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RAPID COMMUNICATION

Optical Properties of Some Novel 2,5-Disubstituted 1,3,4-Oxadiazole Derivatives and their Application as an Efficient Cell Staining Azo Dyes

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Abstract A series of six 2,5-disubstituted 1,3,4-oxadiazole derivatives with various length of conjugation have been synthesized and their optical properties including UV-visible absorption spectra, fluorescence emission spectra, molar absorption co-efficient, Stokes shift and the relative fluorescence quantum yield were measured in a variety of organic solvents. Correlation of the absorption spectra and fluorescence emission response of the 2,5-disubstituted 1,3,4-oxadiazole derivatives with the substituent effect revealed that the optical response can easily be tuned toward red shift by increasing conjugation length. The synthesized compounds were further employed in bioimaging assay in order to investigate their potential as an efficient cell staining agent using L-929 cells under confocal fluorescence microscope which showed scrupulous cell permeability and no toxicity as assessed by MTT assay.

Keywords 1,3,4-oxadiazole · Optical properties · Substituent effect · Bathochromic shift · Cell staining agent · MTT assay

Introduction

The design and development of efficient fluorescent heterocyclic compounds with high fluorescent quantum yield, larger stokes shift, relatively high molar absorption coefficient and facile synthetic strategy is an interesting topic due to their fundamental role in multiple areas such as emitters for electroluminescence devices, molecular probes for biochemical research, in traditional textile and polymer fields, fluorescent whitening agents and photo conducting materials [1]. Generations of electricity employing photo electrochemical cells fabricated with aromatic organic dyes have been considered as an alternative and cheap source for power generation. Dye-sensitized solar cells are attracting academic and commercial interest as an alternate renewable energy source where light absorbing dyes encapsulated on mesoporous semiconductor surface for solar energy storage [2–9]. Among several organic compounds, 1,3,4-oxadiazole derivatives are highly attractive in the research exhibiting potential used in organic electronics as electro-active and opto-active materials due to their high electron accepting properties with improved fluorescent quantum yields [10–14]. Synthetic dyes have been extensively used in numerous industrial processes and the goal of dying process is color durability as well as resistant to physical and chemical degradation [15–17]. Among them organic chromophores including azo compounds are widely used as pigments in many commercial products. Azo dyes attained the widest range of usage by possessing easily tunable structure and simple application procedure [18–20]. Furthermore, azo dyes have been studied widely because of their excellent thermal and optical properties with interesting applications in optical recording medium, toner, ink-jet printing, and oil-soluble light fast dyes [21].

Fluorescent imaging is emerging as a very promising technique for drug discovery and development. Organic dyes have been extensively using for fluorescent labelling of biomolecules and cells in order to investigate the in vivo or in vitro mechanism in living cells and tissues due to their commercial availability and ease of use by incorporating optical properties [22,23].

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The present study concerns the design, synthesis and optical properties of novel 2,5-disubstituted 1,3,4-oxadiazole derivatives in several organic solvent as well as inside biological cells using L-929 cell lines. We have further investigated the effect of increasing conjugation on the optical behavior of synthesized derivatives. The synthesized oxadiazole derivatives showed considerable bathochromic shift by substituting the terphenyl group which indicate that emission color of oxadiazole dyes can easily be tuned from blue to red by increasing conjugation length. Meanwhile, the synthesized oxadiazole compounds exhibited very good cell permeability can be employed as an efficient cell staining agent without any toxicity as assessed by MTT assay. Furthermore, the adopted synthetic methodology was simple which give very good product yield without involvement of complex purification techniques may widely be implemented in low cost azo dyes synthesis and their applications as nontoxic fluorescent molecules for bioimaging.

Experimental

Substrate and Reagents

Substituted aromatic carboxylic acids were purchased from Alfa Aesar and Aldrich. Ethanol, methanol, chloroform, DMSO, acetonitrile, water (Samchun Chemicals, Korea), H_2SO_4, HCl (Jin Chemical & Pharmaceutical Co. Ltd., Korea) were used in these experiments. The major chemicals utilized for biological studies includes MEM (minimum essential media, Wel Gene, Korea), FBS (*fetal bovine serum,* Bio west U.S.A), Tripsin (Thermo scientific, South Loga, Utah), PBS (Wel Gene, Korea) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma Aldrich, U.S.A].

