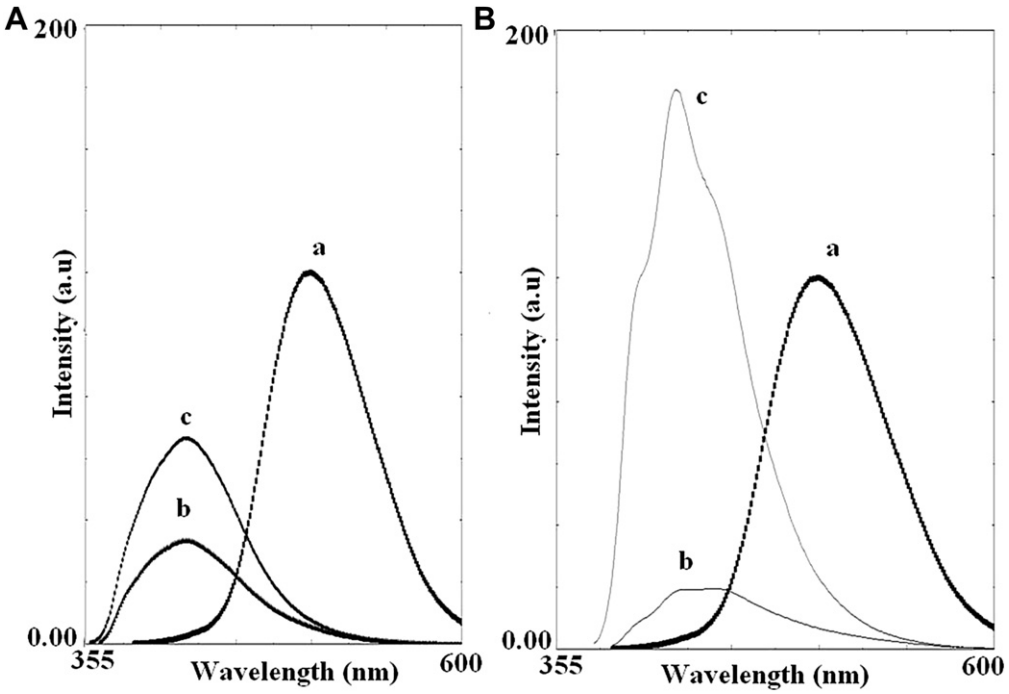


Fig. 4. A. Fluorescence spectra of **VI**-benzaldehyde system in methanol at 25 °C a). **VI** [2.75 × 10^{-5} M] λ_{ex} 378 nm b). **VI** [2.75 × 10^{-5} M] + 3.28 × 10^{-3} M benzaldehyde λ_{ex} 347 nm c). **VIIc** [1.35 × 10^{-5} M] λ_{ex} 347 nm. B: Fluorescence spectra of **VI**-cinnamaldehyde system in methanol at 25 °C. a). **VI** [2.75 × 10^{-5} M] λ_{ex} 378 nm b). **VI** [2.75 × 10^{-5} M] + cinnamaldehyde 3.20 × 10^{-3}M λ_{ex} 366 nm c). **VIId** [3.20 × 10^{-5} M] λ_{ex} 366 nm.

