Synthesis and photophysics of new benzophenoxazine derivatives fused with julolidine

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Abstract: Phenoxazinium salts are mostly used as markers for various purposes. In this context, we report the synthesis of five new benzophenoxazinium derivatives fused with julolidine moiety. These benzophenoxazinium chlorides were obtained by the condensation of anthracen-1-amine, naphthalen-1-amine, *N*-phenylnaphthalen-1-amine, *N*-propylnaphthalen-1-amine and 3-(naphthalen-1-ylamino)propan-1-ol, with 9-nitroso-8-hydroxyjulolidine hydrochloride as blue solids. The photophysical behavior of these compounds in anhydrous ethanol when acidified with TFA or basified with TEAH was also investigated as well as their response in aqueous media.

Keywords: Nile Blue; julolidine; benzophenoxazinium dyes; NIR probes; acid-base equilibrium

1. Introduction

Fluorescent probes are widely used for imaging in cell biology.[1] Nile Blue (NB), one of the most used benzo[*a*]phenoxazinium dyes, and their derivatives emit light in the near infrared (NIR) region of the spectrum and are prominent as indicators for fluorimetric and photometric detection.[2] In general, benzo[*a*]phenoxazinium dyes are well known as markers due to their several interesting spectral properties. These compounds exhibit intense long-wavelength absorption and emission at about 650nm. Hence, they are used as potential candidates for dyeing paper, fiber stuff and laser dyes.[3] Moreover, they are useful as photosensitizers in photodynamic therapy, fluorophor in energy and electron-transfer reactions antitubercolastatic or anticancerogenic agents.[4]

In the previous reports by our research group we have synthesised several NB derivatives,[5-10]

and in the present communication we are interested to explore the properties of a rigid structure in comparison with the unrestricted dye. Herein, we report the synthesis of benzophenoxazinium derivaties fused with julolidine moiety. The photophysical characterization was performed in dry ethanol and aqueous medium. Fluorescence quantum yields and acid-base equilibrium were also examined.

2. Experimental

Typical procedure for the preparation of compounds 1, 2a-d (illustrated for **1**).

To a cold solution (ice bath) of 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol (9-nitroso-8-hydroxyjulolidine hydrochloride) **3** (0.115 g, 0.525 mmol) in ethanol (2 mL), anthracen-1-amine **4** (0.051 g, 0.262 mmol) and concentrated hydrochloric acid (14.0 µL) was added. The mixture were refluxed for 18 hours and monitored by TLC (dichloromethane/methanol, 9.5:0.5). After evaporation of the solvent and column chromatography purification on silica gel with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, 9,10,11,13,14,15-hexahydro-6*H*-naphtho[2,3-*a*]quinolizino[1,9-*hi*]phenoxazin-6-iminium chloride **1** was obtained as a blue solid (0.047 g, 21%). ^{1}H NMR (CD$_3$OD, 400 MHz): δ_H = 1.92-2.05 (m, 4H, 10-H and 14-H), 2.58 (t, J = 6.8 Hz, 2H, 9-H), 2.79 (t, J = 6.4 Hz, 2H, 15-H), 3.42 (t, J = 6.0 Hz, 2H, 11-H), 3.50 (m, 2H, 13-H), 6.49 (s, 1H, 7-H), 7.08 (s, 1H, 16-H), 7.67 (m, 2H, 2-H and 3-H), 8.0 (d, J = 8.0 Hz, 1H, 1-H), 8.04 (d, J = 7.2 Hz, 1H, 4-H), 8.68 (s, 1H, 5-H), 8.90 (s, 1H, 18-H) ppm. ^{13}C NMR (CD$_3$OD, 100.6 MHz): δc = 20.09 (C-14), 20.48 (C-10), 21.61 (C-9), 28.49 (C-15), 51.43 (C-11), 52.03 (C-13), 96.77 (C-7), 106.27 (Ar-C), 121.56 (Ar-C), 124.86 (C-16), 125.63 (Ar-C), 127.13 (C-2), 128.70 (C-3), 128.88 (C-1), 129.82 (C-4), 130.10 (Ar-C), 130.33 (Ar-C), 131.22 (Ar-C), 133.47 (Ar-C), 135.23 (Ar-C), 151.00 (Ar-C), 160.00 (C-6) ppm. IR (KBr 1%, cm^{-1}): v = 3432, 3350, 3096, 2950, 1656, 1634, 1573, 1551, 1517, 1472, 1435, 1410, 1385, 1351, 1333, 1286, 1214, 1166, 1142, 1095, 1052, 1015, 902, 838, 752 cm^{-1}.