Instrumentations

The reaction progress was monitored by thin layer chromatographic (TLC) analysis, and the R_f values were determined by employing pre–coated silica gel aluminum plates, Kieselgel 60 F_{254} from Merck (Germany), using *n*-hexane : ethyl acetate, 1:1 as an eluent. TLC was visualized under a UV lamp (VL–4. LC, France). The melting points were determined on a Fisher Scientific (USA) melting point apparatus and are uncorrected. The FT-IR spectra were recorded in KBr pellets on a SHIMADZU FTIR–8400S spectrometer (Kyoto, Japan). Proton and carbon nuclear magnetic resonance (^{1}H NMR & ^{13}C NMR) spectra were recorded on a Bruker Avance 500 MHz spectrometer with TMS as the internal standard. The chemical shifts are reported as δ values (ppm) downfield from the internal tetramethylsilane of the indicated organic solution. Peak multiplicities are expressed as follows: s, singlet; dd, doublet of doublets; m, multiplet. The coupling constants (*J* values) are given in hertz (Hz). Abbreviations are used as follows: DMSO–d_6, Dimethyl sulfoxide-d_6; FT–IR spectroscopy, Fourier transform infrared spectroscopy; DMEM, Dulbecco's Modified Eagle Medium

General Procedure for Spectroscopic Assay

A 5 mM stock solution of oxadiazole derivative 4a was prepared by dissolving 20 mg of compounds 4a in methanol (total volume 10 mL). For spectroscopic measurements, test solution of 1 mL was prepared with 2 μL of 5 mM stock solution in 1 mL cuvette. The stock solution for rest of oxadiazole derivatives 4b-f were prepared similar as that of 4a. The resulting solution were mixed with relevant solvent before measurement and final volume was fixed as 1 mL for UV-visible and fluorescent measurement using [SCINCO] UV–vis Spectrophotometer "S-3100" and FS–2 Fluorescence spectrometer (SCINCO, Korea), respectively.

Synthetic Protocol

Synthesis of Substituted Aromatic Acid Hydrazides 3a-f

Substituted aromatic acid chlorides 2a-f were synthesized by the reaction of substituted aromatic acids 1a-f (1 mmol) in the presence of 1,2–dichloroethane (12 mL) solvent and phosphorous oxychloride (0.4 mL) as chlorinating agent under reflux for 3 h. Then, the resulting solution was cooled to room temperature, and the solvent was removed under reduced pressure to afford 2a-f, which was directly used in the next step without further purification. Acid chloride 2a-f was dissolved in acetonitrile (80 mL), added dropwise to a solution containing hydrazine hydrate (1 mmol), triethyl amine (TEA, 0.5 mL) and acetonitrile (20 mL) and allowed to reflux for 3 h with monitoring by TLC. After consumption of the starting material, the reaction mixture was cooled to room temperature. Evaporation of the solvent under reduced pressure yielded crude acid hydrazide 3a-f as a white solid on cooling, which was purified by column chromatography and crystallized on methanol [24].

Synthesis of 2,5-Disubstituted-1,3,4-Oxadiazole 4a-f

An equimolar mixture of substituted aromatic acid hydrazides 3a-f and differently substituted aromatic acids were mixed in phosphorous oxychloride and heated under reflux at 107 °C for 3 h. The reaction progress was monitored by thin layer chromatography. After consumption of starting material; the reaction mixture was cooled to room temperature and poured into crushed ice which becomes precipitated. These precipitates was filtered, washed with cold water and diethyl ether, filtered and crystallized on methanol to get 2,5-disubstituted-

1,3,4-oxadiazole 4a-f. The product was purified with column chromatography where needed.

2- [{1,1':4',1'-Terphenyl}-4-yl]-5-(4-Methoxyphenyl) -1,3,4-Oxadiazole (4a)

Brown solid; yield: 71 %; R_f: 0.75 (*n*-hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3029 (sp^2 CH), 2951 (sp^3 CH), 1579 (*C*=N), 1544, 1505 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.78–7.72 (aromatic, 4H, m), 7.54–7.11 (aromatic, 13H, m), 3.56 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3, 164.9, 161.5, 140.5, 139.3, 132.5, 130.0, 128.6, 127.3, 118.0, 114.2, 56.1; Anal. for $C_{27}H_{20}N_2O_2$: C 80.39; H 4.94; N 6.87.

2-(4-Methoxyphenyl)-5-(4-Nitrophenyl)-1,3,4-Oxadiazole (4b)

Yellowish solid; yield: 78 %; R_f: 0.72 (*n*-hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3031 (sp^2 CH), 2944 (sp^3 CH), 1588 (*C*=N), 1548, 1521, 1494 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.74–7.31 (aromatic, 4H, m), 7.15–7.05 (aromatic, 4H, m), 3.54 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.3, 163.9, 160.2, 149.8, 134.5, 132.9, 124.4, 119.2, 114.5, 56.1; Anal. Calcd. for $C_{15}H_{11}N_3O_4$: C 59.87; H 3.78; N 14.19.