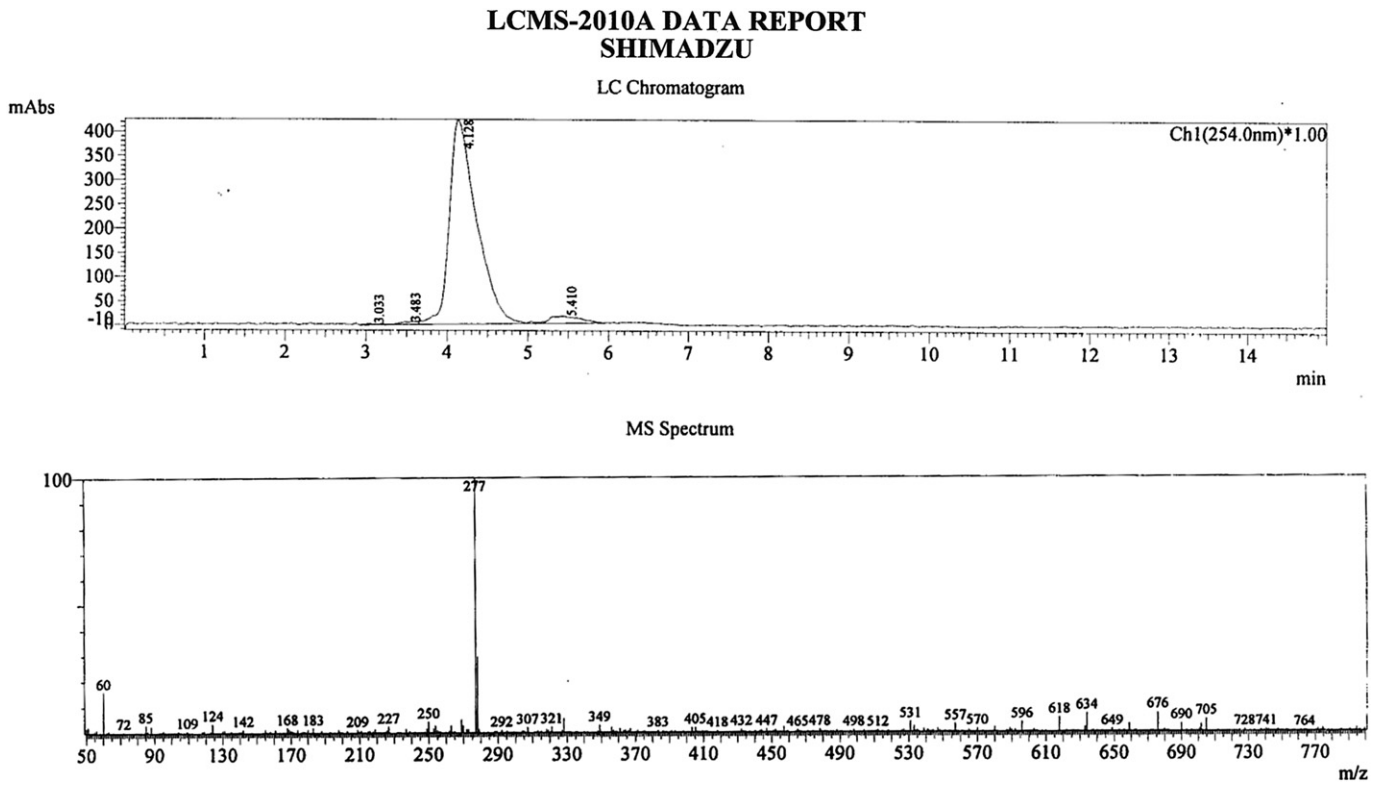


Fig. 5. LC-MS of binary mixture of **VI** and benzaldehyde in methanol solution.

In continuation and to confirm the chemical derivatization, the effect of cinnamaldehyde on the fluorescence of **VI** was investigated. In Fig. 4B, the effect of cinnamaldehyde on the fluorescence spectra of **VI** is shown. From Fig. 4B it can be observed that with addition of cinnamaldehyde, the fluorescence of **VI** was quenched significantly with profound blue shift in its emission maximum. The fluorescence quenching of **VI** and gradual appearance of a new band at lower wavelength with the disappearance of emission maximum at 502 nm with increased cinnamaldehyde is similar to that of the effect of benzaldehyde. Spectrum c in Fig. 4A and B is the emission spectrum of the phenylimidazolocoumarin and styrylimidazolocoumarin. The emission maxima and the excitation maxima of these two imidazolocoumarins are coinciding with the emission and excitation maxima of **VI** in presence of benzaldehyde [3.28×10^{-3} M] and cinnamaldehyde [3.20×10^{-3} M] in solution (Fig. 4). It is interesting to note that when acetaldehyde was added to **VI** and the emission and excitation spectra of the physical mixture were taken, there was no similarity between the emission spectra of latter with that of methylimidazolocoumarin. It is interesting to note that the emission and excitation spectra of the physical mixture of acetaldehyde and **VI** have no similarity with the emission and excitation spectra of methylimidazolocoumarin (**VIIb**). On the other hand the fluorescence emission maximum obtained for **VI**-acetaldehyde binary mixture was at 502 nm, no change in the emission wavelength. The quenching of fluorescence of **VI** in the presence of benzaldehyde and cinnamaldehyde is due to the formation of imidazolocoumarins while there is no such covalent derivatization occurred with the aliphatic aldehydes and benzaldehydes possessing electron withdrawing substituents. Ester and carboxylic acids have no effect on the fluorescence spectrum of **VI**.

