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Absorption and Fluorescence Studies of 3-Ethenylindoles

Anil K. Singh* and Prasanta K. Hota
Department of Chemistry, Indian Institute of Technology, Bombay Powai, Mumbai - 400 076, India

Synthesis, absorption and fluorescence properties of 3-methyl indole (**1**), N-(benzenesulfonyl)-3-(3-oxo-but-1-enyl)-indole (**2**) and 1H-3-(3-oxo-but-1-enyl)-indole (**3**) are described. Extended conjugation at C-3 of indole as in **3** causes moderate resolution of ^1L_a and ^1L_b bands. However, **2** having an electron-withdrawing group at indolic nitrogen shows only the ^1L_a band. While the ^1L_b band largely remains solvent polarity independent, the ^1L_a band undergoes moderate red shift in polar solvents. The fluorescence in **2** and **3** originates from the L_b transition. Additionally, interaction of **2** and **3** with BSA indicates that these compounds bind to the hydrophobic site of BSA with the formation of a highly fluorescent BSA-probe complex.

key words: Indole, Ethenes, Fluorescence, Fluorescence probe, BSA

INTRODUCTION

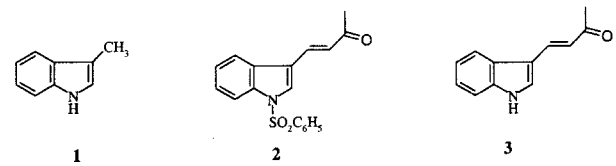
The ground and excited state properties of indole and related compounds have been extensively studied because of the utility of these compounds as fluorescence probes.[1-5] The indole compounds are believed to have two nearly degenerate electronic transitions, namely the ^1L_a and ^1L_b in the first π,π^* excited singlet state.[1, 6-8] The band due to the ^1L_b transition is relatively structured having well-resolved vibronic features. It is solvent independent and appears to be the lowest energy singlet state in nonpolar media. On the other hand, the ^1L_a transition is characterized by broad vibronic band, relatively high dipole moment, and its strong interactions with the solvent molecules.[9-14] It is believed to be a precursor of the singlet excited state (S_1) in polar solvent.[6, 13,14] Further, both the bands are influenced by the substituents.[8,9,15,-19] Thus, 2,3-dimethyl indole[7, 8] and carbazole[20] derivatives show distinct ^1L_a and ^1L_b absorption bands. It is also observed that the presence of an electron donating substituent at 3-position of the indole moiety causes a red shift in ^1L_a absorption band.[14] Similarly the fluorescence properties of these compounds are sensitive to their local environment and are dependent upon the two overlapping excited electronic states, ^1L_a and ^1L_b having different dipole moments.[15]

As the ^1L_a band is broad, and the $^1L_a/^1L_b$ bands are not sufficiently separated, it becomes difficult to measure the exact amount of media and substituent influenced band shifts in indole and its model compounds. This severely limits our understanding of the dynamics of the excited state properties of indole and related molecules. It is desirable to have these bands well resolved in order to clearly understand the excited states of indolic compounds. This warrants design and synthesis of new indole compounds that are capable of exhibiting resolved ^1L_a and ^1L_b bands. In this context we have synthesized indolic ethenes **2** and **3** and examined their electronic absorption and fluorescence properties in comparison to the known 3-methylindole (1) (Figure 1).

MATERIAL AND METHODS

General

3-Formylindole, 3-methylindole, L-tryptophan, quinine sulfate and deuteriated chloroform were of Aldrich Chemical Company, USA make. Other chemicals and reagents, uv grade solvents for spectroscopic studies, and AR grade solvents for synthetic purposes were either from Spectrochem or E. Merck (India), Mumbai. The AR grade solvents were dried and freshly distilled prior to their use. Petroleum ether (60-80°C fraction) procured from local suppliers was distilled prior to its use. Thin layer and column chromatographic analyses were done using silica gel G (Merck). The absorption spectra were measured using a Shimadzu UV-160A spectrophotometer. FTIR spectra in KBr discs were recorded on a Impact Nicolet-400 spectrophotometer. Melting points were determined on a Veego melting point apparatus. The ^{1}H-NMR spectra in $CDCl_3$ using TMS as internal standard were



1 2 3

Figure 1. Structure of compounds **1-3**.

