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N-Alkyl-*N*-[5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]alkan-1-aminium chlorides: synthesis and photophysical studies

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Abstract: Fluorescent benzo[*a*]phenoxazinium chlorides with dipropyl-, dioctyl- didecyl- and didodecyl-chains as the amino substituents of the 9-positions of the tetracyclic system were synthesised in moderate to good yields. All the compounds absorbed in the wavelength range of 638-641 nm and emitted at 673 or 676 nm with quantum yields of 0.17-0.19 in ethanol.

Keywords: Benzo[*a*]phenoxazinium dyes; Near-infrared fluorescence probes; Cationic dyes; Nile Blue derivatives.

1. Introduction

Fluorescent chromophores have gained pivotal importance in life sciences, particularly in detection, labelling, diagnosis and analysis.[1-5] The fluorophores with absorption and emission at longer wavelengths (600-1000) nm are suitable for bio-applications, taking into account their minimum interference from absorption scattering and the natural auto-fluorescence of biological molecules.[6,7] Oxazine derivatives, such as phenoxazines and benzo[*a*]phenoxazines have been reported for various spectroscopic research studies in the near-infrared region.[8]

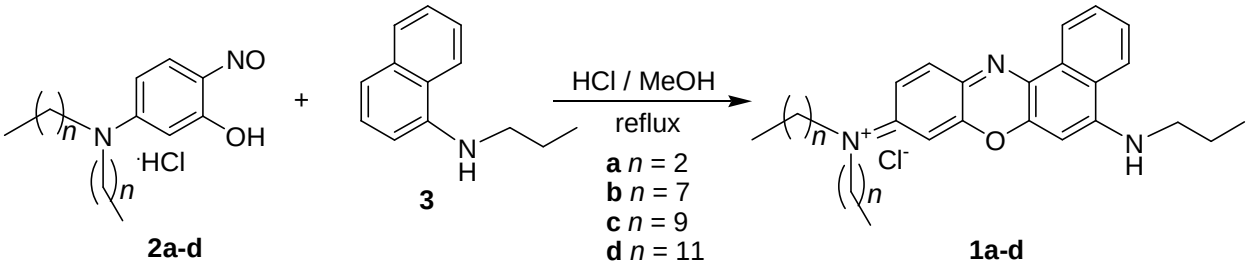
Taking cues from previous results of benzo[*a*]phenoxazinium dyes with long aliphatic chains at the 5-position of the aromatic system,[9] together with our current research interest in the synthesis and characterisation of fluorescent probes,[10] we decided to synthesise new near-infrared labels based on benzo[*a*]phenoxazinium chlorides with double long alkyl side-chains at the 9-position of the aromatic systems, which would function as anchors in biological structures such as lipids, membranes, and proteins.

2. Results and Discussion

Benzo[*a*]phenoxazinium chlorides **1a-d** were synthesised by condensation of 5-(dialkylamino)-2-nitrosophenol hydrochlorides **2a-d** with *N*-propylnaphthalen-1-amine **3** in

acid media. The intermediates **2a-d** were obtained by nitrosation of the corresponding 3-dialkylaminophenol with sodium nitrite in the presence of hydrochloric acid, in a mixture of ethanol-water as the solvent.[11] The 3-dialkylaminophenol derivatives and the *N*-propylnaphthalen-1-amine **3**[9] were respectively obtained by alkylation of 3-aminophenol and naphthalen-1-amine with the corresponding alkyl bromo-derivatives, in ethanol as the solvent, in good to moderate yields.

A cyclisation reaction of 5-(dipropylamino)-2-nitrosophenol hydrochloride **2a**, 5-(dioctylamino)-2-nitrosophenol hydrochloride **2b**, 5-(didecylamino)-2-nitrosophenol hydrochloride **2c**, 5-(didodecylamino)-2-nitrosophenol hydrochloride **2d** with *N*-propylnaphthalen-1-amine **3** occurred in the presence of concentrated hydrochloric acid under reflux with methanol. Purification by silica gel colunm chromatography produced pure *N*-alkyl-*N*-[5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]alkan-1-aminium chlorides **1a-d** as blue solids in yields of about 52 or 73%. (Scheme, Table). All compounds obtained were fully characterised by the usual analytical techniques.



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

Table. Synthesis, UV/Visible and fluorescence data for compounds **1a-d** in ethanol.

Compound	Yield (%)	ε ($M^{-1}cm^{-1}$)	λ_{abs} (nm)	λ_{em} (nm)	Φ_F
1a	73	47761	638	673	0.17
1b	52	48912	641	676	0.17
1c	54	46649	640	676	0.18
1d	52	59969	641	676	0.19

Electronic absorption spectra of 10^{-6} M solutions in degassed absolute ethanol were measured for the synthesised benzo[*a*]phenoxazinium chlorides **1a-d**. As can be seen from the Table, the absorption maxima (λ_{abs}) for all compounds are the same (638-641) nm, though there is a large variation in the alkyl chain-lengths at the 9-amino position ranging from n = 2 to 11. This observation is consistent with the benzo[*a*]phenoxazinium derivatives with a similar

variation in chain-lengths at the 5-position of the ring.[9] Hence, it can be concluded that the value of λ_{abs} is independent from the size of the alkyl chain at the 5- or 9-positions of the tetracyclic ring.

