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Photophysical behavior of a novel 4-aza-indole derivative in different solvents: reverse solvatochromism

Ebru Bozkurt[1] · **Sengul Dilem Dogan**[2]

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Abstract

The photophysical properties of a new 4-aza-indole derivative [ethyl 1-((2-(2-ethoxy-2-oxoethyl)pyridin-3-yl)carbamoyl)-2-hydroxy-1*H*-pyrrolo-[3,2-*b*]pyridine-3-carboxylate, **12**] were determined in different solvents. Compound **12** exhibited an absorbance peak at 340–360 nm with high fluorescence intensity in the wavelength range from 405 to 417 nm in all solvents except *N*,*N*-dimethylformamide (DMF). Compound **12** exhibited reverse solvatochromism behavior depending on the solvent polarity. Furthermore, compound **12** showed very high quantum yield in all solvents independent of their polarity. The results suggest that this novel dye could be used for many applications, e.g., as a labeling agent and in bio- or analytical sensors and/or optoelectronic devices.

Keywords 4-Aza-indole · Absorption · Fluorescence · Solvent · Reverse solvatochromism

Introduction

An aromatic amino acid, tryptophan, is widely used to study segmental elasticity, structure–function relationships, and interactions of proteins due to its intrinsic fluorescence property. However, tryptophan cannot always be used as a probe, since it has complex photophysics. Therefore, synthesis and optical characterization of novel tryptophan analogs is a very interesting research area. In recent years, many azatryptophan derivatives have been synthesized as fluorescence probes to solve this problem. In such investigations of the photophysical properties of azaindole derivatives, the side chain of azatryptophan is mainly the focus

✉ Ebru Bozkurt
ebrubozkurt@atauni.edu.tr

[1] Program of Occupational Health and Safety, Erzurum Vocational Training School, Ataturk University, 25240 Erzurum, Turkey

[2] Department of Pharmaceutical Basic Sciences, Faculty of Pharmacy, Erciyes University, 38039 Kayseri, Turkey

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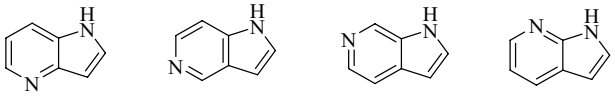
[1, 2]. For this purpose, researchers have focused on azaindole isomers such as 4-azaindole, 5-azaindole, 6-azaindole, and 7-azaindole (Scheme 1). Among these isomers, 4-azaindole has been identified as a promising candidate [1]. Therefore, synthesis of new 4-azaindole derivatives and characterization of their photophysical properties is a very important area of study.

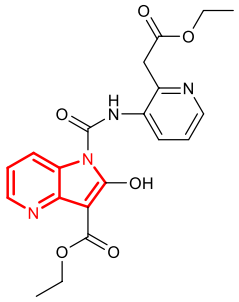
Solvatochromism refers to changes in the absorption and fluorescence spectra of fluorescent molecules depending on factors such as the hydrogen-bonding ability, pH, and polarity of the solvent. Since the ground- and excited-state polarities of fluorescent molecules are different, the stability of these states may change depending on solvent properties, altering the energy gap between them and thereby the position, intensity, and shape of the absorption or fluorescence spectra. A shift in the absorption or fluorescence band maximum to longer wavelength due to the effect of a solvent is called positive solvatochromism, whereas a shift to shorter wavelength is called negative (reverse) solvatochromism [3, 4].

The aim of this study is to investigate the photophysical properties of a novel synthesized aza-indole derivative [ethyl 1-((2-(2-ethoxy-2-oxoethyl)pyridin-3-yl) carbamoyl)-2-hydroxy-1*H*-pyrrolo-[3,2-*b*]pyridine-3-carboxylate, compound **12**] (Scheme 2) in different solvents. To the best of the authors' knowledge, such investigation has not been reported in literature to date. For this purpose, ultraviolet–visible (UV–Vis) absorption spectroscopy, and steady-state and time-resolved fluorescence measurements were carried out on the compound **12** in different solvents. Additionally, photophysical parameters of compound **12**, such as the fluorescence quantum yield, fluorescence lifetime, and radiative (k_r) and non-radiative (k_{nr}) rate constants, were calculated in different solvents. It is hoped that



Scheme 1 Structure of azaindole isomers

Scheme 2 Structure of compound **12**



the results of this study will make an important contribution for new tryptophan analogs.

