New benzo[*a*]phenoxazinium chlorides with chlorinated terminals at 2- and 9-positions

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Abstract: The synthesis of novel benzo[*a*]phenoxazinium salts possessing one, two or three chlorinated terminals at their substituents in 2- and 9- positions of the polycyclic moiety was carried out. These compounds displayed fluorescence emission in ethanol and water at physiological pH between 643 and 665 nm.

Keywords: Chlorinated compounds; Benzo[*a*]phenoxazines; Nile Blue derivatives; Fluorescent labels.

1. Introduction

Several research studies involving fused heteroaromatic dyes based on the oxazine moiety have been focus on their absorption and fluorescence spectroscopic properties.[1,2] These long-wavelength fluorophores find applications as biological probes, namely in covalent labeling of amino acids,[3] proteins,[4] peptides and DNA.[5] Also, more frequently they have been used in non-covalent labeling, such us for staining nucleic acids in a variety of context, in monitoring protein conformation alterations or for therapeutic purposes.[6,7]

Although the interesting photophysical properties, several compounds possessing the phenoxazine nucleus have been stressed owing to their antiproliferative properties with potential applications both as antitumour and as antimicrobial agents.[8–10]

Previously reported work regarding the evaluation of the antifungal activity, using *Saccharomyces cerevisiae* as a model organism, of naphtho[2,3-*a*]phenoxazine and 5,9-diaminobenzo[*a*]phenoxazine derivatives, revealed diverse antimicrobial efficiencies, the most efficient compound possessing cloropropyl as substituent at the 5-amino-position of the tetracyclic system (MIC of 3.75 μM).[11,12]

Considering our promising results, and having in mind future evaluation of biological activity, we decided to synthesise new benzo[*a*]phenoxazinium salts having chlorinated terminals at their substitutes in 2- and 9- positions. Furthermore, these compounds can also be used as covalent and non-covalent probes, and consequently the photophysical properties were also studied in ethanol and water in simulated physiological conditions.

2. Experimental

2.1. Typical procedure for the synthesis of 1a-c (described for **1b**): To a solution of 5-((3-chloropropyl)amino)-2-nitrosophenol **2b** (0.055 g, 2.2×10^{-4} mol) in ethanol (3 mL), concentrated hydrochloric acid (2.26×10^{-3} mL) was added followed by the 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3** (0.031 g, 1.1×10^{-4} mol). The reaction mixture was refluxed for 1h and monitored by TLC (dichloromethane/methanol, 8.5:1.5). After evaporation of the solvent and purification by column chromatography on silica gel with dichloromethane and dichloromethane/methanol 8.5:1.5, as the eluent, 3-chloro-*N*-(2-(3-chloropropoxy)-5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)propan-1-aminium **1b** was obtained as a blue solid (0.011 g, 19%). Mp = 128.3-130.8 °C. R_f = 0.33 (dichloromethane/methanol, 9:1). FTIR (KBr 1%): ν_{max} 3389, 2924, 2853, 1640, 1590, 1548, 1461, 1419, 1331, 1281, 1222, 1151, 1127, 1036, 909, 815, 781, 717, 666 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz): δ 1.14 (3H, t, J = 7.2 Hz, $NHCH_2CH_2\mathit{CH_3}$), 1.85-1.95 (2H, m, $NHCH_2\mathit{CH_2}CH_3$), 2.10-2.24 (2H, m, $NHCH_2\mathit{CH_2}CH_2Cl$), 2.30-2.41 (2H, m, $OCH_2\mathit{CH_2}CH_2Cl$), 3.64-3.80 (4H, m, $NHCH_2CH_2\mathit{CH_2}Cl$ and $NH\mathit{CH_2}CH_2CH_3$), 3.87 (2H, t, J = 6.4 Hz, $OCH_2CH_2\mathit{CH_2}Cl$), 4.26 (2H, t, J = 6.8 Hz, $NH\mathit{CH_2}CH_2CH_2Cl$), 4.38 (2H, t, J = 6.0 Hz, $O\mathit{CH_2}CH_2CH_2Cl$), 6.70 (1H, d, J = 2.0 Hz, H-8), 6.90 (1H, s, H-6), 7.11 (1H, dd, J = 7.8 Hz and 1.6 Hz, H-3), 7.32-7.38 (1H, m, H-10), 7.75 (1H, d, J = 9.2 Hz, H-11), 8.18 (1H, d, J = 2.4 Hz, H-1), 8.24 (1H, d, J = 9.2 Hz, H-4) ppm. ^{13}C NMR (CD_3OD, 100.6 MHz): δ 11.73 ($NHCH_2CH_2\mathit{CH_3}$), 23.31 ($NHCH_2\mathit{CH_2}CH_3$), 31.21 ($NHCH_2\mathit{CH_2}CH_2Cl$), 33.19 ($OCH_2\mathit{CH_2}CH_2Cl$), 41.59 ($NH\mathit{CH_2}CH_2CH_2Cl$), 42.21 ($OCH_2CH_2\mathit{CH_2}Cl$), 43.17 ($NHCH_2CH_2\mathit{CH_2}Cl$), 47.53 ($NH\mathit{CH_2}CH_2CH_3$), 66.47 ($O\mathit{CH_2}CH_2CH_2Cl$), 94.18 (C-6), 97.39 (C-8), 108.00 (C-1), 115.60 (C-3), 118.50 (C-Ar), 120.31 (C-10), 126.33 (C-4), 130.31 (C-Ar), 133.79 (C-Ar), 134.96 (C-11), 136.42 (C-Ar), 149.16 (C-Ar), 153.63 (C-Ar), 155.16 (C-9), 159.93 (C-5), 163.33 (C-2) ppm. HRMS: m/z (ESI): calcd. for $C_{25}H_{28}{}^{35}Cl_2N_3O_2$ [M^++1] 472.15531; found 472.15354. Calcd. for $C_{25}H_{28}{}^{35}Cl^{37}ClN_3O_2$ [M^++1] 474.15252; found 474.15016. Calcd. for $C_{25}H_{28}{}^{37}Cl_2N_3O_2$ [M^++1] 476.14951; found 476.15235.