Similarly, the evaluation of fluorescent properties of these fluorophores in ethanol, using Oxazine 1 as a standard (fluorescence quantum yield, $\Phi_F = 0.11$ in ethanol[12]) and excitation at 590 nm revealed that the emission maxima for all the fluorophores **1a-d** are the same (673 or 676) nm with fluorescence quantum yields of 0.17-0.19 (Table).

3. Conclusion

In summary, 5,9-diaminobenzo[*a*]phenoxazinium dyes **1a**–**d**, possessing dialkylamino-substituents at the 9-amino positions of the tetracyclic aromatic systems were efficiently synthesised. These cationic dyes, with high absorption and emission in the near-infrared region in ethanol are potential fluorescence probes for bio-applications. The double alkyl chains make these fluorochromophores specially suitable for biomembrane non-covalent labelling as the phospholipids that comprise the biological membrane also have double alkyl chains between twelve and eighteen carbon atoms. The synthesised compounds possessing double aliphatic chains of different lengths are expected to probe different depths of the biomembrane. In order to elucidate the capability of these novel benzo[*a*]phenoxazinium derivatives as biomembrane fluorescence labels, photophysical studies in model biological membranes are underway.

4. Experimental

4.1. Typical procedure for the synthesis of 1a-d (described for **1a**): To an ice cold solution of 5-(dipropylamino)-2-nitrosophenol hydrochloride **2a** (0.222 g, 1×10^{-3} mol) in methanol (3 mL), concentrated hydrochloric acid (0.04 mL) was added followed by the *N*-propylnaphthalen-1-amine **3** (0.185 g; 1×10^{-3} mol). The reaction mixture was refluxed for 12h and monitored by TLC (chloroform/methanol, 95:5). After evaporation of the methanol and purification by colunm chromatography on silica gel with chloroform and chloroform/methanol, mixtures of increasing polarity, as the eluent, *N*-propyl-*N*-[5-(propylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]propan-1-aminium chloride **1a** was obtained as a blue solid (0.307 g, 73%). Mp = 240-242 °C. $R_f = 0.19$ (chloroform/methanol, 94:6). FTIR (KBr 1%): ν_{max} 3392, 3175, 3051, 2967, 2931, 2872, 1640, 1588, 1547, 1495, 1452, 1435, 1384, 1330, 1278, 1258, 1196, 1168, 1124, 1073, 1005, 945, 884, 855, 824, 809, 783, 769, 730 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz): δ 1.08 (t, *J* 7.6 Hz, 6 H, 2×$NCH_2CH_2CH_3$),

1.14 (t, *J* 7.2 Hz, 3 H, $NHCH_2CH_2\mathit{CH_3}$), 1.75-1.85 (m, 4 H, $2\times NCH_2\mathit{CH_2}CH_3$), 1.86-1.96 (m, 2 H, $NHCH_2\mathit{CH_2}CH_3$), 3.61 (t, *J* 8.0 Hz, 4 H, $2\times N\mathit{CH_2}CH_2CH_3$), 3.67 (t, *J* 7.2 Hz, 2 H, $NH\mathit{CH_2}CH_2CH_3$), 6.81 (d, *J* 2.0 Hz, 1 H, 8-H), 6.88 (s, 1 H, 6-H), 7.17-7.27 (m, 1 H, 10-H), 7.73-7.83 (m, 2 H, 11-H, 3-H), 7.88 (t, *J* 7.6 Hz, 1 H, 2-H), 8.31 (d, *J* 8.0 Hz, 1 H, 4-H), 8.79 (d, *J* 8.0 Hz, 1 H, 1-H) ppm. ^{13}C NMR (CD_3OD, 100.6 MHz): δ 11.45 ($2\times NCH_2CH_2\mathit{CH_3}$), 11.77 ($NHCH_2CH_2\mathit{CH_3}$), 21.81 ($2\times NCH_2\mathit{CH_2}CH_3$), 23.08 ($NHCH_2\mathit{CH_2}CH_3$), 47.54 ($NHCH_2CH_2\mathit{CH_3}$), 54.56 ($2\times N\mathit{CH_2}CH_2CH_3$), 94.51 (C-6), 97.17 (C-8), 116.65 (C-10), 123.86 (C-4), 124.88 (Ar-C), 125.55 (C-1), 130.90 (C-3), 131.41 (Ar-C), 132.53 (Ar-C), 132.91 (C-2), 133.92 (C-11), 135.15 (Ar-C), 149.50 (Ar-C), 153.09 (Ar-C), 155.96 (C-9), 159.36 (C-5) ppm. The assignments were supported by HMBC and HMQC techniques. HRMS: m/z (ESI): calcd. for $C_{25}H_{30}N_3O$ [M^+] 388.23774; found 388.23834.