In Fig. 6 the effect of concentration of benzaldehyde on the fluorescence of **VI** is given. From Fig. 6 it is clear that, 1.64×10^{-3} M concentration of benzaldehyde is the minimum concentration at which the phenomenon of shifting in the excitation and emission wavelengths. Spectrum e of Fig. 6 shows the quenched and altered fluorescence spectrum of the benzaldehyde [1.64×10^{-3} M] − **VI** [2.20×10^{-5} M] physical mixture. At this concentration of benzaldehyde [1.64×10^{-3} M], the fluorescence emission maximum of **VI** occurred was at 424 nm and with further increase in benzaldehyde concentration the fluorescence intensity increased experiencing a little blue shift. However, at [3.28×10^{-2} M] of benzaldehyde, the new emission maximum attained maximum intensity and the additional amount of benzaldehyde led to the quenching of perspective imidazolocoumarin, respectively. The isoemissive point observed at 454 nm and the emission curves from a to f in Fig. 6 reveals that **VI** is gradually

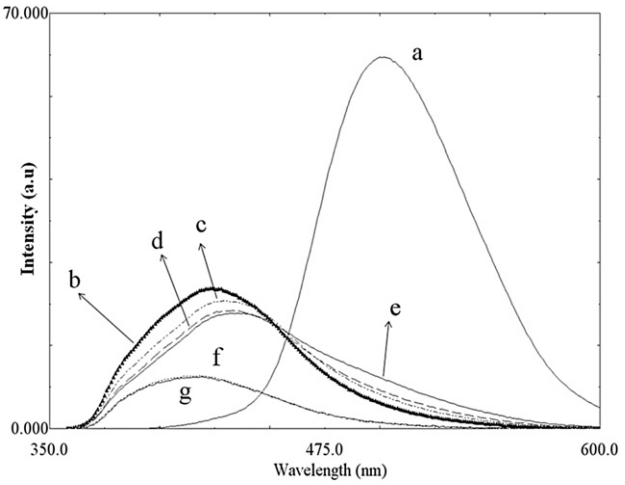


Fig. 6. Fluorescence response of **VI** in methanol to benzaldehyde at 25 °C a. **VI** [2.20×10^{-5} M], b–g. **VI** [2.20×10^{-5} M] + 3.28×10^{-2} M, 1.64×10^{-2} M, 3.28×10^{-3} M, 1.64×10^{-3} M, 6.56×10^{-2} M and 9.84×10^{-2} M benzaldehyde.

converted to phenylimidazolocoumarin with addition of benzaldehyde and these two have identical fluorescence intensity at 450 nm. But at greater than [3.28×10^{-2} M] of benzaldehyde, the **VI** has been completely and irreversibly converted to phenylimidazolocoumarin. The R_f value of the compound formed in the solution is found to be identical with the R_f value of VIIC (on Merck silica gel 60 F_{254} aluminum plates using chloroform:methanol (9.5:0.5 v/v) as eluent).

The mechanism of fluorescence quenching of **VI** with the third category of carbonyls is due to the formation of imidazolocoumarins. It was further noticed that when the electron donating groups are substituted at *ortho* position and or if the electron donating group is a poor electron donor like methyl group, the rate of formation of imidazolocoumarin is slow. In Fig. 7, the time course for the rate of quenching of fluorescence of **VI** in presence of benzaldehyde and 4-methyl benzaldehyde is provided. From Fig. 7 it can be observed that when the rate of diminishing of fluorescence of **VI** was monitored at a 5 s time interval, the rate of quenching of fluorescence of **VI** in presence of benzaldehyde is faster than that in presence of *para* tolualdehyde. A similar trend was observed with other less effective electron donating groups like isopropyl, *meta* tolualdehyde and *ortho* tolualdehyde. This was further confirmed by the second order rate constants, k_2. Under the condition [**VI**] = [aldehyde], plots of [1/*A*] vs. time were linear with a positive slope and an intercept on the ordinate indicating that the reaction follows overall second-order kinetics. In Fig. 8, a typical plot between 1/[*A*] vs *t* (s) is shown. [*A*] is the concentration of **VI** and was derived from the fluorescence emission intensity in presence of respective aldehyde. The k_2 values obtained were 1826.13 $M^{-1}S^{-1}$, 457 $M^{-1}S^{-1}$, 399 $M^{-1}S^{-1}$, 85 $M^{-1}S^{-1}$, 43.66 $M^{-1}S^{-1}$, 36.50 $M^{-1}S^{-1}$ and 29.36 $M^{-1}S^{-1}$ benzaldehyde, cinnamaldehyde, 4-methoxy benzaldehyde, 4-methyl benzaldehyde, 4-isopropyl benzaldehyde, 3-methyl benzaldehyde and 2-methyl benzaldehyde respectively. The rate of fluorescence quenching with the unsubstituted benzaldehyde was faster than all the aldehydes investigated. The next efficient quenching occurred with cinnamaldehyde. Everything being equal, the benzaldehydes having electron donating group at *para* position was faster than the electron donating group at *ortho* position. Greater is the electron donating ability faster is the quenching. The reason for the faster formation of imidazolocoumarins, especially with benzaldehyde and cinnamaldehyde over the other ring activating groups is not immediately known. Therefore, **VI** is a selective fluorophore that can yield a covalent derivative with a few selected benzaldehydes at different rates of time intervals leading to its fluorescence quenching.