2.2. Procedure for the preparation of 3: To a solution of 5-(propylamino)naphthalen-2-ol (0.070 g, 3.48×10^{-4} mol) in acetonitrile (2 mL), 1-bromo-3-chloropropane (0.038 mL, 3.83×10^{-4} mol) and cesium carbonate (0.554 g, 1.70×10^{-3} mol) were added, and the resulting mixture was heated at 60 ºC for 1h30min. The progress of the reaction was monitored by TLC (ethyl acetate/light petroleum 1:5). The excess of base was filtered out, the solvent was

evaporated and the crude mixture was purified by column chromatography on silica gel using ethyl acetate/light petroleum 1:5, as the eluent. 6-(3-Chloropropoxy)-*N*-propylnaphthalen-1-amine **3** was obtained as a light brown solid (0.09 g, 93%). Mp = 97.1-100.2 °C. TLC (ethyl acetate/light petroleum 1:5): R_f = 0.67. FTIR (neat): ν_{max} 3405, 2959, 2926, 2872, 1623, 1587, 1529, 1476, 1460, 1432, 1378, 1341, 1284, 1267, 1224, 1181, 1151, 1070, 1047, 974, 912, 865, 837, 818, 779, 743 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ 0.96 (3H, t, J = 7.6 Hz, $NHCH_2CH_2\mathit{CH_3}$), 1.64-1.76 (2H, m, $NHCH_2\mathit{CH_2}CH_3$), 2.17-2.28 (2H, m, $OCH_2\mathit{CH_2}CH_2Cl$), 3.15 (2H, t, J = 7.6 Hz, $NH\mathit{CH_2}CH_2CH_3$), 3.82 (2H, t, J = 6.4 Hz, $OCH_2CH_2\mathit{CH_2}Cl$), 4.18 (2H, t, J = 6.0 Hz, $O\mathit{CH_2}CH_2CH_2Cl$), 6.48 (1H, broad s, H-2), 7.00-7.13 (2H, m, H-4 and H-7), 7.20 (1H, d, J = 2.4 Hz, H-5), 7.24 (1H, t, J = 8.0 Hz, H-3), 8.09 (1H, d, J = 9.2 Hz, H-8) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz,): δ 11.67 ($NHCH_2CH_2\mathit{CH_3}$), 21.20 ($NHCH_2\mathit{CH_2}CH_3$), 31.73 ($OCH_2\mathit{CH_2}CH_2Cl$), 42.04 ($OCH_2CH_2\mathit{CH_2}Cl$), 45.92 ($NH\mathit{CH_2}CH_2CH_3$), 64.19 ($O\mathit{CH_2}CH_2CH_2Cl$), 103.00 (C-2), 107.36 (C-5), 116.00 (C-4), 116.21 (C-7), 118.52 (C-8a), 123.48 (C-8), 127.34 (C-3), 135.57 (C-4a), 143.06 (C-1), 156.14 (C-6) ppm. HRMS: m/z (EI): calcd. for $C_{16}H_{20}NO^{35}Cl$ [M^+] 277.1233; found 277.1235; calcd. for $C_{16}H_{20}NO^{37}Cl$ [M^+] 279.1204; found 279.1208.