Experimental

Materials

H_2SO_4 (98 % purity), all solvents (99 % purity), and quinine sulfate were purchased from Sigma (USA), Merck (Germany), and Fluka (USA), respectively, and used without further purification. Stock solution of compound **12** at 1.0×10^{-3} M was prepared in ethanol. A certain amount of compound **12** was obtained from this stock solution by evaporating the solvent. For all measurements, the concentration of compound **12** was 1.0×10^{-6} M. All experiments were performed at room temperature.

Instruments

UV–Vis absorption and fluorescence spectra of the samples were recorded using a PerkinElmer Lambda 35 UV–Vis spectrophotometer (USA) and Shimadzu RF-5301PC spectrofluorophotometer (Japan), respectively. Fluorescence and absorption measurements were taken for compound **12** at room temperature. For steady-state fluorescence measurements, all samples were excited at 350 nm and the fluorescence intensity was recorded between 360 nm and 600 nm. Fluorescence spectra were measured using slit width of 1.5–3 and corrected using Origin 8.0 software. Fluorescence lifetime measurements were carried out using a LaserStrobe model TM3 spectrofluorophotometer from Photon Technology International (PTI, USA) with a combined pulsed nitrogen laser/tunable dye laser for excitation at 366 nm. Decay curves were collected over 200 channels using a nonlinear time scale with the time increment increasing according to an arithmetic progression. The fluorescence decays were analyzed using the lifetime distribution analysis software supplied by the instrument company. Fit quality was assessed by χ^2 values and weighted residuals [5]. Good results typically produce χ^2 values of 0.9 to 1.2. In our study, the values were between 0.8 and 1.16.

The fluorescence quantum yields of the molecule were calculated using the Parker–Rees equation:

$$\phi_s = \phi_r \left(\frac{D_s}{D_r}\right)\left(\frac{\eta_s^2}{\eta_r^2}\right)\left(\frac{1-10^{-OD_r}}{1-10^{-OD_s}}\right), \quad (1)$$

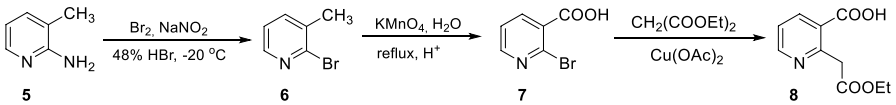
where D is the integrated area under the corrected fluorescence spectrum, n is the refractive index of the solution, and OD is the optical density at the excitation wavelength (λ_{ex} = 350 nm). The subscripts "s" and "r" refer to the sample and reference solution, respectively [6]. Quinine sulfate in 0.5 M H_2SO_4 solution was used as reference. The fluorescence quantum yield of quinine sulfate was 0.55 in 0.5 M H_2SO_4 solution [7].

The rate constants of radiative (k_r) and nonradiative (k_{nr}) deactivation were calculated by using the following equations:

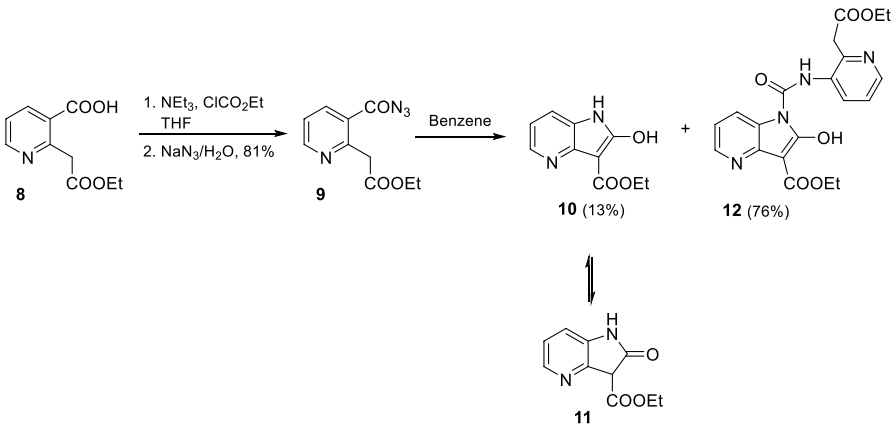
$$k_r = \frac{\boldsymbol{\Phi}}{\tau_{av}}, \tag{2}$$

$$\frac{1}{\tau_{av}} = k_r + k_{nr}, \tag{3}$$

where Φ is the fluorescence quantum yield and τ_{av} is the average fluorescence lifetime of samples [8].



