

New benzo[*a*]phenoxazines bearing the (4,6-dichloro-1,3,5-triazin-2-yl)amino group: synthesis and photophysical properties

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Abstract: Synthesis of new benzo[*a*]phenoxazinium chlorides possessing the (4,6-dichloro-1,3,5-triazin-2-yl)amino at 5-amino function of the heterocycles is described. The preliminary photophysical properties of these compounds in anhydrous ethanol when acidified with TFA or basified with TEAH is also investigated, as well as their response in aqueous media. These benzo[*a*]phenoxazinium chlorides exhibited fluorescence with maximum emission wavelengths between 628 and 676 nm, in anhydrous ethanol and water.

Keywords: Benzo[*a*]phenoxazinium dyes; Nile Blue; 2,4,6-trichloro-1,3,5-triazine; acid-base equilibrium.

1. Introduction

Fluorescent probes such as small organic molecules used for optical imaging purposes have emerged as an important tool in various fields of sciences.¹ Among the fluorescent probes, benzophenoxazinium derivatives are preferred due to their high wavelength of emission maxima, good molar extinction coefficients, photochemical stability and long shelf lives.² Due to their interesting optical properties, benzo[*a*]phenoxazinium chlorides are used as biomarkers for labeling of amino acids, proteins and also utilized for various other analytical applications.³ In addition, these compounds are medicinally important as antifungal⁴ and antimalarial agents.⁵ Phenoxazine core compounds also serve as electron donors for dye sensitized solar cells,⁶ enhance electro-optic activity in second-order nonlinear optical chromophores⁷ and function as efficient sensitizers for photodynamic therapy (PDT).⁸

Owing to the importance and our research activity towards Nile Blue (NB) derivatives,⁹⁻¹¹ we herein describe the synthesis of new benzo[*a*]phenoxazinium chlorides possessing the (4,6-dichloro-1,3,5-triazin-2-yl)amino at 5-position of the polycyclic system. The photophysical characterization in anhydrous ethanol and aqueous media along with the addition of acid and base is discussed.

2. Experimental

Typical procedure for the preparation of compounds 1 and 2 (illustrated for 1).

To an ice cold solution of 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **4a** (50 mg, 0.23 mmol) in ethanol (1 mL), was added concentrated hydrochloric acid (0.6 μ L) followed by 4,6-dichloro-*N*-(naphthalen-1-yl)-1,3,5-triazin-2-amine **3** (34 mg, 0.12 mmol) and was refluxed for 14 hrs. The progress of the reaction was monitored by TLC (dichloromethane/methanol: 95:5). After evaporation of the solvent and column chromatography purification on silica gel with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, *N*-(5-((4,6-dichloro-1,3,5-triazin-2-yl)amino)-10-methyl-9*H*-benzo[*a*]phenoxazin-9-ylidene)ethanaminium chloride **1** was obtained as a blue solid (0.027 g, 24%). ¹H NMR (CD₃OD, 400 MHz): δ_{H} = 1.37 (t, *J* = 7.2 Hz, 3H, NCH₂CH₃), 2.27 (s, 3H, CH₃), 3.47 (q, *J* = 7.2 Hz, 2H, NCH₂CH₃), 6.70 (s, 1H, 8-H), 6.75 (s, 1H, 6-H), 7.52 (s, 1H, 11-H), 7.76 (broad s, 1H, 2-H), 7.88 (t, *J* = 7.6 Hz, 1H, 3-H), 8.21 (d, *J* = 7.2 Hz, 1H, 1-H), 8.78 (d, *J* = 7.6 Hz, 1H, 4-H) ppm. ¹³C NMR (CD₃OD, 100.6 MHz): δ_{C} = 14.14 (NCH₂CH₃), 17.66 (CH₃), 39.70 (NCH₂CH₃), 94.48 (C-8), 97.17 (C-6), 123.96 (Ar-C), 124.51 (C-1), 125.45 (C-4), 128.38 (C-10), 130.61 (C-2), 131.61 (Ar-C), 132.77 (C-11), 132.94 (Ar-C), 133.18 (C-3), 134.32 (Ar-C), 149.02 (Ar-C), 151.57 (Ar-C), 152.46 (Ar-C), 156.57 (2 \times Ar-C), 161.94 (2 \times Ar-C) ppm.