Typical procedure for the preparation of intermediates of 5a-d (illustrated for **5b**).

To a solution of naphthalen-1-amine (1.0 g, 6.98 mmol) in ethanol (3 mL), chlorobenzene (0.783 g, 6.98 mmol) was added, and the resulting mixture was refluxed for 6 hours. The progress of reaction was monitored by TLC (dichloromethane/methanol, 9.5:0.5). After completion of the reaction, the solvent was evaporated and the mixture was purified by column chromatography on silica using

dichloromethane and dichloromethane/methanol (99:1), as the eluent. *N*-Phenylnaphthalen-1-amine **5b** was obtained as violet solid (1.357 g, 88%). TLC (dichloromethane/methanol, 9.0:1.0): R_f = 0.40. Mp 59-61 °C. ^{1}H NMR (CDCl$_3$, 400 MHz): δ_H = 6.81 (dd, *J* = 6.8 and 1.6 Hz, 2H, 2-H, 4'-H), 7.32-7.41 (m, 4H, 2-H, 3-H, 2'-H and 6'-H), 7.48-7.54 (m, 4H, 6-H, 7-H, 3'-H and 5'-H), 7.83-7.88 (m, 3H, 4-H, 5-H and 8-H) ppm. ^{13}C NMR (CD$_3$OD, 100.6 MHz): δc = 109.61 (C-4), 118.89 (C-2' and C-4'), 120.74 (C-4 and C-8), 123.59 (Ar-C), 124.78 (C-7), 125.78 (C-6), 126.28 (C-3, Ar-C and C-5'), 128.48 (C-5), 134.33 (C-4a and C-1'), 142.02 (C-1) ppm.

3. Results and discussion

Benzophenoxazinium chlorides **1**, **2a-d** were synthesised by condensation of 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol **3** with anthracen-1-amine, naphthalen-1-amine and *N*-alkylated naphthalen-1-amines **5a-d** in acid media. Intermediates **5b-d** were obtained by alkylation of naphthalen-1-amine with chlorobenzene, 1-bromopropane and 3-bromopropan-1-ol in ethanol as the solvent, in low to moderate yields. The required 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol **3** was obtained by nitrosation of the corresponding 1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol with sodium nitrite in the presence of hydrochloric acid, in a mixture of ethanol-water as the solvent.[11]
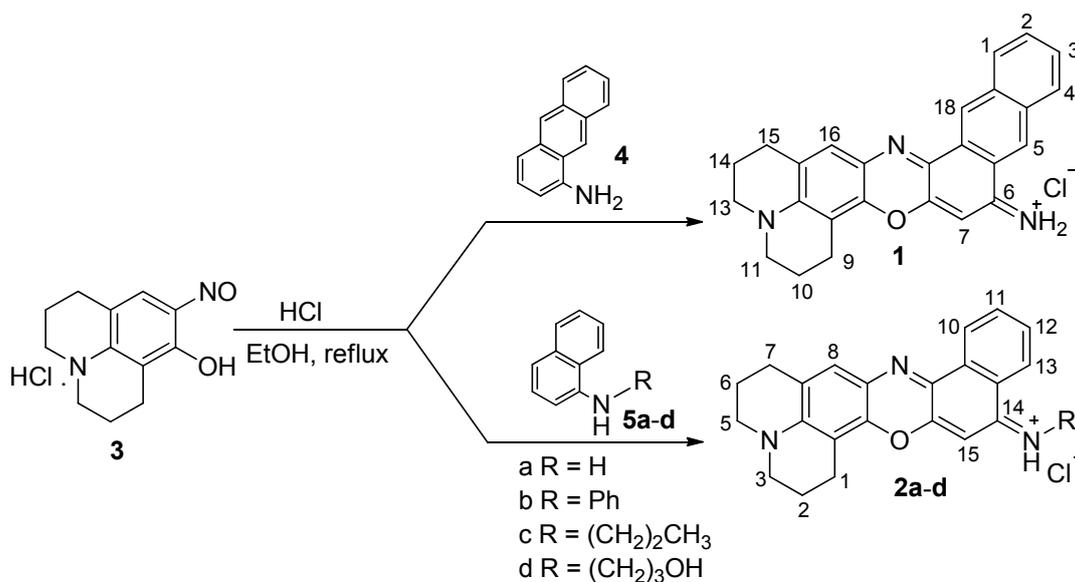
Scheme 1. Synthesis of benzophenoxazinium chlorides **1** and **2a-d**.