*To whom correspondence should be addressed.
E-mail : Retinal@chem.iitb.ac.in
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recorded either on a Varian 60 MHz (EM 360L) or on a VXR 300 MHz FTNMR instrument. The GC-mass spectra were measured on a GCD 1800-A Hewlett Packard GC-Mass spectrometer. The fluorescence spectra were recorded on a Perkin Elmer LS-55 spectrofluorimeter, keeping the emission and excitation slit width 10 nm. The fluorescence emission spectra were recorded by exciting at the absorption maximum ($\lambda_{ab\ max}$) and the excitation spectra were obtained by using the fluorescence emission maximum ($\lambda_{em\ max}$) of the respective compounds. Fluorescence quantum yields (Φ_f) were determined against tryptophan in phosphate buffer, pH=7.2 ($\Phi_{f\ ref}$ = 0.12)[21] and quinine sulfate in 0.1N H_2SO_4 ($\Phi_{f\ ref}$ = 0.51)[22] as standard. Time-resolved fluorescence measurements were carried out using a high repetition rate picosecond laser coupled to a time-correlated single photon counting (TCSPC) spectrometer (Hamamatsu-2809). Sample was excited in the absorption band of the compounds at 315 nm by vertically polarized pico second laser pulses (frequency doubled Ti-sapphire laser). The emission was collected at the peak in 390-430 nm region. The fluorescence decays were fitted to single or multi exponential functions. The quality of the exponential fits was evaluated by the reduced χ^2 value (~1.2). In protein systems, phosphate buffer, pH = 7.4 were used through out the experiment. The binding constant (K) with BSA were determined according to the literature procedure[23] and equation 1.

$$(\Phi/\Phi_a\text{-}1)^{-1}=(\Phi_b/\Phi_a\text{-}1)^{-1}(1+1/KC) \qquad \text{equation 1.}$$

Where, Φ: fluorescence quantum yield of compound in the presence of BSA, Φ_a: fluorescence quantum yield of compound in absence of BSA, Φ_b: fluorescence quantum yield of compound-BSA complex, C: concentration of BSA, K: binding constant.

The Stern-Volmer quenching constant was determined by using the equation 2[24].

$$F_0/F\text{ -}1 = K_{sv}[Q] \qquad \text{equation 2.}$$

Where, F_0: Fluorescence intensity of protein emission in the absence of quencher, F: Fluorescence intensity of protein emission in the presence of quencher, K_{sv} : Stern-Volmer quenching constant, [Q] : quencher concentration. For the determination of K_{sv} values, the quencher concentrations up to 10×10^{-6} M in case of compound **2** and 18×10^{-6} M in case of compound **3** were considered.

For all the electronic spectroscopic (absorption, fluorescence excitation and emission) studies, 2.0×10^{-5} M solutions of the respective compounds were used.

Synthesis

3-Formyl-N-benzene sulfonyl indole-3-Formyl-N-benzene sulfonylindole was synthesized according to the literature procedure by the sulfonation of 3-formylindole in the presence of benzenesulfonyl chloride, potassium carbonate as a base and acetone as a solvent in 89 % yield.[25] It showed the following physico-chemical properties: m.p.155-156°C (Lit.[25] m.p. 157-158°C); Uv-vis (MeOH) λ_{max}/nm (ε, l mol^{-1}cm^{-1}): 285 (18333); IR (KBr) $\nu_{max,}$ cm^{-1}: 1678, 1179; ^{1}H-NMR (60 MHz): δ 10.5 (1H, s, -CHO), 7.7-8.1 (3H, m), 8.2 (1H, s), 7.2-7.6(6H, m).