2-(4-Methoxybenzyl)-5-(4-Nitrophenyl)-1,3,4-Oxadiazole (4c)

Faint yellow solid; yield: 79 %; mp 144–146 °C; R_f: 0.71 (*n*-hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3036 (sp^2 CH), 2948 (sp^3 CH), 1587 (*C*=N), 1551, 1522, 1499 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.51–7.34 (aromatic, 4H, m), 7.21–6.96 (aromatic, 4H, m), 3.55 (s, 3H, OCH_3), 3.37 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.1, 165.0, 160.5, 139.4, 135.4, 129.7, 124.1, 118.2, 114.7, 56.2, 35.1; Anal. for $C_{16}H_{13}N_3O_4$: C 61.69; H 4.24; N 13.61.

2-(4-Methoxyphenethyl)-5-(4-Nitrophenyl)-1,3,4-Oxadiazole (4d)

Off white solid; yield: 78 %; mp 151–152 °C; R_f: 0.75 (*n*-- hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3029 (sp^2 CH), 2944 (sp^3 CH), 1588 (*C*=N), 1546, 1511, 1478 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.84–7.58 (aromatic, 4H, m), 7.24–7.19 (aromatic, 2H, m), 7.05–6.96 (aromatic, 2H, m), 3.56 (s, 3H, OCH_3), 2.86 (t, 2H, *J*=6.0 Hz, CH_2), 2.86 (t, 2H, *J*=6.0 Hz, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.2, 165.3, 160.2, 149.3, 134.2, 131.1, 126.5, 118.3, 38.2, 33.1, 56.0; Anal. for $C_{17}H_{15}N_3O_4$: C 62.78; H 4.67; N 12.89.

2-(4-Fluorobenzyl)-5-(4-Nitrophenyl)-1,3,4-Oxadiazole (4e)

Antique white solid; yield: 80 %; mp 151–153 °C; R_f: 0.72 (*n*-hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3033 (sp^2 CH), 2951 (sp^3 CH), 1589 (*C*=N), 1544, 1502 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.72–7.49 (aromatic, 4H, m), 7.31–7.14 (aromatic, 4H, m), 3.38 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.9, 165.8, 162.1, 149.7, 133.1, 130.4, 122.4, 121.0, 114.7, 34.1; Anal. for $C_{15}H_{10}FN_3O_3$: C 60.19; H 3.39; N 14.02.

2-(4-Fluorophenethyl)-5-(4-Nitrophenyl)-1,3,4-Oxadiazole (4f)

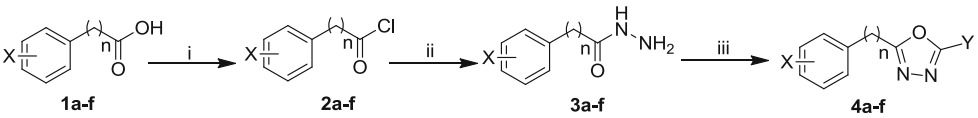
Misty rose like solid; yield: 80 %; mp 163–165 °C; R_f: 0.70 (*n*-hexane : ethyl acetate, 1:1); FT–IR (υ /cm^{-1}): 3037 (sp^2 CH), 2947 (sp^3 CH), 1589 (*C*=N), 1550, 1510, 1489 (*C*=C of phenyl ring); ^{1}H NMR (500 M Hz, DMSO-d_6) δ 7.74–7.58 (aromatic, 4H, m), 7.41–7.14 (aromatic, 4H, m), 2.87 (t, 2H, *J*=6.0 Hz, CH_2), 2.86 (t, 2H, *J*=6.0 Hz, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.0, 165.3, 162.4, 148.9, 136.1, 134.2, 130.4, 129.7, 122.5, 119.0, 35.1; Anal. for $C_{16}H_{12}FN_3O_3$: C 61.37; H 3.84; N 13.39.

Results and Discussions

Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles 4a-f, is illustrated in Scheme 1. Briefly, the substituted aromatic acid chloride 2a-f was synthesized by the reaction of corresponding substituted aromatic acids 1a-f in the presence of phosphorus oxychloride under reflux conditions. The substituted aromatic acid hydrazides 3a-f were synthesized by refluxing the corresponding substituted aromatic acid chlorides 2a-f with hydrazine hydrate (80 %) in the presence of triethyl amine in acetonitrile solvent. The coupling of aromatic acid hydrazides 3a-f with several substituted aromatic acids were carried out by refluxing with phosphorus oxychloride at 107 °C which afford corresponding 2,5-disubstituted 1,3,4-oxadiazole derivatives 4a-f. The synthesized oxadiazole derivatives were purified by crystallization and column chromatography and evaluated their optical properties in several organic solvents.