The condensation reaction of anthracen-1-amine **4**, naphthalen-1-amine **5a**, *N*-phenylnaphthalen-1-amine **5b**, *N*-propylnaphthalen-1-amine **5c** and 3-(naphthalen-1-ylamino)propan-1-ol **5d**, with 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol **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 methanol/dichloromethane as eluents. The benzophenoxazinium chlorides **1**, **2a-d** were obtained as blue solids (Scheme 1). All compounds obtained were fully characterized by the usual analytical techniques.

The ^{1}H NMR spectra (**2a-d**) showed the signals of aliphatic protons from the methylenic groups of 1-H and 7-H as multiples (δ 2.51 to 2.98 ppm), 2-H and 6-H (δ 1.98 to 2.22 ppm) and methylene protons close to the nitrogen atom 3-H and 5-H appeared as multiplets (δ 3.50 to 3.67 ppm). Similarly, the methylenic groups of substituents at 14-position, directly linked to the nitrogen atom NH*CH$_2$* appeared as a multiplet or a triplet (**2c**, **2d**) (δ 3.63 to 3.75 ppm), as well as groups close to the same atom, NHCH$_2$*CH$_2$*, showed as multiplets at (δ 1.30 to 2.14 ppm). The terminal methyl group exhibited a triplet (δ 1.13 ppm) and methylene protons adjacent to hydroxyl functionality (**2d**) showed a triplet (δ 3.82 ppm). In addition, spectra showed the aromatic protons of the polycyclic system, in particular 8-H (δ 7.16 to 7.47 ppm), and 15-H (δ 6.77 to 6.92 ppm), which appeared in the form of singlets.

The ^{13}C NMR spectra showed the signals of methylenic groups of C-1 and C-7 (δ 28.38 to 20.60 ppm), C-2 and C-6 (δ 21.47 to 21.66) and close to the nitrogen atom C-3 and C-5 (δ 51.88 to 52.47 ppm). The groups of substituents at 14-position, directly linked to the nitrogen atom NH*CH$_2$* (**2c**, **2d**) (δ 42.97 to 47.08 ppm), as well as groups close to the same atom, NHCH$_2$*CH$_2$*, (δ 30.75 to 32.27 ppm). In addition, there was the presence of carbons of the methyl group (**2a**, δ 11.78 ppm) and the carbon proximity to hydroxyl functionality (**2d**, δ 60.54 ppm). Spectra showed the aromatic carbons, in particular C-8 (δ 130.03 to 130.27 ppm) and C-15 (δ 93.35 to 96.70 ppm), respectively.

Fundamental photophysical studies and the fluorescent properties of benzophenoxazinium chlorides **1**, **2a-d** were carried out in dry ethanol, and distilled water.

Previous studies on this type of compounds showed that the photophysical properties in proton-accepting solvents is influenced by acid-base equilibria mainly located at the 5-amino position.[9,10] In ethanol media the absorption spectra are dominated by an acidic form (AH[+]) and a ~100nm blue shifted neutral form (A).[12] The fluorescence of the basic form is broad and centred at around 600nm while the acid form (AH[+]) shows a band centred above 660nm with a much higher quantum yield.[12] The later

reaches 0.4 when the 9-amino position is mono-alkylated and varies between 0.1 and 0.2 when it is di-alkylated.[9,10,12]