5-(Propylamino)naphthalen-2-ol

To a solution of 5-aminonaphthalen-2-ol (0.030 g, 1.89×10^{-3} mol) in methanol (2 mL), 1-bromopropane (0.182 mL, 1.98×10^{-3} mol) was added, and the resulting mixture was refluxed for 34h. The progress of the reaction was monitored by TLC (ethyl acetate/light petroleum 1:3). The solvent was evaporated and the crude mixture was purified by column chromatography on silica gel using ethyl acetate/light petroleum 1:3, as the eluent. 5-(Propylamino)naphthalen-2-ol was obtained as a grey solid (0.124 g, 33%). Mp = 88.9-91.3 °C. TLC (ethyl acetate/ light petroleum 1:3): R_f = 0.47. FTIR (neat): ν_{max} 3308, 3100, 2963, 2936, 2875, 1636, 1584, 1513, 1454, 1424, 1384, 1350, 1277, 1224, 1152, 1125, 1078, 1004, 956, 907, 878, 855, 814, 783, 765, 750, 711, 677, 635, 609 cm^{-1}. ^1H NMR (DMSO-d_6, 300 MHz): δ 0.96 (3H, t, J = 7.5 Hz, $NHCH_2CH_2\mathit{CH_3}$), 1.60 - 1.73 (2H, m, $NHCH_2\mathit{CH_2}CH_3$), 3.12 - 3.15 (2H, m, $NH\mathit{CH_2}CH_2CH_3$), 5.92 (1H, broad s, NH), 6.25 (1H, d, J = 7.8 Hz, H-6), 6.83 (1H, d, J = 8.1 Hz, H-8), 6.89 (1H, dd, J = 9.15 and 2.7 Hz, H-3), 6.94 (1H, d, J = 2.4 Hz, H-1), 7.13 (1H, t, J = 8.1 Hz, H-7), 7.99 (1H, d, J = 9.0 Hz, H-4), 9.49 (1H, s, OH) ppm. ^{13}C NMR (DMSO-d_6, 75.4 MHz,): δ 11.83 ($NHCH_2CH_2\mathit{CH_3}$), 21.49 ($NHCH_2\mathit{CH_2}CH_3$), 45.00 ($NH\mathit{CH_2}CH_2CH_3$), 100.28 (C-6), 109.18 (C-1), 113.79 (C-8), 115.79 (C-3), 117.51 (C-4a),

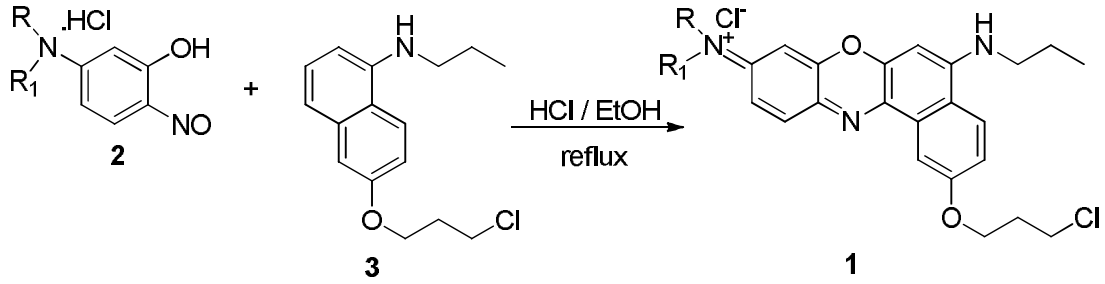
123.34 (C-4), 127.23 (C-7), 135.83 (C-8a), 144.38 (C-5), 154.97 (C-2) ppm. HRMS: m/z (EI): calcd. for $C_{13}H_{15}NO$ [M^+] 201.1154; found 201.1160.

