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New NIR dyes based on quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides: synthesis, photophysics and antifungal activity

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ABSTRACT

A series of new quinolizino[1,9-*hi*]phenoxazinium dyes built on julolidine and naphthalen-1-amine derivatives or anthracen-1-amine were prepared. The *N*-terminal of these quinolizino[1,9-*hi*]phenoxazinium chlorides contains aromatic or aliphatic substituents, along with the functionalities such as chloro, hydroxyl and carboxyl. The photophysical behaviour of these compounds was studied in anhydrous ethanol and aqueous medium under acidic and basic conditions. These fluorophores display absorption and emission maxima up to 675 and 712 nm, respectively, can serve as alternative sensing tools in biological assays.

All the quinolizino[1,9-*hi*]phenoxazinium chlorides were evaluated against the yeast *Saccharomyces cerevisiae* in a broth microdilution assay. It was found that their antifungal activity depended on the substituent at 14-amino position in benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chlorides, and also on the addition of a fused benzene ring, which occurs in naphtho[2,3-*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chloride. The highest activity, with a MIC of 0.78 μM, was obtained for benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chloride with a 3-chloropropyl substituent at the 14-amino position of the heterocycle core.

1. Introduction

Small fluorescent molecules serve as central tools in the field of biosciences [1]. The emerging need of fluorescent probes requires design and strategic synthesis of new fluorescent dyes. Nile Blue (NB) and their derivatives are studied and used as markers due to their fluorescent and solvatochromic characteristics [2–9]. In this context, benzophenoxazinium dyes are structurally compact with high molar extinction coefficients and exhibit strong fluorescence in the near-infrared (NIR) region with high photochemical stability, which indicates the efficacy of these dyes as fluorophores for biological applications [10]. Benzophenoxazinium chlorides function as potential photosensitizers for photodynamic therapy [11,12], pH sensors for simultaneous far-red and near-infrared live bioimaging [13], promising drugs for malaria [14] and reversing vinca alkaloid resistance in multidrug-resistant cancer cells [15], among other promising biological applications [16–18].

Julolidine based dyes serve as important tools in photochemical, biological systems due to low toxicity, displaying good chemical and thermal stability [19,20]. These compounds are used as sensitizers in dye sensitized solar cells due to their large π-conjugated system and high electron donating property [21]. In addition, they are also used as photoconductive materials [22], chemiluminescence substances [23], chromogenic substrates in analytical redox reactions [24,25], nonlinear optical materials [26], phototriggers in the release of neurotransmitters [27], potential anti-depressants and tranquilizers [28]. Julolidine derivatives function as chemosensors for the selective detection of metals such as Cu^{2+} [29], Fe^{3+} [30], Al^{3+} [31], Zn^{2+} [32], and also act as fluorescent molecular rotors [33]. Moreover, 8-hydroxyjulolidine was used for the synthesis of bridged phenoxazinium salts and some of these compounds function as acid-base indicators as reported by Kanitz et al. [34]. However, Kanitz publication is the only one so far using julolidine system in the preparation of phenoxazinium dyes.

Keeping in mind the importance and in continuation of our research interest towards the synthesis and applications of benzophenoxazinium salts [2–8,12,17,18,35–37], we herein report a new series of benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium and naphtho[2,3-*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chlorides obtained by

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condensation of nitroso derivative of 8-hydroxyjulolidine with naphthalen-1-amine derivatives and anthracen-1-amine, respectively. The introduction of the julolidine nucleous into the polycyclic system is expected to result in maxima absorption and emission wavelengths of fluorophores higher than those obtained from similar unbridged anilines [34].

The new compounds synthesised possesses aromatic or aliphatic substituents, along with the functionalities such as chloro, hydroxyl and carboxyl at 14-amino position of the heterocyclic system. The choice of these substituents, namely the propyl and chloropropyl groups, was based on the fact that benzo[*a*]phenoxazines previously reported by our research group possessing these groups on the 5-amino positions displayed the best biological activities against the yeast *Saccharomyces cerevisiae* [17,18]. On the other hand, the presence of chloro as well as hydroxyl and carboxyl as side chain termini will increase the versatility of these compounds, particularly in terms of fluorescent labeling, allowing their use as covalent markers of biomolecules, in addition to their intrinsic ability as non-covalent markers due to the ionic character of their structures.