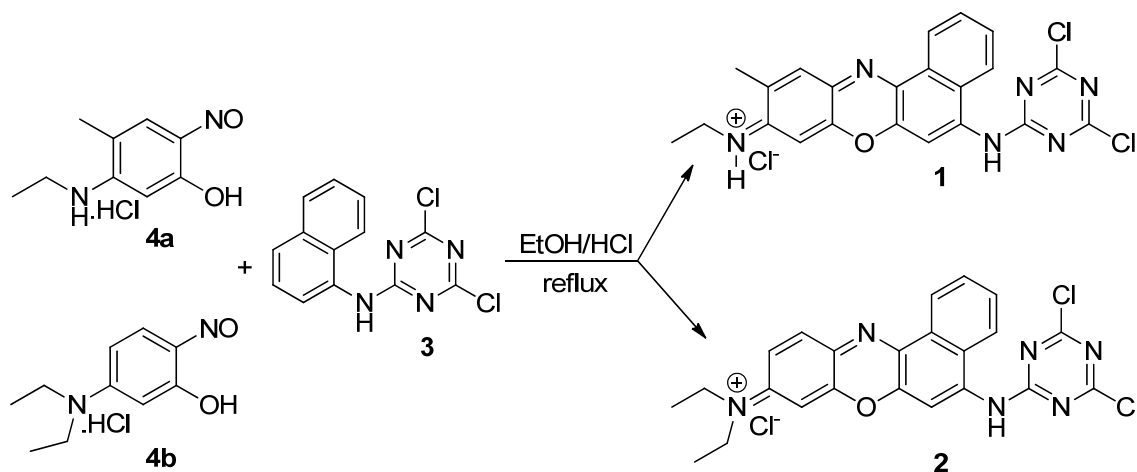
Procedure for the *N*-alkylation of naphthalen-1-amine with 2,4,6-trichloro-1,3,5-triazine.

A solution of 2,4,6-trichloro-1,3,5-triazine (0.500 g, 2.71 mmol) in acetone (3 mL) was added with stirring to a cold solution (0-5 °C) of sodium hydrogenocarbonate (0.600 g) in water (6 mL), in a three-necked flask (250 mL) equipped with a magnetic stirrer. This resulted in the formation of slurry of cyanuric chloride. A solution of 1-naphthylamine (0.777 g, 2.71 mmol) in acetone (1 mL) was added to the cold slurry of cyanuric chloride. After sometime the reaction mixture was neutralized by saturated solution of sodium hydrogenocarbonate. The mixture was stirred for 2 hr at 0-5 °C. The pale violet solid was filtered and recrystallized from ethanol to obtain 4,6-dichloro-*N*-(naphthalen-1-yl)-1,3,5-triazin-2-amine **3** in good yield (0.125 g, 79%). TLC (dichloromethane/methanol, 9.5:0.5): *R*_f = 0.53.

Mp 149-151 °C. ^1H NMR (CD_3OD , 400 MHz): $\delta_{\text{H}} = 7.51-7.63$ (m, 2H, 2-H and 3-H), 7.65-7.72 (m, 2H, 6-H and 7-H), 7.94-8.02 (m, 2H, 4-H and 5-H), 8.05-8.14 (m, 1H, 8-H) ppm. ^{13}C NMR (CD_3OD , 100.6 MHz): $\delta_{\text{C}} = 123.46$ (C-4), 125.39 (Ar-C), 126.39 ($\text{C}_{\text{nap-2}}$), 127.04 ($\text{C}_{\text{nap-6}}$), 127.64 ($\text{C}_{\text{nap-3}}$), 127.92 ($\text{C}_{\text{nap-7}}$), 129.04 ($\text{C}_{\text{nap-5}}$), 129.50 ($\text{C}_{\text{nap-8}}$), 135.71 (Ar-C), 148.61 (Ar-C), 164.49 (Ar-C), 164.95 (Ar-C), 166.96 (Ar-C) ppm. IR (KBr 1%, cm^{-1}): $\nu = 3126, 3063, 2921, 2788, 2615, 1667, 1631, 1588, 1543, 1509, 1392, 1373, 1346, 1313, 1267, 1236, 1171, 1092, 1055, 1020, 995, 957, 886, 846$ cm^{-1} .