The absorption spectra of compounds 4a-f (1×10^{-5} mol L^{-1}) and the relevant spectral characteristics are shown in Fig. 1, Fig. 2 and the corresponding optical data are summarized in Table 1 and Table 2. The results indicate that the absorption spectra of 4a and 4b are red shifted as compared to rest of derivatives 4c-f. The compound 4a contain 4-OCH_3 group as substituent X and terphenyl group as substituent Y while compound 4b contain 4-OCH_3 group as substituent X and 4-NO_2 phenyl group as substituent Y. The reasons for bathochromic shift for both of these compounds

Scheme. 1 Synthesis of 2,5-disubstituted-1,3,4-oxadiazole 4**a-f:** Reagents and conditions: (i) $POCl_3$, $ClCH_2CH_2Cl$, reflux 3 h; (ii) hydrazine hydrate, TEA, MeCN, reflux 3 h; (iii) substituted aromatic acid, $POCl_3$, reflux, 3 h



Comp.	X	n	Y	Comp.	X	n	Y
4a	4-OCH_3	0	4-Terphenyl	**4d**	4-OCH_3	2	4-Nitrophenyl
4b	4-OCH_3	0	4-Nitrophenyl	**4e**	4-F	1	4-Nitrophenyl
4c	4-OCH_3	1	4-Nitrophenyl	**4f**	4-F	2	4-Nitrophenyl

are due to extensive conjugation which ultimately reduces the band gap energy between HOMO to LUMO and facilitates the rapid electronic transition. The non-bonding electron pair of oxygen and nitrogen in case of compound 4a initiate the delocalization of electronic cloud. The nonbonding electron of oxygen atom in the electron donating methoxy group pushes its electron toward the aromatic ring which ultimately transferred to electron withdrawing nitro group by passing through oxadiazole skeleton. This electron movement induces the low energy electronic transition at remarkably longer wavelength.

Further investigation of substituent effect on the bathochromic shift of molecule was carried out by substituting terphenyl group to the oxadiazole ring as substituent Y, interestingly; there was a considerable red shift which reflects the low energy transition due to extensive delocalization of electronic cloud in relatively longer pathway as shown in Fig. 1. In virtue of introduction of sp^3 carbon atom in the conjugated pathway, the absorption maxima of low energy absorption band was remarkably blue shifted. In case of compound 4c and 4d, there was a reduction of electronic delocalization pathway as there was one (in case of 4c) and two (in case of