Fundamental photophysical studies of these cationic fluorophores were carried out in anhydrous ethanol and aqueous medium under acidic and basic conditions.

The antifungal activity of these phenoxazinium chlorides was assesd by using the yeast *Saccharomyces cerevisiae* as a model organism. Comparison of MIC values of all the compounds revealed that benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chloride with a 3-chloropropyl substituent at the 14-amino position of the heterocycle core exhibits the best activity.

2. Experimental section

2.1. Synthesis general

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analysis was carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel 60F_{254}), and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer. NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 °C. All chemical shifts are given in ppm using δ_H Me_4Si = 0 ppm as reference and *J* values are given in Hz. Assignments were made by comparison of chemical shifts, peak multiplicities and *J* values, and were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Mass spectrometry analysis were performed at the "CACTI - Unidad de Espectrometria de Masas", at University of Vigo, Spain. All commercial reagents were used as received.

2.2. Synthetic method for the preparation of 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-ij]quinolin-8-ol hydrochloride 1

1,2,3,5,6,7-Hexahydropyrido[3,2,1-*ij*]quinolin-8-ol (8-hydroxyjulolidine) (0.300 g, 1.58 mmol) was weighed in a round bottom flask and dissolved in ethanol (5 mL) which was placed in an ice bath with continuous stirring. After a period of 15 min, concentrated hydrochloric acid (0.419 mL) was added. A solution of sodium nitrite (0.123 g, 1.73 mmol) in water (1 mL) was prepared and added to the ice cold acidic solution over a period of 30 min. The reaction mixture turns brown and stirring was continued for more 5 h and then filtered with the sintered glass funnel. To avoid excess of acid it was washed with small amounts of water and ethanol. The precipitate was dried to get a fine brownish red powder (0.345 g), whose ^{1}H NMR spectrum suggested the presence of compound **1** in a mixture with the corresponding isomer, 10-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol hydrochloride, in the 7:3 ratio. ^{1}H NMR (DMSO, 400 MHz): δ_H 1.79–1.87 (m, 4 H, 2-H and 6-H, isomer), 1.88–2.0 (m, 4 H, 2-H and 6-H), 2.45 (t, *J* = 5.6 Hz, 2 H, 1-H or 7-H, isomer), 2.58 (t, *J* = 5.6 Hz, 2 H, 1-H or 7-H), 2.65 (t, *J* = 5.6 Hz, 2 H, 1-H or 7-H, isomer), 2.71 (t, *J* = 5.6 Hz, 2 H, 1-H or 7-H), 3.40–3.50 (m, 4 H, 3-H and 5-H, isomer), 3.73–3.80 (m, 4 H, 3-H and 5-H), 6.79 (s, 1 H, 9-H, isomer), 7.30 (s, 1 H, 10-H) ppm.

2.3. Synthetic method for the preparation of N-phenylnaphthalen-1-amine 2b

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 h. The reaction progress was monitored by TLC (dichloromethane/methanol, 9.5:0.5 vol). After completion of the reaction, 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 **2b** was obtained as pink solid (1.357 g, yield 88%). Mp 59–61 °C. R_f = 0.40 (dichloromethane/methanol, 9.0:1.0 vol): ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 6.81 (dd, *J* = 6.8 and 1.6 Hz, 2 H, 2-H and 4-H Ph), 7.32–7.41 (m, 4 H, 3-H Ph, 5-H Ph, 2-H Ph and 6-H Ph), 7.48–7.54 (m, 3 H, 4-H, 6-H, 3-H), 7.82–7.89 (m, 3 H, 7-H, 5-H and 8-H) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ_C 109.61 (C-2 and 1 × Ar–C), 118.89 (2 × Ar–C), 120.74 (2 × Ar–C), 123.59 (C-8a), 124.78 (1 × Ar–C), 125.78 (1 × Ar–C), 126.28 (3 × ArC), 128.48 (1 × Ar–C), 134.33 (C-4a and C-1 Ph), 142.02 (C-1) ppm.