N-(Benzenesulfonyl)-3-(3-oxo-but-1-enyl)-indole (**2**)-

N-(benzenesulfonyl)-3-(3-oxo-but-1-enyl)-indole (**2**) was synthesized by the condensation reaction of 3-formyl-N-benzenesulfonylindole and acetone in presence of pyridine and pipiridine. The reaction mixture was heated at 100°C for 8 hours. The progress of the reaction was monitored by thin layer chromatography (TLC) in 20 % ethyl acetate in petroleum ether as solvent (product R_f = 0.2). The reaction mixture was cooled to room temperature, poured in ice-cold water and was treated with 100 mL of diluted hydrochloric acid to remove excess of pyridine from the reaction mixture. The crude product was filtered and purified by column chromatography using 5-10% ethyl acetate in petroleum ether as the eluting solvent, when the desired compound was obtained in 25% yield. m.p. 140-142°C; Uv-vis (MeOH) λ_{max} nm (ε, λ mol^{-1}cm^{-1}): 316 (26250); IR (KBr) $\nu_{max,}$ cm^{-1}: 1657 (C=O), 1617 (C=C), 1183 (S=O) cm^{-1}; ^{1}H-NMR (300 MHz): δ 8.01(1H, m,-C_2), 7.88-7.93(3H, m, -near sulfonyl-proton and -C_4), 7.81(1H, m, -C_7),7.63 (1H, d, J = 18 Hz, -CH=C-$COCH_3$), 7.45-7.59 (3H, m, -benzene protons -C_4), 7.31-7.42 (2H, m, -C_5C_6), 6.82 (1H, d, J = 18 Hz, -C=CH-$COCH_3$), 2.38 (3H, s, -CH_3); GC-Mass: R_t, 18.88 min (column HP-5, flow rate of helium gas 0.7 mL/min, electron ionization detector, temperature 280°C), m/z (% rel int): 325 (18, M^+), 184 (100, $[M\text{-}SO_2C_6H_5]^+$), 77 (61, $[C_6H_5]^+$), 43 (24, $[CH_3CO]^+$).

1H-3-(3-oxo-but-1-enyl)-indole (**3**)-

1H-3-(3-oxo-but-1-enyl)-indole (**3**) was synthesized in 80 % yield by the hydrolysis of N-(benzene-sulfonyl)-3-(3-oxo-but-1-enyl)-indole (**2**) in the presence of aqueous sodium hydroxide. The reaction mixture was heated for 3~4 hours. The progress of the reaction was monitored by TLC in 20 % ethyl acetate in petroleum ether as solvent (product R_f = 0.1). The reaction mixture was cooled to room temperature and neutralized with dil. hydrochloric acid. The product was filtered and purified by column chromatography using 5-20% ethyl acetate in petroleum ether as the eluting solvent, when the desired compound was obtained in 80% yield. m.p. 152-154°C; Uv-vis (MeOH) λ_{max} nm (ε, λ mol^{-1}cm^{-1}): 295 (17000), 354 (4250); IR (KBr) $\nu_{max,}$ cm^{-1}: ν 3434 (NH), 2927, 2861 (CH), 1657 (C=O), 1637 (C=C). cm^{-1}; ^{1}H-NMR (300 MHz): δ 8.69 (1H, s, br,-NH), 7.90-7.96 (1H, m, -C_2), 7.79 (1H, d, J = 18 Hz, -C=CH-$COCH_3$), 7.53(1H, d, -C_4), 7.40-7.46(1H, m, -

C_7), 7.23-7.32 (2H, m, -C_5C_6), 6.81(1H, d, J = 18 Hz, -CH=C-$COCH_3$), 2.39(3H, s,-CH_3).