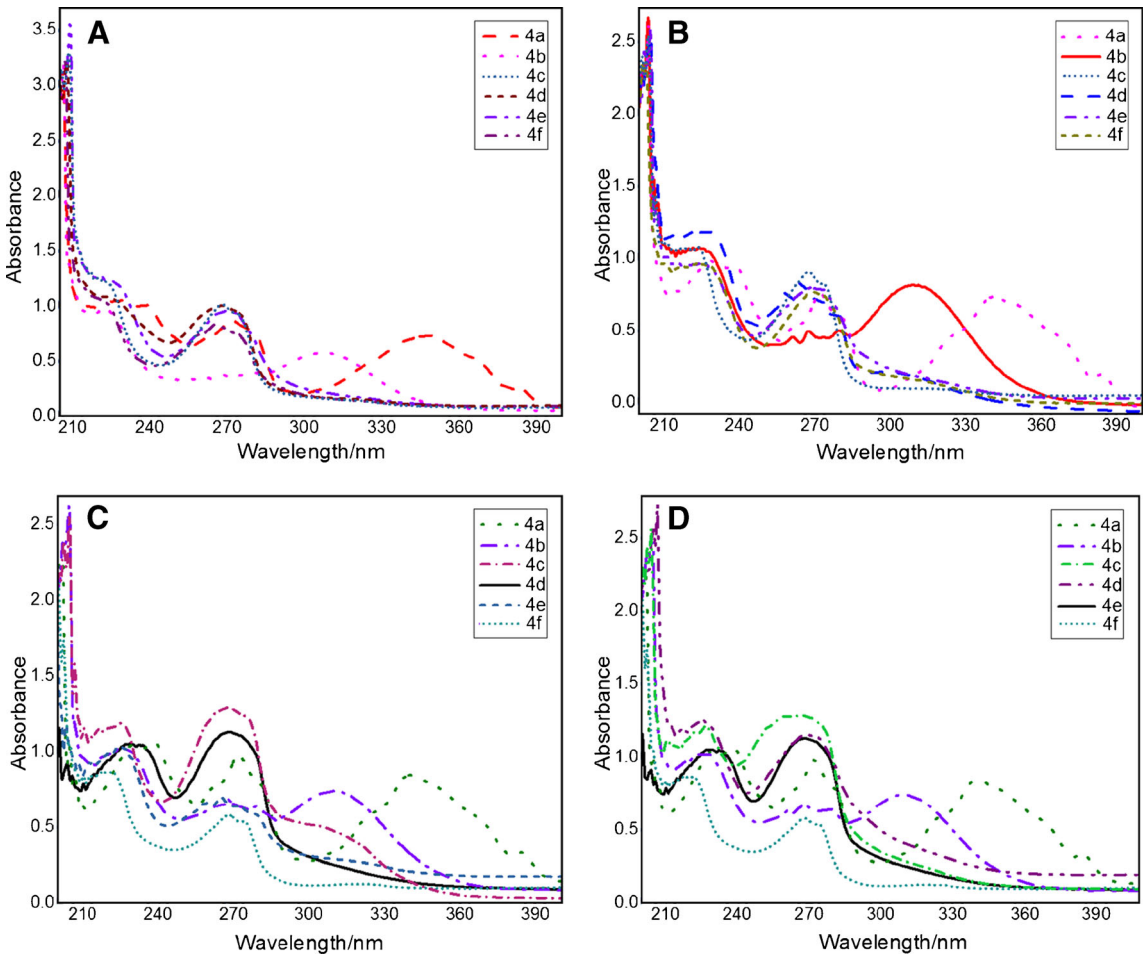


Fig. 1 UV-visible absorption spectra of 2,5-disubstituted 1,3,4-oxadiazole derivatives 4**a**-f in various organic solvents of different polarities including (**a**) acetonitrile; (**b**) dimethyl sulfoxide; (**c**) ethanol; (**d**) chlororom

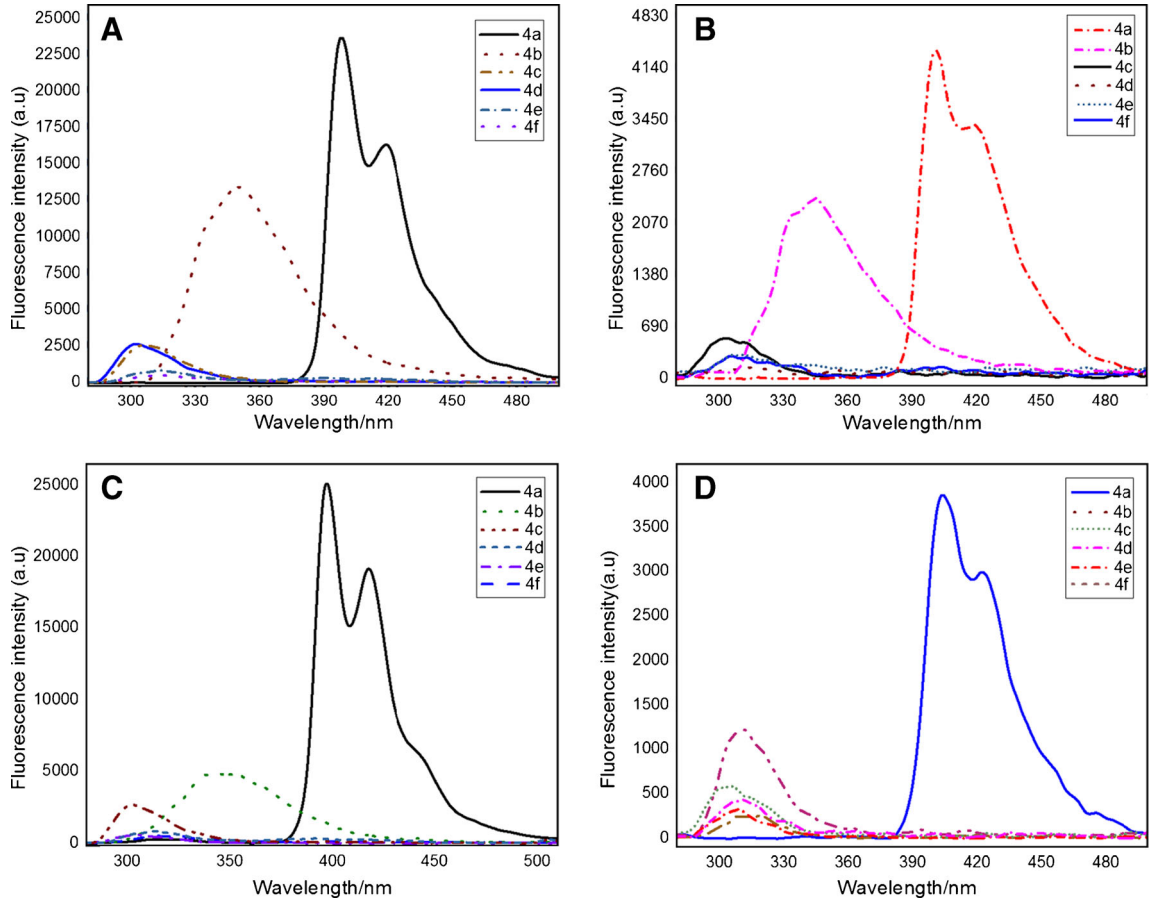


Fig. 2 Fluorescence emission spectra of 2,5-disubstituted 1,3,4-oxadiazole derivatives 4**a-f** in various organic solvents of different polarities including (**a**') acetonitrile; (**b**') dimethyl sulfoxide; (**c**') ethanol; (**d**') chlororom

4d) extra carbon atoms in between X substituted aryl ring and oxadiazole ring. While same high energy electronic transition was observed in case of compound 4e and 4f which contains electron withdrawing 4-fluoro group as substituent X instead of electron donating 4-methoxy group meanwhile both of the derivatives contains extra carbon atom in between oxadiazole ring and side coupled group.