At 470nm the basic form is mostly excited with a small fraction of acidic form. At higher wavelengths the situation is reversed. In Figures 1 and 2 the absorption and fluorescence of compounds **1**, **2a-d** are shown either in ethanolic or aqueous media.
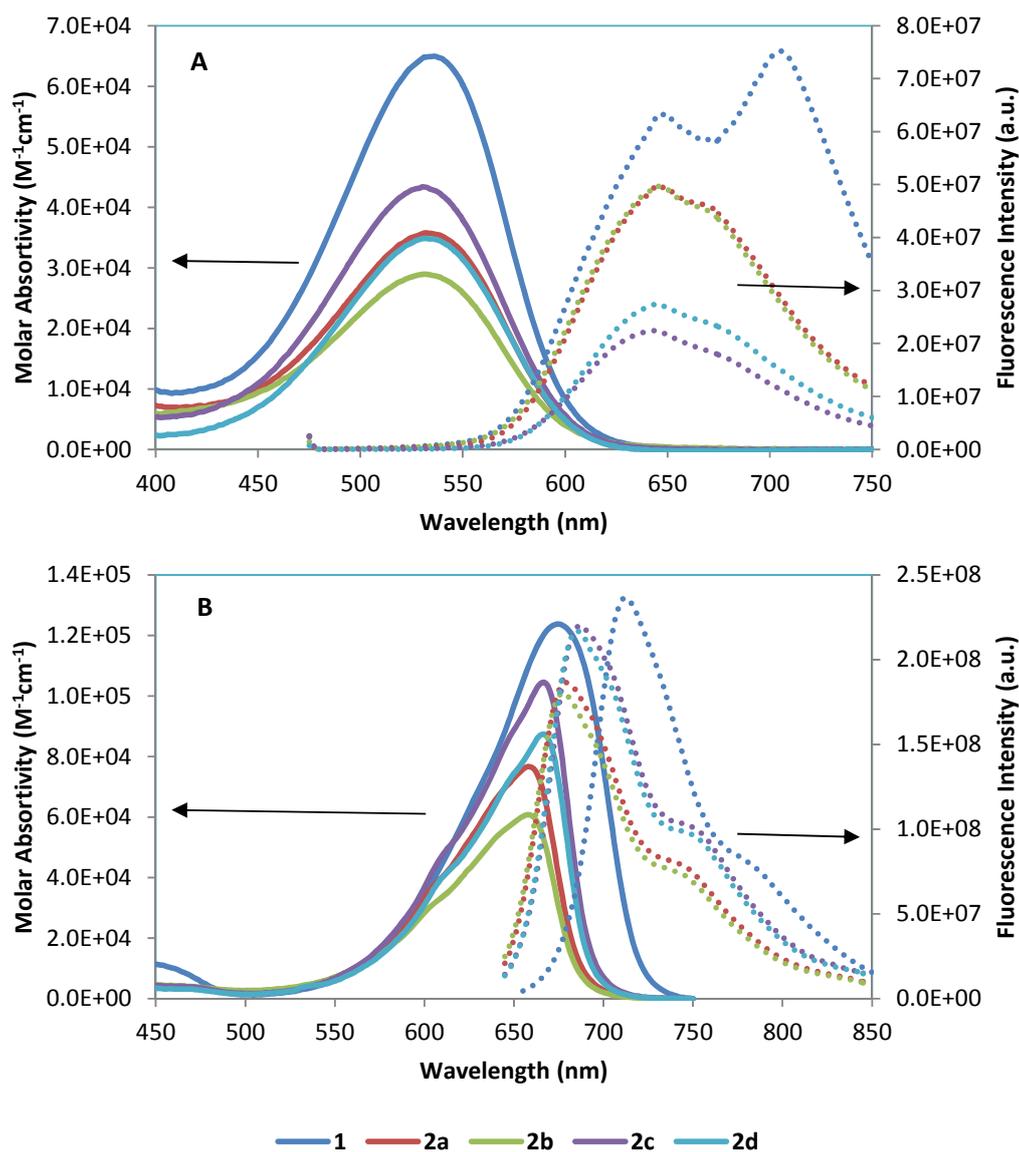
Figure 1. Absorption and emission spectra of compounds **1** and **2a-d** in either basified (panel **A**) or acidified (panel **B**) dried ethanol. Emission of basic form at 470nm excitation and emission of acid form at 640nm excitation.
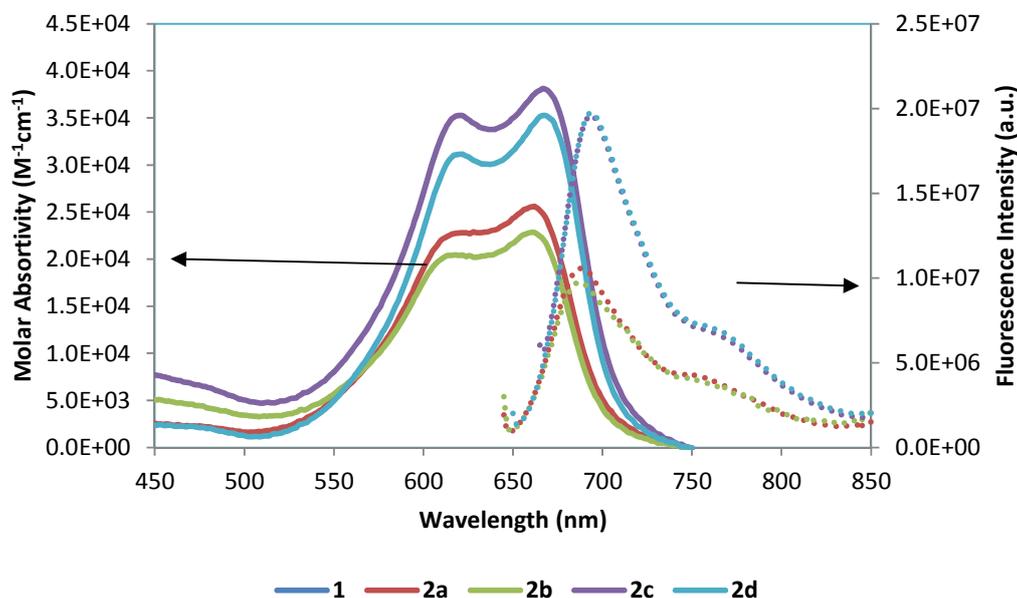
Figure 2. Absorption and emission spectra of compounds **1** and **2a-d** in acidified water. Emission at 640nm excitation.