Scheme 3 Synthesis of the key compound, 2-(2-ethoxy-2-oxoethyl) nicotinic acid (**8**)



Scheme 4 Synthesis of compound **12**

Ethyl 1-((2-(2-ethoxy-2-oxoethyl)pyridin-3-yl) carbamoyl)-2-hydroxy-1*H*-pyrrolo-[3,2-*b*]pyridine-3-carboxylate (12)

Compound **12** was synthesized and characterized as described in our previous study [9] as summarized in Schemes 3 and 4. White solid from chloroform, m.p. (decomposition) 264–265 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.83 (s, 1H), 11.48 (s, 1H), 8.42 (dd, J = 7.7, 0.7 Hz, 1H), 8.29 (dd, J = 4.7, 1.5 Hz, 1H), 8.26 (dd, J = 8.2, 1.4 Hz, 1H), 7.55 (t, J = 5.8 Hz, 1H), 7.20 (dd, J = 8.0, 5.0 Hz, 1H), 6.86 (dt, J = 7.1, 1.6 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 4.16 (q, J = 7.1 Hz, 2H), 3.97 (s, 2H), 1.33 (t, J = 7.1 Hz, 3H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 165.9, 164.8, 151.1, 147.0, 145.4, 145.3, 132.8, 130.7, 129.5, 127.3, 122.7, 121.8, 113.5, 83.4, 61.2, 60.4, 40.7, 14.6, 14.1. ν_{max} (ATR): 3287, 2979, 1723, 1592, 1557, 1413, 1383, 1329, 1264, 1179, 1131, 1077, 1021. HRMS: m/z $[M\text{-}H]^-$ calcd. for $C_{20}H_{19}N_4O_6$: 411.13101; found: 411.13273.

Results and discussion

Absorption measurements of compound **12** (1.0×10^{-6} M) were taken in 14 different solvents, such as toluene, diethyl ether, 1,4-dioxane, tetrahydrofuran (THF), ethyl acetate, chloroform, dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetonitrile (ACN), isopropanol (i-PrOH), ethanol (EtOH), methanol (MeOH), and water, at room temperature (Fig. 1a–c). Compound **12** exhibited two absorption peaks at 250–280 nm and 340–360 nm in all solvents (except DMF). The bands at 250–280 nm and 340–360 nm were attributed to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in compound **12**, respectively. The changes in the absorption peaks of compound **12** depending on the solvent polarity are presented in Table 1. Blue-shifts in both peaks were observed. The magnitude of the shifts was related to the strength of the hydrogen bond formed [10].

The steady-state fluorescence and normalized fluorescence spectra of compound **12** are presented in Figs. 2 and 3. As shown in Fig. 2, compound **12** displayed a high-intensity fluorescence band between 405 and 417 nm in all the solvents. In addition, formation of shoulders in the fluorescence band of compound **12** was observed in some solvents. It is known that these shoulders can be attributed to π^*–π transitions from different vibrational levels of the first singlet excited state to the ground state [11, 12]. Blue-shifts in the fluorescence maxima were observed with increase in the solvent polarity (Table 1). Depending on the solvent polarity, the blue-shifts in the absorption peaks of compound **12** were lower than those of the fluorescence peaks. It is suggested that compound **12** has no charge transfer in the ground state and that solvent–solute interactions are greater in the excited state [13]. This negative solvatochromism observed with increasing solvent polarity indicates that the ground-state molecule is better stabilized by solvation than the molecule in the excited state [3, 14, 15]. The formation of