In order to investigate the solvent effect on the absorption and emission spectra of synthesized compounds 4a-f, the absorption and emission spectra were recorded in variety of organic solvent with different polarities including chloroform, ethanol, acetonitrile and DMSO. The absorption spectra of compounds were slightly red shifted in the presence of acetonitrile and DMSO while there was slight blue shift in case of

Table 1 Electronic spectral data of conjugated oxadiazole derivatives 4a-f in various organic solvents of different polarities

S.No.	λ_{max} (nm)											
	Chloroform			Ethanol			Acetonitrile			DMSO		
	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	[a]Em	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	[a]Em	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	[a]Em	$\pi\rightarrow\pi^*$	$n\rightarrow\pi^*$	[a]Em
4a	230, 272	340	397	231, 272	343	397	239, 272	348	401	237, 271	348	403
4b	227, 272	310	312	226	311	345	223	310	350	225, 268	311	350
4c	226	265	302	223	267	303	227	269	306	225	270	305
4d	226	269	308	228	263	310	221	269	311	231	268	313
4e	230	267	303	225	269	305	227	269	313	228	268	309
4f	221	271	315	226	269	313	222	266	315	220	270	315

[a] position of fluorescence emission maxima, nm

Table 2 Optical characteristics of the newly synthesized oxadiazole derivatives 4a-f including molar absorption coefficient, Stokes shift and relative fluorescence quantum yield in several variable polarities organic solvents

S.No	$CHCl_3$				EtOH				MeCN				DMSO			
	[a]ε 10^3 (M^{-1} cm^{-1})		[b]S. Shift	[c]ΦFL	[a]ε 10^3 (M^{-1} cm^{-1})		[b]S. Shift	[c]ΦFL	[a]ε 10^3 (M^{-1} cm^{-1})		[b]S. Shift	[c]ΦFL	[a]ε 10^3 (M^{-1} cm^{-1})		[b]S. Shift	[c]ΦFL
	π→π*	n→π*	(cm^{-1})		π→π*	n→π*	(cm^{-1})		π→π*	n→π*	(cm^{-1})		π→π*	n→π*	(cm^{-1})	
4a	20.6, 19.6	17.0	4222	0.21	19.6, 15.0	14.8	3965	0.26	20.0, 17.0	14.8	3797	0.27	21.0, 19.2	17.0	3921	0.19
4b	20.4, 13.0	14.8	206	0.16	21.4,	14.4	3168	0.25	19.4	11.6	3686	0.24	20.6, 13.6	14.8	3582	0.14
4c	24.2	26.0	4623	0.13	21.6	18.2	4449	0.16	2.4	19.2	4494	0.19	23.6	25.8	4250	0.12
4d	25.0	23.0	4707	0.14	23.6	16.8	5764	0.14	24.8	20.2	5020	0.19	20.8	22.6	5364	0.10
4e	21.0	22.6	4449	0.11	19.2	16.0	4387	0.09	21.2	20.0	5225	0.13	19.4	13.8	4950	0.09
4f	17.2	11.0	5154	0.10	1.9	15.2	5225	0.06	21.2	16.8	5847	0.06	17.2	10.8	5291	0.02

[a] Molar absorption coefficient calculated by using Beer Lambert law

[b] Stokes shift calculated by using equation 1

[c] Relative fluorescence quantum yield calculated by equation 2

ethanol and chloroform. The compound 4a exhibited absorption maxima at 340 nm, 343 nm, 348 nm and 348 nm in various organic solvent including chloroform, ethanol, acetonitrile and DMSO, respectively. There was about 8 nm bathochromic shift in case of compound 4a by increasing solvent polarity. This shift is due to the larger orientation polarizability of polar solvent, which is a result of its dipole moment.

Almost all of the compounds showed two absorption maxima, the absorption maxima obtained at longer wavelength that was attributed due to n→π* electronic transition of the conjugated system while high energy transition at shorter wavelength was considered due to π→π* electronic transition as band gap between π→π* energy level are relatively high as compare to n→π*. The exact values for n→π* and π→π* electronic transition for all the synthesized derivatives 4a-f in several organic solvent are tabulated in Table 1.