3. Results and discussion

Benzophenoxazinium chlorides **1** and **2** were synthesized by condensation of nitroso derivatives, namely 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **4a** and 5-(diethylamino)-2-nitrosophenol hydrochloride **4b** with 4,6-dichloro-*N*-(naphthalen-1-yl)-1,3,5-triazin-2-amine **3** in acidic conditions. 4,6-Dichloro-*N*-(naphthalen-1-yl)-1,3,5-triazin-2-amine **3** was obtained by *N*-alkylation of naphthalen-1-amine with 2,4,6-trichloro-1,3,5-triazine in acetone under ice cold conditions. The other intermediates such as 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **4a**, 5-(diethylamino)-2-nitrosophenol hydrochloride **4b** were obtained by the nitrosation of 3-(ethylamino)-4-methylphenol and 3-(diethylamino)phenol with sodium nitrite in the presence of hydrochloric acid, in a mixture of ethanol-water as the solvent under ice cold conditions.¹²



Scheme 1. Synthesis of benzo[*a*]phenoxazinium chlorides **1** and **2**.

The condensation reaction of nitroso derivatives 5-(ethylamino)-4-methyl-2-nitrosophenol hydrochloride **4a**, 5-(diethylamino)-2-nitrosophenol hydrochloride **4b** with 4,6-dichloro-*N*-(naphthalen-1-yl)-1,3,5-triazin-2-amine **3** was carried out in the presence of concentrated hydrochloric acid under reflux conditions. The progress of the reaction was checked with TLC and upon completion; the crude reaction mixture was purified with dichloromethane and mixtures of dichloromethane/methanol as eluents. The benzo[*a*]phenoxazinium chlorides **1** and **2** were obtained as blue solids (Scheme 1). All compounds obtained were fully characterized by the usual analytical techniques.

The ¹H NMR spectra (**1** and **2**) exhibited a triplet for the terminal methyl groups (δ 1.36 ppm) and methylene protons proximity to the nitrogen atom appeared as quartets at (δ 3.47 to 3.72 ppm). The methyl group of 10-H for the compound **1** showed at a sharp singlet at (δ 2.27 ppm). The aromatic protons of the polycyclic system showed singlets for 6-H (δ 6.75 or 6.92 ppm), and either singlet or doublet for 8-H (δ 6.70 or 6.99 ppm). Similarly, for the other aromatic protons 11-H (δ 7.52 or 7.95 ppm), 2-H (δ 7.76 or 7.87 ppm), 1-H (δ 8.21 or 8.37 ppm) and 3-H (δ 7.88 or 7.99 ppm) appeared in form of singlets doublets, triplets and multiplets.

The ¹³C NMR spectra showed the carbons of the methyl groups (**1** and **2**) (δ 12.86 to 14.14 ppm) and methylene carbons adjacent to the nitrogen (39.70 to 46.97 ppm). The methyl group attached to C-10 for the compound **1** at (δ 17.66 ppm). The aromatic signals for the carbons C-6 and C-8 (δ 97.17 or 94.48 ppm), C-2 and C-11 (δ 130.61 to 134.29 ppm) and C-3 (δ 133.18 or 133.50 ppm) were exhibited.

Electronic absorption and emission spectra of 4×10^{-6} M solutions in anhydrous ethanol and water were measured for synthesized benzo[*a*]phenoxazinium chlorides (Table 1). The relative fluorescence quantum yields (Φ_F) were determined using Oxazine 1 as a standard ($\Phi_F = 0.11$ in ethanol).¹³

Table 1. Preliminary photophysical studies of compounds **1** and **2** in anhydrous ethanol and water after the addition of TFA.