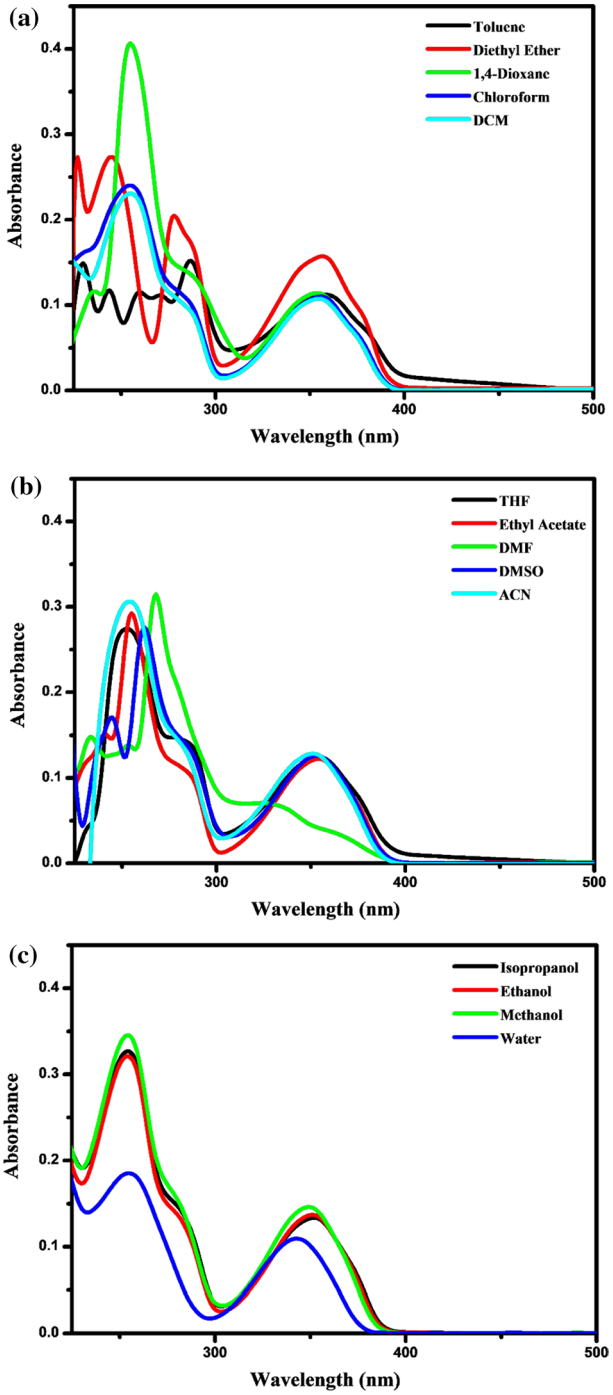


Fig. 1 Absorption spectra of compound **12** in **a** aprotic, **b** polar aprotic, and **c** polar protic solvents

Table 1 Spectroscopic and photophysical properties of compound **12** in selected solvents

Solvent	ε (M^{-1} cm^{-1})	λ_{abs} (nm)	λ_{em} (nm)	Φ	τ_{av} (ns)	$k_r \times 10^{-9}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
Toluene	1520	287/358	417	0.72	6.540	0.1104	0.0425
Diethyl ether	2048	278/357	410	0.59	8.024	0.0735	0.0512
1,4-Dioxane	4065	254/354	417	0.57	5.403	0.1052	0.0799
THF	2744	253/354	414	0.63	6.868	0.1060	0.0341
Ethyl acetate	2925	255/355	411	0.71	6.705	0.1336	0.0053
Chloroform	2402	255/356	411	0.76	7.140	0.0915	0.0541
DCM	2307	255/355	413	0.96	7.199	0.1053	0.0439
DMF	3146	268	414	0.84	7.464	0.1123	0.0217
DMSO	2760	262/353	416	0.82	7.486	0.1090	0.0246
ACN	3064	254/351	414	0.71	7.382	0.0967	0.0388
i-PrOH	3269	254/352	407	0.90	7.046	0.1277	0.0142
EtOH	3212	254/351	407	0.77	7.051	0.1091	0.0327
MeOH	3455	254/349	409	0.76	7.583	0.1000	0.0319
Water	1854	225/343	405	0.67	8.624	0.0775	0.0385

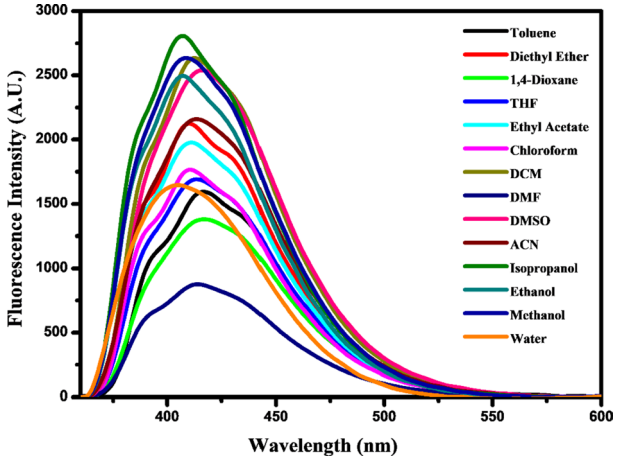


Fig. 2 Fluorescence spectra of compound **12** in selected solvents

hydrogen bonds with increasing polarity ensures that the molecule is stable in the ground state [16].