RESULTS AND DISCUSSION

The electronic absorption spectral data of **1-3** in different solvents are summarized in Table 1, and as typical example, the spectra in methyl cyclohexane are shown in Figure 2. The vibronic bands near 290 nm correspond to the ^1L_b transition as previously reported for **1**.[6] In comparison to **1**, the absorption spectra of **2** and **3** are characterized by a rather broad band in ~300-360 nm range, and the spectrum below ~300 nm lacks fine structures. The peaks near 290 nm for **3** can be due to the ^1L_b transition. The maximum near 313 nm for **2** and 334 nm for **3** corresponds to the ^1L_a transition. The red-shift in the ^1L_a band as compared to the ^1L_b band can be due to the presence of the ene conjugation at C-3 position of the indole moiety. The other bands near 240 and 237 nm in compounds **2** and **3** are due to their second singlet excited state (B_a). Such a band for **1** appears at 226 nm.[6] While, the absorption spectrum of **1** is largely insensitive to the solvent polarity, a moderate solvent polarity effect on the absorption spectra of **2** and **3** is observed. On changing the solvent from a relatively non-polar *n*-heptane to polar dimethylformamide (DMF), the ^1L_a band of **2** shifts only by 2 nm, and of **3** only by 8 nm. However, in hydrogen bond donor solvent like methanol, the red shift is much more pronounced in **3** as compared to **2**. The ^1L_a transition in **3** is ten times more sensitive to hydrogen bond interaction than it is in **2**. This can be due to strong interaction of the NH-group of indole moiety in **3** with the solvent molecules. Thus, the position of ^1L_a band in **2** and **3** is influenced by the nature of the substituent at indole nitrogen, solvent polarity as well as by the hydrogen bond capability of the solvent.

Table 1. Absorption spectral data of 1-3 in solvents of varying polarity

Solvents	1	2		3	
	L_b(nm)	L_b(nm)	L_a(nm)	L_b(nm)	L_a(nm)
Methyl cyclohexane	290	-	313	291	334-354
n-Heptane	290	-	313	291	334
1,4-Dioxane	290	-	313	291	334
Methanol	290	-	316	295	354
Dimethylformamide	291	-	315	290	342
Acetonitrile	290	-	313	291	331

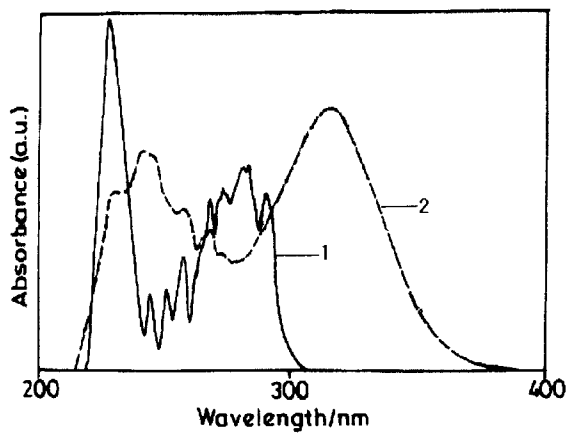


Figure 2a. Uv-visible absorption spectra of **1** and **2** in methyl cyclohexane.

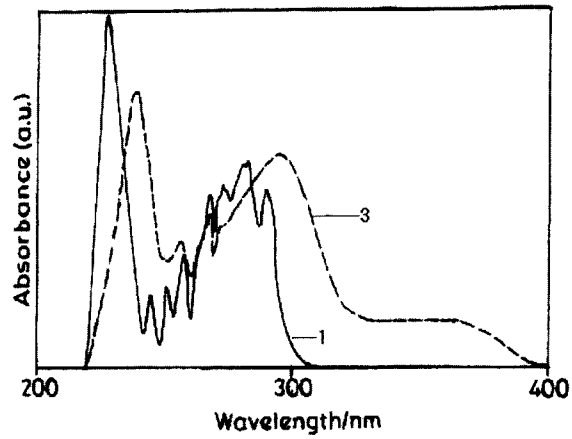


Figure 2b. Uv-visible absorption spectra of **1** and **3** in methyl cyclohexane.