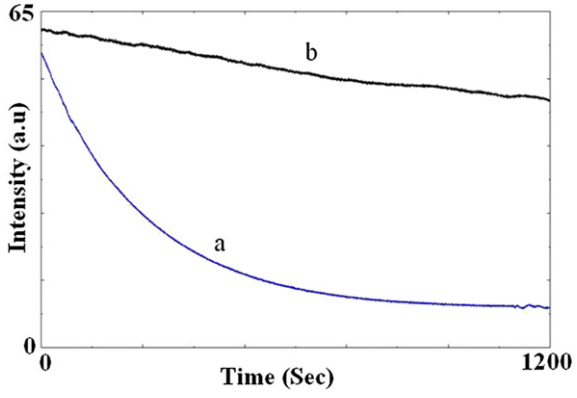


Fig. 7. Fluorescence decay of **VI** in presence of aldehydes in methanol solvent at 25 °C (λ_{em} 502 nm, λ_{ex} 378 nm), a) **VI** [2.75×10^{-5} M] + benzaldehyde [1.64×10^{-2} M], b. **VI** [2.75×10^{-5} M] + 4-methyl benzaldehyde [1.86×10^{-2} M].

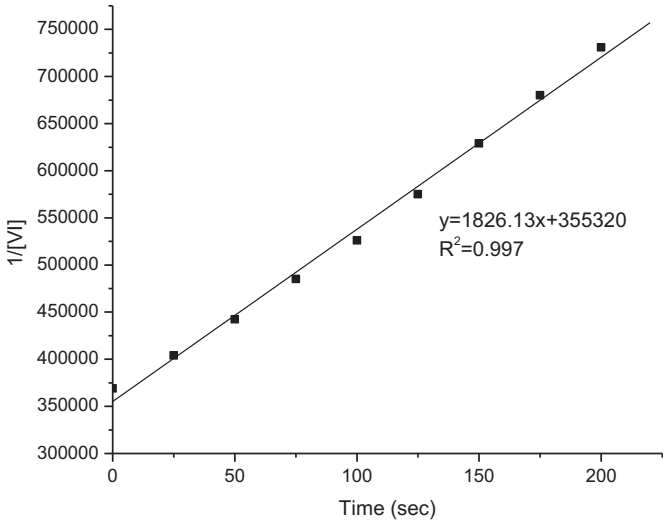


Fig. 8. Second order kinetics plot for the fluorescence quenching of **VI** in presence of benzaldehyde.

4. Conclusion

Akin to the 7-aminocoumarin, the new 6,7-diamino-4-methylcoumarin, **VI** is highly fluorescent. It exhibits remarkable solvatochromism and its fluorescence is sensitive to a set of aldehydes. In the presence of benzaldehyde, benzaldehydes with electron donating groups and cinnamaldehyde, **VI** developed to imidazolocomarins which are less fluorescent. The decreased fluorescence by the formation of derivatives leads to their selective detection by fluorometry.

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Appendix A. Supplementary data

Supplementary data related to this article can be found in the online version at http://dx.doi.org/10.1016/j.dyepig.2012.08.021.

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