4.2. Typical procedure for the preparation of 2a-d (described for **2a**): To an ice-cold solution of the 3-dipropylaminophenol (0.540 g; 2.80×10^{-3} mol) in ethanol (5 mL), concentrated hydrochloric acid (1.2 mL) was added and stirred until the reaction mixture became homogenous. A solution of sodium nitrite (0.225 g; 1.16×10^{-3} mol) in water (0.6 mL) was then added drop-wise within an interval of 20 min. The resulting reaction mixture was stirred for 1h30min and progress of the reaction was monitored by TLC (dichloromethane/methanol, 95:5). After completion of the reaction, precipitate was filtered. The product was obtained as a yellow solid (0.609 g) and was characterised by ^{1}H and ^{13}C NMR, which showed isomers *ortho* and *para* (the nitro and the hydroxyl groups) in the ratio 60:40. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.84 (t, *J* 7.2Hz, 6 H, $2\times CH_2CH_2\mathit{CH_3}$ *ortho*), 0.90-1.00 (m, 6 H, $2\times CH_2CH_2\mathit{CH_3}$ *para*), 1.50-1.70 (m, 8 H, $2\times CH_2\mathit{CH_2}CH_3$ *ortho*, $2\times CH_2\mathit{CH_2}CH_3$ *para*), 3.43 (br s, 4 H, $2\times\mathit{CH_2}CH_2CH_3$ *ortho*), 3.70 (br s, 4 H, $2\times\mathit{CH_2}CH_2CH_3$ *para*), 5.73 (d, *J* 2.4 Hz, 1 H, 2-H *para*), 6.73 (br s, 1 H, 6-H *ortho*), 6.90 (dd, *J* 10.0 and 2.4 Hz, 1 H, 6-H *para*), 7.21 (dd, *J* 10.4 and 2.4 Hz, 1 H, 4-H *ortho*), 7.27 (d, *J* 10 Hz, 1 H, 5-H *para*), 7.49 (d, *J* 10.4 Hz, 1 H, 3-H *ortho*) ppm. ^{13}C NMR (DMSO-d_6, 100.6 MHz,): δ 10.77 ($NCH_2CH_2\mathit{CH_3}$ *para*, $NCH_2CH_2\mathit{CH_3}$ *ortho*), 16.34 ($NCH_2\mathit{CH_2}CH_3$ *para*), 22.12 ($NCH_2\mathit{CH_2}CH_3$ *ortho*), 53.89 ($N\mathit{CH_2}CH_2CH_3$ *para*), 54.48 ($N\mathit{CH_2}CH_2CH_3$ *ortho*), 95.36 (C-2 *para*), 97.84 (C-6 *ortho*), 115.60 (C-6 *para*), 119.70 (C-4 *ortho*), 122.63 (C-3 *ortho*), 134.25 (C-5 *para*), 147.31 (C-1 *ortho*), 149.04 (C-3 *para*), 157.65 (C-4 *para*), 161.49 (C-2 *ortho*), 166.38 (C-5 *ortho*), 168.57 (C-1 *para*) ppm.

3-(dipropylamino)phenol

To a solution of 3-aminophenol (2.0 g, 1.83×10^{-3} mol) in ethanol (8 mL), 1-bromopropane (2.50 mL, 2.75×10^{-2} mol) was added and the resulting mixture was refluxed for 4h30min. The progress of the reaction was monitored by TLC (dichloromethane). The crude product obtained by evaporation of the ethanol was purified by column chromatography on silica gel using dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent. 3-(Dipropylamino)phenol was obtained as a brown solid (0.715 g, 20%). Mp = 99.7-101.7 °C. TLC (dichloromethane): R_f = 0.22. FTIR (KBr 1%): ν_{max} 3208, 3188, 3053, 2966, 2880, 2711, 2672, 2636, 2508, 1613, 1508, 1488, 1471, 1456, 1431, 1417, 1386, 1340, 1318, 1276, 1227, 1204, 1169, 1147, 1126, 1116, 1086, 1037, 1012, 999, 991, 965, 890 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ 0.84 (t, *J* 7.6 Hz, 6 H, 2×$NCH_2CH_2\mathit{CH_3}$), 1.59 (br s, 4 H, 2×$NCH_2\mathit{CH_2}CH_3$), 3.30 (t, *J* 8.0 Hz, 4 H, 2×$N\mathit{CH_2}CH_2CH_3$), 6.87 (br s, 2 H, 4-H and 6-H), 7.05 (br s, 1 H, 2-H), 7.17 (t, *J* 8.0 Hz, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 10.85 (2×$NCH_2CH_2\mathit{CH_3}$), 18.86 (2×$NCH_2\mathit{CH_2}CH_3$), 58.17 (2×$N\mathit{CH_2}CH_2CH_3$), 107.61 (C-2), 113.60 (C-4 and C-6), 130.63 (C-5), 141.31 (C-3), 158.22 (C-1) ppm. The assignments were supported by HMBC and HMQC techniques. HRMS: m/z (EI): calcd. for $C_{12}H_{19}NO$ [M^+] 193.1467; found 193.1474.

In addition to the 3-(dipropylamino)phenol, 3-(propylamino)phenol was also isolated as a brown oil (1.410 g, 51%) and spectroscopic data confirmed its structure.

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