The steady state fluorescence emission and excitation spectra of **2** and **3** are shown in Figures 3 and 4 respectively, and the fluorescence emission and excitation maxima, fluorescence quantum yield and Stokes shift of **1-3** in organic solvents of varying polarity are summarized in Table 2. Compound **1** shows single fluorescence emission maximum in all the solvents, which gets red shifted with increasing

Table 2. Fluorescence emission maximum ($\lambda_{f\,max}$), excitation maximum ($\lambda_{ex\,max}$), fluorescence quantum yield (Φ_f) and Stokes' shift values for 1-3

Compound	Solvent	$\lambda_{f\,max}$ (nm)	$\lambda_{ex\,max}$ (nm)	Stokes Shift (cm^{-1})	Φ_f
1	*n*-Heptane	337	280	5157	0.236
	1,4-Dioxane	337	281	4809	0.199
	Methanol	361	280	6782	0.337
	DMF	365	286	6967	0.463
	Acetonitrile	354	278	6234	0.210
2	*n*-Heptane	344,427	282	7910	0.008
	1,4-Dioxane	344,429	284	7852	0.002
	Methanol	348,428	282	8970	0.001
	DMF	425	295	7766	0.029
	Acetonitrile	344,403	282	9375	0.001
3	*n*-Heptane	369,422	286	5844	0.025
	1,4-Dioxane	373,403	283	6466	0.014
	Methanol	381,425	285	4496	0.104
	DMF	420	306	6210	0.044
	Acetonitrile	381,427	279	5765	0.013

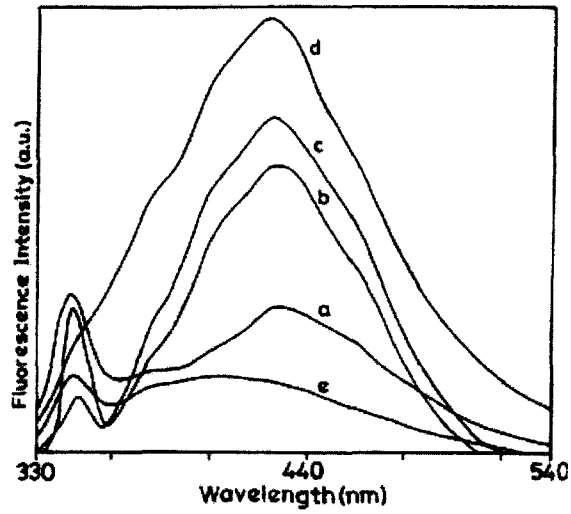


Figure 3a. Fluorescence emission spectra of **2** in (a) heptane, (b) 1, 4-dioxane, (c) methanol, (d) DMF and (e) acetonitrile.

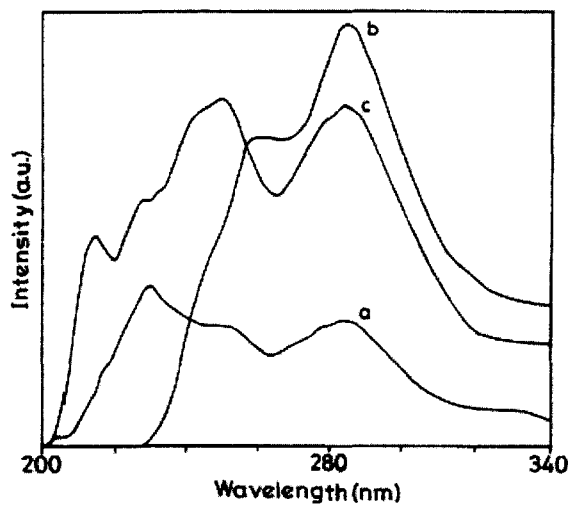


Figure 3b. Fluorescence excitation spectra of **2** in (a) *n*-heptane, (b) 1,4-dioxane, (c) methanol.