The above characteristics are confirmed in ethanol media when acidified with trifluoroacetic acid (TFA) or basified with tetraethylammonium hydroxide (TEAH). In contrast, the acid-base behavior is different from what we obtained in our earlier reports for similar compounds but without the julolidine moiety[7,8] as, using the same amount of TEAH, the equilibrium is not completely shifted towards the basic form (Fig.1). For the acid form it seems possible to completely displace the equilibrium with the same amount of TFA previously used, but, particularly for compound **1**, the absorption spectra are broader.

The absorption spectra of these type of compounds, but with an amino group at the 9-position, in water usually evidences the presence of non-fluorescent H-aggregates through a ~40nm blue shifted shoulder.[12] The relative amount of that shoulder obviously depends on dye concentration. In the present case of the studied julolidine fused compounds the spectrum at 4×10^{-6}M is similar to the ones obtained for compounds without the julolidine moiety at 5×10^{-5}M. This clearly indicates the much higher tendency for aggregation of the synthesised benzophenoxazines fused with julolidine.

Table 1 shows absorption and emission maxima, Stokes shift and fluorescence quantum yield for the acid form in dried ethanol and in water.

Table 1. Preliminary photophysical studies of compounds **1**, **2a-d** in dry ethanol, water and after the addition of TFA and TEAH.

	Dry ethanol + TFA					Distilled water + TFA				
Cpd	λ_{abs} (nm)	ε(M^{-1}cm^{-1})	λ_{em} (nm)	$\Delta\lambda$ (nm)	Φ_F	λ_{abs} (nm)	ε(M^{-1}cm^{-1})	λ_{em} (nm)	$\Delta\lambda$ (nm)	Φ_F
1	675	123850	701	26	0.16	663	-	-	-	-
2a	658	76650	679	21	0.17	662	25450	687	25	0.024
2b	658	61250	677	19	0.19	661	22900	685	24	0.026
2c	667	104425	685	18	0.19	667	38200	694	27	0.027
2d	666	87300	684	18	0.21	668	36050	694	26	0.032

Through the stiffening of 9-amino position, it was expected that the fluorescence quantum yield in acidified ethanol should be higher than the values previously obtained for benzophenoxazines without a fused julolidine moiety and di-alkylated compounds (between 0.1 and 0.2).[9,10,12] In fact, the improvement in fluorescence quantum yield was not observed which can be explained by the possible presence of aggregates in ethanolic media that was evidenced in the broader spectrum of the studied compounds in acidified ethanol. Nevertheless, it seems that the 5-amino position (6- or 14-positions in **1** and **2a-d**, respectively) is the main pathway of excited state nonradiative desactivation.

Comparing compounds **1** and **2a** significant red shifts were observed both in absorption (17nm) and emission (22nm). The origin of this shift is certainly the higher π conjugation system that results from the fusion of anthracene with the phenoxazine moiety instead of naphthalene.

Also worth mentioning is the much lower fluorescence quantum yield in aqueous media. This is easily understandable by the fact that H-aggregates are non-fluorescent and they are very prominent for the studied compounds.

4. Conclusions

Five benzophenoxazinium chlorides derivatives with fused julolidine bearing different terminal groups at the amino position were synthesised. The photophysics of the acid and basic forms were studied in dried ethanolic media, by adding either a strong acid or a strong base. The acid form was also followed in water. The reported compounds were found to have higher tendency to aggregate than

similar compounds without the fused julolidine. This tendency can possibly account for a lower than expected fluorescence quantum yield for the studied compounds.

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