Cpd	Dry ethanol + TFA					Water + TFA				
	λ_{abs} (nm)	$\epsilon(\text{M}^{-1}\text{cm}^{-1})$	λ_{em} (nm)	$\Delta\lambda$ (nm)	Φ_F	λ_{abs} (nm)	$\epsilon(\text{M}^{-1}\text{cm}^{-1})$	λ_{em} (nm)	$\Delta\lambda$ (nm)	Φ_F
1	615	97750	639	24	0.73	616	59250	644	28	0.29
2	628	74400	663	35	0.33	636	35025	676	40	0.06

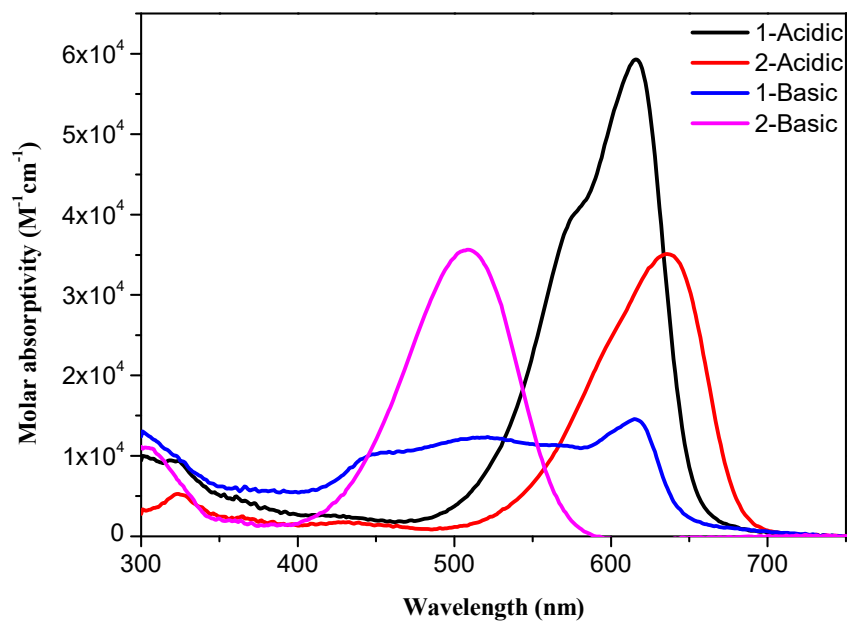


Figure 1. Absorption spectra of compounds **1** and **2** in acidified and basified water.

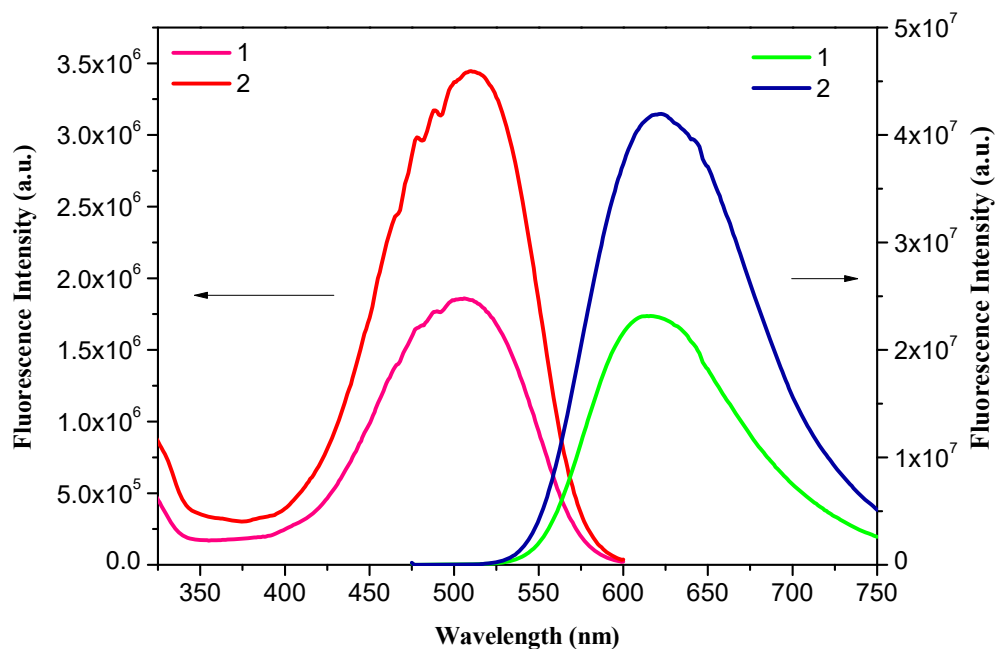


Figure 2. Spectra in basified dry ethanol at 470 nm excitation and the excitation spectra were collected at 605 nm emission wavelength.