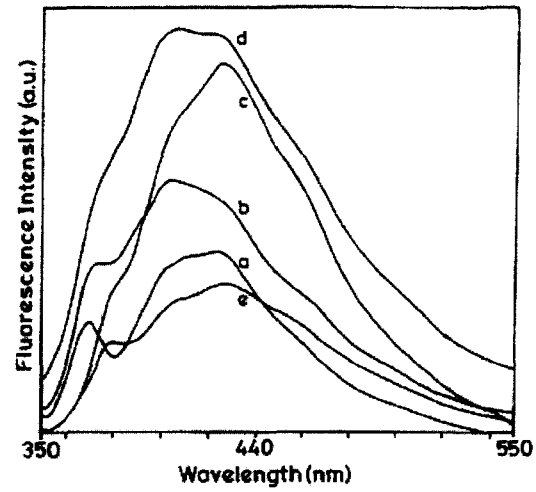


Figure 4a. Fluorescence emission spectra of **3** in (a) *n*-heptane, (b) 1,4-dioxane, (c) methanol, (d) DMF and (e) acetonitrile.

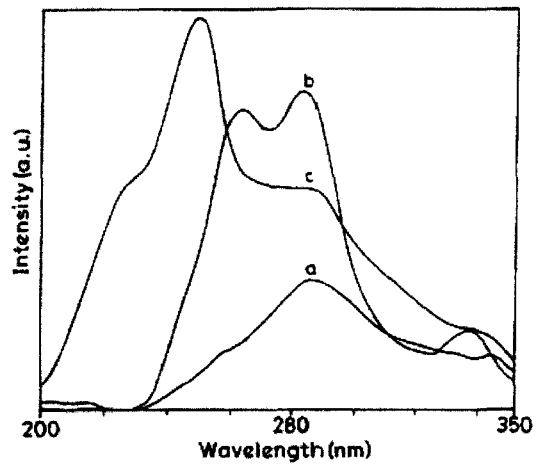


Figure 4b. Fluorescence excitation spectra of **3** in (a) *n*-heptane, (b) 1,4-dioxane, (c) methanol.

solvent polarity. Compounds **2** and **3** on the other hand show two fluorescence emission maxima in all the solvents, except in DMF in which the fluorescence band is rather broad, and centered around 425 nm. The position of fluorescence emission maximum of **2** and **3** does not significantly change by changing the solvent otherwise. The fluorescence excitation spectra of **2** and **3** are characterized by the presence of a prominent peak near 280 nm (rather than at 300-350 nm). This indicates that the fluorescence emission in these compounds originates from the L_b transition and not from the L_a transition. As compared to **1**, compounds **2** and **3** have relatively low fluorescence quantum yield. The large Stokes' shifts observed indicate that the singlet-excited state of these compounds is relatively polar in nature. Such polarity for these compounds can arise due to intramolecular charge transfer in their excited state. Such intramolecular charge transfer can occur at many sites in these molecules, including conformational relaxations in acyclic single as well as double bonds. Both the compounds show multi-exponential fluorescence decay (Figure 5), and have at least two excited species, one having a shorter lifetime (0.01-0.9 ns) and the other one having a relatively longer lifetime (3.3-5.6 ns) (Table 3). In polar solvent like methanol the shorter-lived species becomes dominant (~83-99%). The longer and shorter lifetimes can respectively be attributed to L_a and L_b states. In polar solvent, the energy gap between the L_a and L_b band increases, hence, more number of molecules are in the L_b state rather than in the L_a state. Based on the results, an energy level diagram for the electronic absorption and fluorescence emission energy states of 3-substituted indolic ethenes **2** and **3** is suggested in Scheme 1.