The fluorescence quantum yield and lifetime values of compound **12** were also determined depending on solvent polarity. These values for compound **12** in different solvents are listed in Table 1. The fluorescence quantum yields were rather high in all the investigated solvents. It was observed that there was no linear relationship between the fluorescence quantum yield values and the solvent polarity. It is generally observed that the quantum yield increases or decreases with increasing polarity [17–19]. It is apparently difficult to obtain high quantum yield

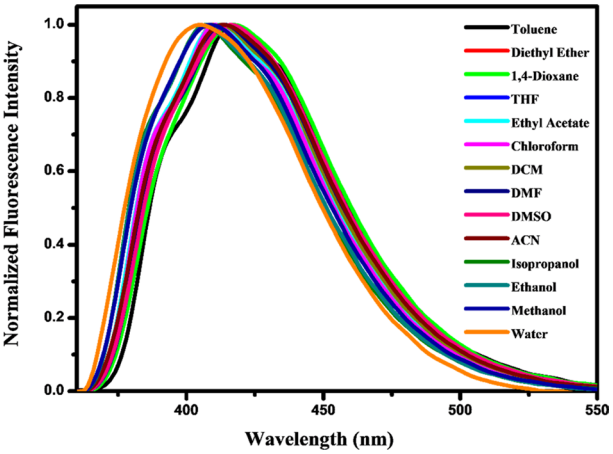


Fig. 3 Normalized fluorescence spectra of compound **12** in selected solvents

Table 2 E_T (dye) values for compound **12** in selected solvents and the $E_T(30)$ values

Solvent	$E_T(30)$ (kcal mol^{-1})	E_T(dye) (kcal mol^{-1})
Toluene	33.9	68.56
Diethyl ether	34.5	69.73
1,4-Dioxane	36.0	68.56
THF	37.4	69.06
Ethyl acetate	38.1	69.56
Chloroform	39.1	69.56
DCM	40.7	69.23
DMF	43.2	69.06
DMSO	45.1	68.76
ACN	45.6	69.06
i-PrOH	48.4	70.25
EtOH	51.9	70.25
MeOH	55.4	69.90
Water	63.1	70.60

in all solvents [20]. As observed for the quantum yield, the fluorescence lifetime values of compound **12** were also found to be very high in all solvents (Table 1).

The solvatochromism of compound **12** was investigated in 14 solvents with different polarity. Table 2 presents the E_T (dye) values for each solvent, calculated from the fluorescence spectra as E_T (dye) = 28,591/E_{max}, as well as the $E_T(30)$

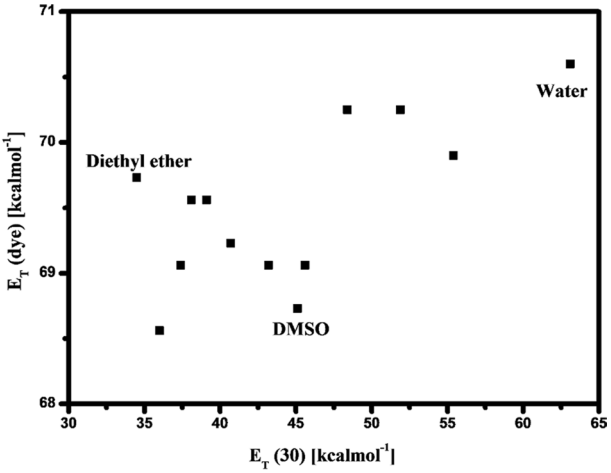


Fig. 4 E_T (dye) values for selected solvents as a function of Reichardt's $E_T(30)$ parameter

values of the solvents [14, 21]. Figure 4 shows a plot of E_T (dye) as a function of $E_T(30)$ for compound **12**. As seen in Fig. 4, there are two regions corresponding to less and more polar solvents. Starting with diethyl ether, the E_T values for compound **12** decrease until DMSO then increase with increasing solvent polarity from DMSO to water. It is believed that these changes indicate that reverse solvatochromism occurs in compound **12** with increasing solvent polarity [22, 23].

Conclusions

The photophysical behavior of a new 4-aza-indole derivative [ethyl 1-((2-(2-ethoxy-2-oxoethyl)pyridin-3-yl)carbamoyl)-2-hydroxy-1*H*-pyrrolo-[3,2-*b*]pyridine-3-carboxylate, **12**] were determined in different solvents. Compound **12** exhibited high fluorescence intensities at wavelengths ranging from 405 to 417 nm in all solvents except DMF. Compound **12** displayed reverse solvatochromism behavior depending on the solvent polarity. Compound **12** exhibited very high quantum yield values in all solvents, representing a rare result in literature. The results suggest that this novel dye could be used in many applications, e.g., as a labeling agent and in bio- or analytical sensors and/or optoelectronic devices.

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