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 as 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 chloropropyl 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-propynaphthalen-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)-9H-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. Rf = 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}. {\textsuperscript{1}H NMR (CD_{3}OD, 400 MHz): } \δ 1.14 (3H, t, J = 7.2 Hz, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 1.85-1.95 (2H, m, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 2.10-2.24 (2H, m, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 2.30-2.41 (2H, m, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 3.64-3.80 (4H, m, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl and NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 3.87 (2H, t, J = 6.4 Hz, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 4.26 (2H, t, J = 6.8 Hz, NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 4.38 (2H, t, J = 6.0 Hz, OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 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. {\textsuperscript{13}C NMR (CD\textsubscript{3}OD, 100.6 MHz): } \δ 11.73 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 23.31 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 31.21 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 33.19 (OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 41.59 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 42.21 (OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 43.17 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 47.53 (NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 66.47 (OCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}Cl), 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): calcld. for C\(_{25}\)H\(_{28}\)Cl\(_2\)N\(_3\)O\(_2\) [M\(^+\)] 472.15531; found 472.15354. Calcd. for C\(_{25}\)H\(_{28}\)Cl\(_2\)N\(_3\)O\(_2\) [M\(^+\)] 474.15252; found 474.15016. Calcd. for C\(_{25}\)H\(_{37}\)Cl\(_2\)N\(_3\)O\(_2\) [M\(^+\)] 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 °C 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-propynaphthalen-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): Rf = 0.67. FTIR (neat): νmax 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-d6, 400 MHz): δ 0.96 (3H, t, J = 7.6 Hz, CH₃), 1.64-1.76 (2H, m, NHCH₂CH₂CH₃), 2.17-2.28 (2H, m, OCH₂CH₂CH₂Cl), 3.15 (2H, t, J = 7.6 Hz, NHCH₂CH₂CH₃), 3.82 (2H, t, J = 6.4 Hz, OCH₂CH₂CH₂Cl), 4.18 (2H, t, J = 6.0 Hz, OCH₂CH₂CH₂Cl), 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.

13C NMR (DMSO-d6, 100.6 MHz): δ 11.67 (NHCH₂CH₂CH₃), 21.20 (NHCH₂CH₂CH₃), 31.73 (OCH₂CH₂CH₂Cl), 42.04 (OCH₂CH₂CH₂Cl), 45.92 (NHCH₂CH₂CH₃), 64.19 (OCH₂CH₂CH₂Cl), 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 C₁₆H₂₀N⁵Cl [M⁺] 277.1233; found 277.1235; calcd. for C₁₆H₂₀N⁵Cl [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): Rf = 0.47. FTIR (neat): νmax 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-d6, 300 MHz): δ 0.96 (3H, t, J = 7.5 Hz, NHCH₂CH₂CH₃), 1.60 - 1.73 (2H, m, NHCH₂CH₂CH₃), 3.12 - 3.15 (2H, m, NHCH₂CH₂CH₃), 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.

13C NMR (DMSO-d6, 75.4 MHz): δ 11.83 (NHCH₂CH₂CH₃), 21.49 (NHCH₂CH₂CH₃), 45.00 (NHCH₂CH₂CH₃), 100.28 (C-6), 109.18 (C-1), 113.79 (C-8), 115.79 (C-3), 117.51 (C-4a), 123.48 (C-8a), 127.34 (C-3), 135.57 (C-4a), 143.06 (C-1), 156.14 (C-6) ppm.
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-propynaphthalen-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 60°C and using cesium carbonate as base yielding the 6-(3-chloropropoxy)-N-propynaphthalen-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)-9H-benzo[a]phenoxazin-9-yldene)propan-1-aminium chloride hydrochloride 1a, 3-chloro-N-(2-(3-chloropropoxy)-5-(propylamino)-9H-benzo[a]phenoxazin-9-yldene)propan-1-aminium 1b and 3-chloro-N-(2-(3-chloropropoxy)-5-(propylamino)-9H-benzo[a]phenoxazin-9-yldene)-N-(3-chloropropyl)propan-1-aminium 1c. After purification by silica gel column 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 (1H and 13C) spectroscopy.

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

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 C13H15NO [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-propynaphthalen-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

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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 ($\lambda_{\text{abs}}$) for all compounds was from 614 to 645 nm, and in compounds 1b and 1c a considerable bathochromic 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, $\Phi_F = 0.11$ in ethanol\textsuperscript{15}), and excitation at 590 nm. The results showed that the emission maxima ($\lambda_{\text{em}}$) for fluorophores 1a-c were in the range 643-665 nm with fluorescence quantum yields of 0.02-0.34. Comparison of $\lambda_{\text{em}}$ values in ethanol and at pH 7.4 showed a bathochromic shift in the aqueous solutions for compounds 1a and 1c. It was also found that in both solvents $\lambda_{\text{em}}$ was superior for compounds 1b and 1c.

Table 1. Photophysical data for compounds 1a-c in ethanol and at physiological pH. \textsuperscript{a}in nm.

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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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