3. Results and Discussion

The synthesis of benzo[*a*]phenoxazinium chlorides **1a-c** was achieved by condensation of the 2-nitrosophenol precursors **2a-c** with 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3** in acidic media. The required 5-(3-aminopropylamino)-2-nitrosophenol di-hydrochloride **2a**, 5-((3-chloropropyl)amino)-2-nitrosophenol hydrochloride **2b** and 5-(bis(3-chloropropyl)amino)-2-nitrosophenol hydrochloride **2c** were synthesized by usual procedure involving treatment of 3-((3-aminopropyl)amino)phenol hydrobromide, 3-((3-chloropropyl)amino)phenol and 3-(bis(3-chloropropyl)amino)phenol, respectively, with sodium nitrite in acid solution.[13,14]

By alkylation of 5-aminonaphthalen-2-ol with the 1-bromopropane in ethanol, under reflux, followed by column chromatography purification in silica gel 5-(propylamino)naphthalen-2-ol was obtained. This derivative was then reacted with 1-bromo-3-chloropropane, in acetonitrile, heating at 60ºC and using cesium carbonate as base yielding the 6-(3-chloropropoxy)-*N*-propylnaphthalen-1-amine **3**, also after column chromatography purification.

Reaction of nitrosophenols **2a-c** with precursor **3** in ethanol and concentrated hydrochloric acid, under reflux, produced 3-amino-*N*-(2-(3-chloropropoxy)-5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)propan-1-aminium chloride hydrochloride **1a**, 3-chloro-*N*-(2-(3-chloropropoxy)-5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)propan-1-aminium **1b** and 3-chloro-*N*-(2-(3-chloropropoxy)-5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene)-*N*-(3-chloropropyl)propan-1-aminium **1c**. After purification by silica gel colunm chromatography compounds **1a-c** were obtained as blue solids in moderate to good yields (Scheme 1), and were fully characterised by high-resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy.



a R = H, $R_1 = (CH_2)_3NH_2.HCl$ **b** R = H, $R_1 = (CH_2)_3Cl$ **c** $R = R_1 = (CH_2)_3Cl$

Scheme 1. Synthesis of benzo[*a*]phenoxazinium chlorides **1a-c**.

Electronic absorption spectra of 10^{-5} (**1a**) or 10^{-6} M solutions in degassed absolute ethanol and water at physiological pH (adjusted with 0.2 M boric acid, 0.05 M citric acid and 0.1 M sodium phosphate) were measured for the synthesised benzo[*a*]phenoxazinium chlorides **1a-c** (Table 1). The absorption maxima (λ_{abs}) for all compounds was from 614 to 645 nm, and in compounds **1b** and **1c** a considerable batochromic shift was observed at pH 7.4.

Fluorescent properties of compounds **1a-c** were also evaluated in ethanol and water (pH 7.4), using Oxazine 1 as a standard (fluorescence quantum yield, Φ_F = 0.11 in ethanol[15]), and excitation at 590 nm. The results showed that the emission maxima (λ_{em}) for fluorophores **1a-c** were in the range 643-665 nm with fluorescence quantum yields of 0.02-0.34. Comparison of λ_{em} values in ethanol and at pH 7.4 showed a batochromic shift in the aqueous solutions for compounds **1a** and **1c**. It was also found that in both solvents λ_{em} was superior for compounds **1b** and **1c**.

Table 1. Photophysical data for compounds **1a-c** in ethanol and at physiological pH. [a]in nm.

	Ethanol				**pH 7.4**			
Cpd	λ_{abs}[a]	λ_{em}[a]	Φ_F	$\Delta\lambda$[a]	λ_{abs}[a]	λ_{em}[a]	Φ_F	$\Delta\lambda$[a]
1a	617	643	0.32	26	614	648	0.27	34
1b	626	654	0.19	28	639	649	0.02	10
1c	628	659	0.34	31	645	665	0.04	20

4. Conclusion

Fluorescent benzo[*a*]phenoxazinium chlorides with one, two or three chlorine atoms as terminals of their substituents at 2- and 9-positions were obtained. The presence of chlorine atoms increased the possibility of being potential biologically active compounds; studies will be carried out in the near future. Owing to their structural and photophysical properties, these cationic dyes can also be used as non-covalent and covalent probes (mainly compound **1a**) of biomolecules.

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