Compounds **2** and **3** were used as a probe to examine

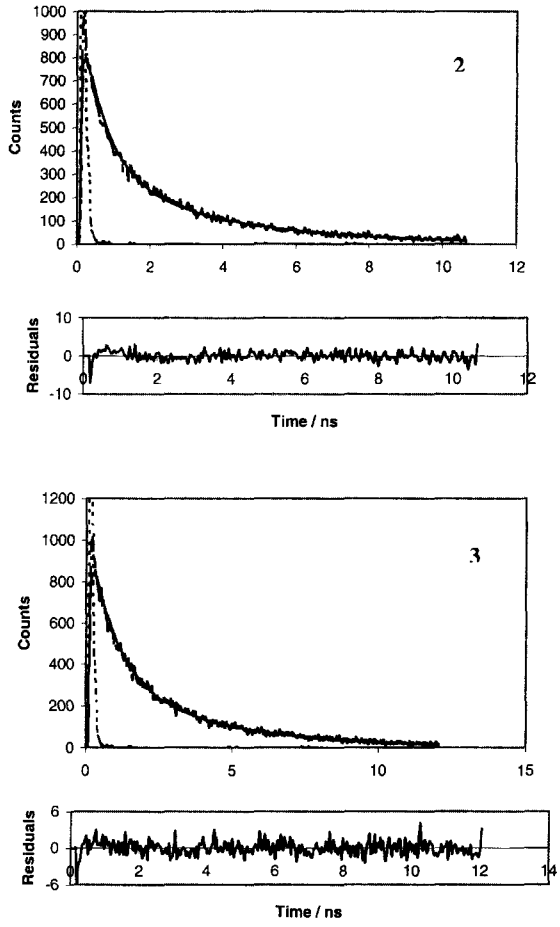


Figure 5. Fluorescence decay profiles of **2** and **3** in *n*-heptane.

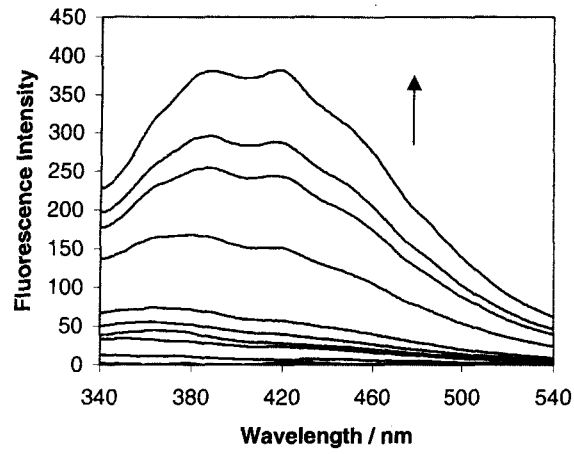
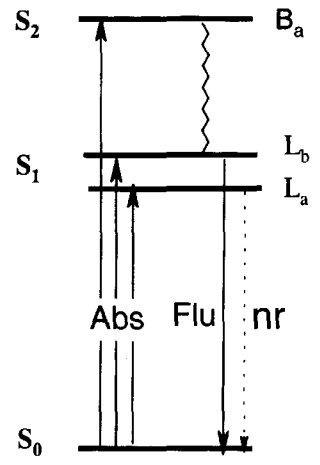


Figure 6. Fluorescence spectra of **2** on increasing BSA concentration (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 x 10^{-6} M).



Scheme 1. Energy level diagram for electronic absorption and fluorescence emission energy states for 3-ethenylindoles **2** and **3** (Abs: absorption, Flu: fluorescence, nr: nonradiative decay process).

interaction between probe and protein bovine serum albumin (BSA). Parameters like, binding constant, Stern-Volmer quenching constant of these molecule with BSA are determined. Typical fluorescence spectra of compound **2** on increasing concentration of BSA are shown in Figure 6. The emission maximum of both the compounds is blue shifted upon binding to BSA (Table 4). It is found that fluorescence quantum yield of BSA-bound complex of **2** and **3** is enhanced 20 to 40 times more as compared to the unbound compound. This indicates that these compounds are strongly interacting with protein environment. A high value of protein-probe binding constant (K~10^6) is calculated for both the compounds.

Further, the fluorescence quenching study of tryptophan in BSA is done with increasing concentration of compounds **2** and **3.** The Stern-Volmer quenching constant is calculated and presented in Table 5. The fluorescence quenching spectra of tryptophan in BSA by **2** is shown in Figure 7. A linear Stern-Volmer plot is obtained in the presence of quencher **3**, whereas the plot deviates from linearity in the presence of **2**.