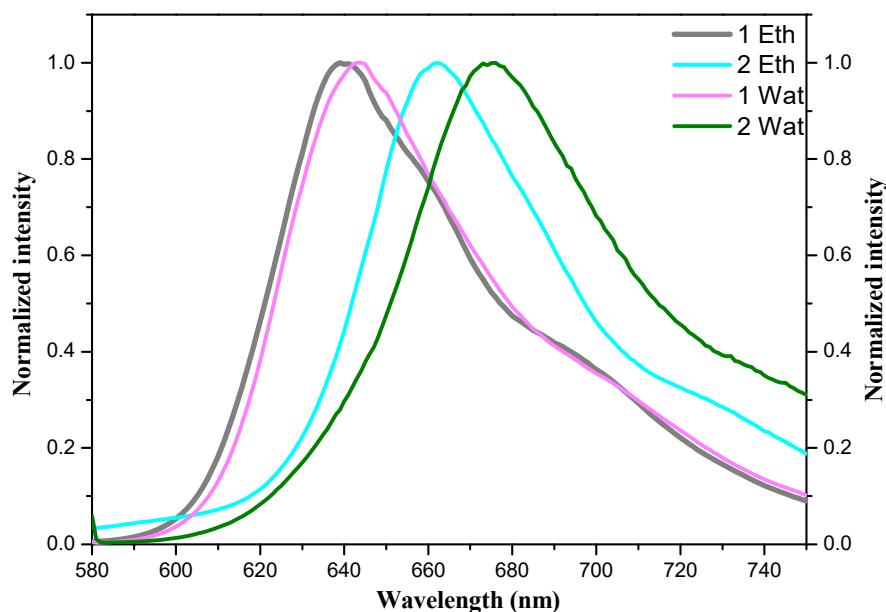


Figure 3. Emission spectra of compounds **1** and **2** in dry ethanol and aqueous media at 575 nm excitation.

In our previous studies,^{14,15} it was found that acid-base equilibrium of benzo[*a*]phenoxazinium chlorides in proton accepting solvents is influenced by the substituents mainly attached at 5-amino position of the heterocyclic system. In ethanol media the emission spectra of the basic form is broad and centered at around 600 nm while the acid form (AH^+) shows a band above 660 nm with a much higher quantum yield.¹⁶ The variation in fluorescence quantum yields with change in substitution at 9-amino position of the benzophenoxazine is monoalkylated 0.4 and 0.1-0.2 for the dialkylated compounds of similar types.¹⁴

In comparison, compound **2** exhibited significant bathochromic shifts both in absorption (13 nm) and emission (24 nm) (Table 1). This is mainly, due to the di-alkylation at the 9-amino position as previously observed. Similarly, compound **1** showed the highest fluorescence efficiency in ethanoic media. On the other hand, greater Stokes shift (40 nm) in aqueous media was observed for compound **2**, which clearly indicates the stronger solvent fluorophore interactions. In acidified water, compound **1** shows the highest absorption and compound **2** decreases in its absorption value with a bathochromic shift of 20 nm (Figure 1). The maximum emission was registered by compound **2** in acidic water (λ_{em} 676 nm). At 470 nm excitation the basic form is mostly excited with a small fraction of acidic form.

Figure 2 shows the spectra in basified dry ethanol at 470 nm excitation and the excitation spectra were collected at 605 nm emission wavelength. Similarly, figure 3 shows the emission spectra at 575 nm excitation, where only the acidic AH⁺ form is excited.

4. Conclusion

Benzo[*a*]phenoxazinium chlorides possessing the (4,6-dichloro-1,3,5-triazin-2-yl)amino at 5-position function of the heterocycle were synthesized. The acidic and basic forms were studied in anhydrous ethanol and water by the addition of either strong acid or base. These compounds have tendency towards aggregation in aqueous medium. This type of fluorescent dyes can be used as synthetic intermediates and extension of the studies are in progress.

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