The experimental values for optical properties of compounds including molar absorption coefficient, Stokes shift and relative fluorescent quantum yield in different organic solvents are tabulated in Table 2. Almost all the compounds showed the considerable Stokes shift with enhanced values of molar absorption coefficient. The Stokes shift of oxadiazole derivatives was calculated by using equation 1 [25] and molar absorption coefficient was calculated by using Beer Lambert law [26].

$$(\upsilon A - \upsilon \mathrm{F}) = \left(\frac{1}{\lambda A} - \frac{1}{\lambda \mathrm{F}} \times 10^7\right) \tag{1}$$

The fluorescent quantum yield was calculated by using equation 2 [27]. All the compounds exhibited very good fluorescent quantum yield and the experimental values are tabulated in Table 2. Anthracene (0.05 M stock solution in chloroform) for 4a, 2-amino pyridine (0.05 M stock solution in 0.1 N sulfuric acid) for 4b, and benzene solution in hexane for 4c-f were used as reference standard [28–30] for relative fluorescence quantum yield determination of oxadiazole derivatives.

$$\Phi_{\mathrm{unk}} = \Phi_{\mathrm{std}}(I_{\mathrm{unk}}/A_{\mathrm{unk}})(A_{\mathrm{std}}/I_{\mathrm{std}})(n_{\mathrm{unk}}/n_{\mathrm{std}})^2 \tag{2}$$

Where Φ_{unk} is the fluorescence quantum yield of the sample, Φ_{std} is the quantum yield of the standard, I_{unk} and I_{std} are the integrated fluorescence intensities of the sample and the standard, respectively, A_{unk} and A_{std} are the absorbance's of sample and the standard at the absorption wavelength, respectively, n_{unk} and n_{std} are the refractive indices of corresponding solvents.

From the fluorescent quantum yield data (Table 2), the result indicates that the quantum yield of the substituent

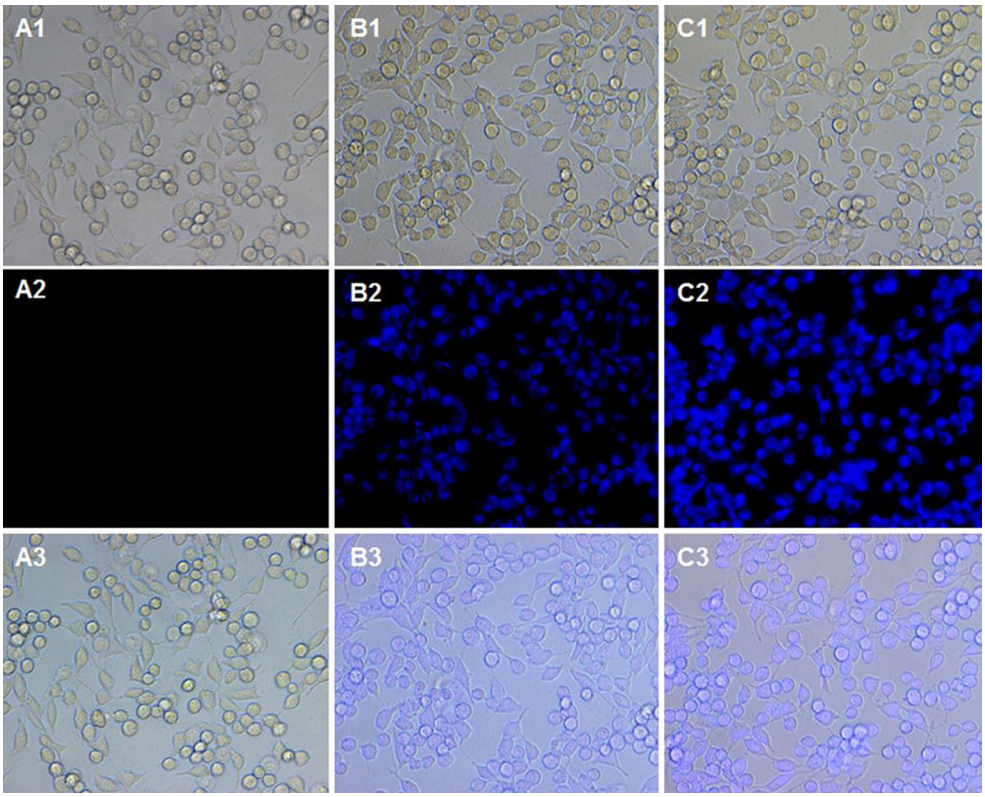


Fig. 3 Confocal fluorescence microscopic images for L-929 cells; (A1-A3) L-929 cells incubated with 4**a** (0 μM); (B1-B3) L-929 cells incubated with 4**a** (3.71 μM); (C1-C3) L-929 cells incubated with 4**a** (7.42 μM). A1-C1: bright field images; A2-C2: fluorescence images: A3-C3: merged images

having electron donating group (OCH_3) is higher than that of the substituent with electron withdrawing fluoro group. This is due to the substituent effect of the molecular structure.