Table 3. Excited state lifetime and amplitude data for 2 and 3 in organic solvents

Compound	Solvent	Life time (τ/ns) /amplitude (α)				τ_{av} (ns)
		τ_1	α_1	τ_2	α_2	
2	*n*-Heptane	0.677	0.653	3.374	0.347	1.614
	1,4-Dioxane	0.336	0.852	3.392	0.148	1.795
	MeOH	0.012	0.998	3.482	0.002	0.020
3	*n*-Heptane	0.917	0.634	3.729	0.366	1.946
	1,4-Dioxane	0.684	0.786	5.490	0.214	1.711
	MeOH	0.562	0.833	5.680	0.167	1.791

Table 4. Absorption (λ_{abs} max) and fluorescence emission maximum (λ_{em} max) of 2 and 3 in phosphate buffer and in BSA.

Compounds	$\lambda_{abs\ max}$/nm	$\lambda_{em\ max}$/nm		Φ_f	
		buffer	BSA-probe	buffer	BSA-probe
2	320	445*	388/418	0.006	0.12
3	353	440*	421	0.003	0.14

*: negligible fluorescence

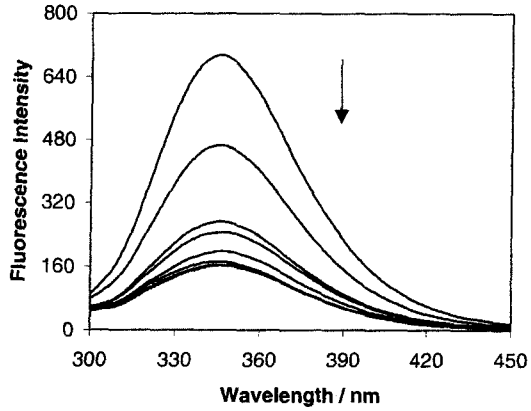


Figure 7. Fluorescence quenching spectra of tryptophan in BSA on increasing concentration of **2** (0, 2, 4, 6, 8, 10, 12×10^{-6}M).

Table 5. Binding constant (K) to BSA and Stern-Volmer quenching constant (K_{SV}) of tryptophan fluorescence in BSA by 2 and 3.

Compound	K (10^6)	R^2	K_{SV} (10^4) Liter/moles	R^2
2	4.28	0.99	27.67	0.91
3	5.49	0.96	17.83	0.98

This indicates that compound **2** (bearing hydrophobic aryl substituent) is interacting with more than one class of binding sites, whereas compound **3** is interacting with the similar types of binding site. It is further found that compound **2** quenches the tryptophan fluorescence 1.5 times more as compared to **3**. It is known that one of the tryptophan residue of BSA (Trp-134, subdomain IB) is present in the hydrophobic region which is responsible for the binding of aromatic components, whereas the other tryptophan (Trp-212, subdomain IIA) is present in the hydrophilic environment of BSA[26]. The present results suggest that **3** binds to subdomain IIA, whereas **2** binds to both subdomain IB and subdomain IIA. This indicates towards role of aryl substituent of **2** for binding to BSA.

In summary, we have shown that indolic compounds bearing ene/enonic substituent at 3-position of the indole moiety can have relatively resolved ^1L_a and ^1L_b states. The ^1L_a transition is relatively more sensitive to solvent polarity than the ^1L_b transition. These compounds show multi-exponential fluorescence decay, and have at least two singlet excited species. In polar solvent like methanol the shorter-lived species becomes the dominant excited species. Further, in polar solvent, the energy gap between the L_a and L_b band increases, hence, more number of molecules is in the L_b state rather than in the L_a state. The fluorescence in **2** and **3** originates predominantly from the ^1L_b state and not from the ^1L_a state. Further, **2** and **3** strongly interact with BSA. The BSA-probe complex is highly fluorescent as compared to the unbound probe.

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