The fluorescent emission spectra of 4a-f are shown in Fig. 2. Similar substituent effect can be seen in case of fluorescent emission spectra. The compound 4a exhibited emission spectrum in the range from 370 nm-500 nm in various solvent. Beside substituent, the solvent polarity also exerts slight effect on the position of emission maxima of the compounds 4a-f. The compound 4a showed fluorescence emission maxima of 397 nm, 397, 401 nm and 403 nm in chloroform, ethanol, acetonitrile and DMSO, respectively. There was a slight bathochromic shift of about 6 nm on increasing the solvent polarity which was demonstrated that the fluorescence emission maxima correlated with substituent attached and solvent polarities. The substituent with extensive conjugation will abruptly give large bathochromic shift while slight red shift also observed by increasing solvent polarity as shown in Fig. 2.

In particular, the incorporation of electron donating group (OCH_3) induced the increment in electronic delocalization which ultimately lowers the band gap energy and shifts the emission toward longer wavelength. Similarly, the terphenyl group with longer delocalization pathway also contributes in the increment of fluorescence emission wavelength. Meanwhile, the electron withdrawing group (fluoro group in case of 4e and 4f) shift the fluorescence emission maxima toward higher energy; concurrently, the same effect (hypsochromic shift) can be seen by the relatively less polar solvent including chloroform and ethanol as compared to acetonitrile and DMSO.

In view of exploring the excellent live cell staining performance of synthesized azo dyes with oxadiazole skeleton, one of the derivatives 4a was analyzed through bioimaging experiment using L-929 cell lines (mouse fibroblast cells) under confocal fluorescence microscope. For staining, cells were incubated with various concentrations of 4a (0 μM, 3.71 μM and 7.42 μM) in complete DMEM (dulbecco's modified eagle medium) for 6 h at 37 °C. After incubation, samples were washed twice with PBS (phosphate buffered saline) and

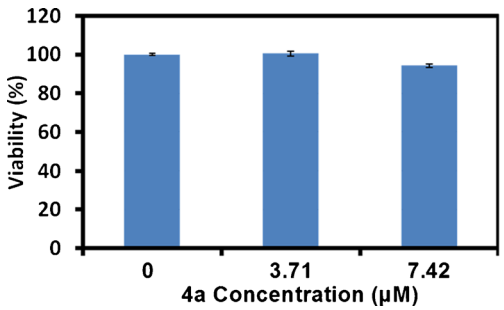


Fig. 4 The L-929 cells viability cultured in complete media with 4**a** (0 μM, 3.71 μM, 7.42 μM) while the control cells were cultured in the medium without ligand. The cells only in DMEM medium without 4**a** represent the control sample

observed under florescent microscope. As shown in Fig. 3, the fluorescent images displayed dye concentration dependence: the cells treated with higher concentrations of 4a showed stronger florescence. These results demonstrated that the synthesized oxadiazole derivatives can be used for live object staining such as cells in biological system, might be employed as fluorescent biomarkers with tunable fluorescent properties e.g., by increasing conjugation length or nature of the substituent attached to oxadiazole moiety, the fluorescent behavior can be switched toward red or blue side of spectrum.

The safety of selected derivatives 4a was assessed after 24 h treatment to the cells by MTT assay. The results showed no toxicity for L-929 cells up to 3.71 μM while at higher concentration (7.42 μM) the viability was reduced to 94.3 % as showed in Fig. 4. The non-toxic behavior of 4a and its ability to introduce intracellular fluorescence in living cells might be utilized as biomarkers for in vivo and in vitro cell studies.

Conclusion

A series of six oxadiazole containing azo dyes with varying conjugation length has been synthesized, and evaluated their optical properties including absorption spectra, emission spectra, molar absorption coefficient, Stokes shift and relative fluorescent quantum yield in a variety of organic solvent with different polarity in order to investigate their potential as an efficient fluorescent dyes with maximum light harvesting potential. The spectral properties of the compounds were significantly dependent on the conjugation length of coupled group as well as their electron donating or withdrawing potential. The absorption and fluorescent spectra were red shifted by incorporating the group with longer conjugation path length and by electron donating group while hypsochromic shift were observed by reducing the conjugation path length and incorporating electron withdrawing substituent. Meanwhile, polar organic solvent induce slight bathochromic shift in the fluorescence and absorption spectra while there was slightly blue shifted in comparative less polar organic solvent. We further investigate the toxicity and cell staining property of synthesized fluorescent dyes by employing L-929 cell lines under confocal fluorescent microscopic experiment. The results showed very good cell permeability of synthesized derivatives with maximum cell viability toward tested compound as assessed by MTT assay. We expect that the interesting substituent and media dependent tunable fluorescent properties and efficient cell viability of the synthesized compounds may have potential used in low cost azo dyes development and as biomarker with promising cell staining potential.

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