2.4. General procedure for the synthesis of quinolizino[1,9-hi]phenoxazin-6-iminium chlorides 4a-f and 5

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) **1** (2 equiv) in ethanol (2–3 mL), precursors **2a-f** or **3** (1 equiv) and concentrated hydrochloride acid (0.25 equiv) were added. The reaction mixture was refluxed during the time mentioned below, and monitored by TLC. Upon completion, the solvent was evaporated under reduced pressure and column chromatography purification was performed on silica gel with dichloromethane and dichloromethane/methanol, mixtures of different polarity, as the eluents and dyes **4a-f** or **5** were obtained as green blue solids.

2.4.1. 2,3,6,7-Tetrahydro-1H-benzo[a]quinolizino[1,9-hi]phenoxazin-14(5H)-iminium chloride 4a

The product of the reaction of **1** (0.115 g, 0.525 mmol) in ethanol (1 mL) and concentrated hydrochloric acid (0.014 mL) with naphthalen-1-amine **2a** (0.037 g, 0.262 mmol) (reflux time 19 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.0:1.0, to give compound **4a** as a green blue solid (0.037 g, yield 19%). Mp 218–221 °C. R_f = 0.34 (dichloromethane/methanol, 9:1 vol). FTIR (KBr 1%): ν_{max} 3417, 3122, 2920, 2927, 2856, 1641, 1586, 1532, 1473, 1437, 1385, 1354, 1317, 1286, 1215, 1175, 1140, 1098, 1033, 778 cm^{-1}. ^{1}H NMR δ_H (CD_3OD, 400 MHz), 1.98–2.08 (m, 4H, 2-H and 6-H), 2.75 (t, *J* = 6.4 Hz, 2H, 1-H), 2.83 (t, *J* = 6.0 Hz, 2H, 7-H), 3.50–3.60 (m, 4H, 5-H and 3-H), 6.62 (s, 1H, 15-H), 7.16 (s, 1H, 8-H), 7.70–7.74 (m, 1H, 12-H), 7.80 (t, *J* = 7.2 Hz, 1H, 11-H), 8.17 (d, *J* = 8.4 Hz, 1H, 13-H), 8.62 (d, *J* = 8.0 Hz, 1H, 10-H) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 20.07 (C-1), 20.38 (C-6), 21.47 (C-2), 28.38 (C-7), 51.88 (C-3), 52.41 (C-5), 96.55 (C-15), 106.54 (Ar–C), 124.20 (C-13), 124.97 (C-10), 128.48 (Ar–C), 129.78 (C-12), 130.03 (C-8), 131.39 (Ar–C), 132.08 (C-11), 132.75 (Ar–C), 132.92 (C–Ar), 133.41 (Ar–C), 144.43 (Ar–C), 151.43 (Ar–C), 152.47 (Ar–C), 159.94 (C-14) ppm. HRMS: *m/z* (ESI): Found $[M+1]^+$: 378.1375; $C_{22}H_{20}ClN_3O$ requires $[M+1]^+$: 378.1375.

2.4.2. N-(2,3,6,7-Tetrahydro-1H-benzo[a]quinolizino[1,9-hi]phenoxazin-14(5H)-ylidene) benzenaminium chloride 4b

The product of the reaction of **1** (0.115 g, 0.525 mmol) in ethanol (1 mL) and concentrated hydrochloric acid (0.014 mL) with *N*-phenylnaphthalen-1-amine **2b** (0.057 g, 0.262 mmol) (reflux time 17 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **4b** as green blue solid (0.047 g, yield 20%). Mp 118.7–120 °C. R_f = 0.44 (dichloromethane/methanol, 9:1 vol). FTIR (KBr 1%): ν_{max} 3439, 3000, 1643, 1469, 1284, 1205, 1141, 1098, 1033, 770 cm^{-1}. ^1H NMR δ_H (CD_3OD, 400 MHz), 2.04–2.18 (m, 4H, 2-H and 6-H), 2.51 (t, J = 6.4 Hz, 2H, 7-H), 2.92–3.0 (m, 2H, 1-H), 3.60–3.70 (m, 2H, 3-H), 3.82 (t, J = 6.4 Hz, 2H, 5-H), 6.83 (s, 1H, 15-H), 7.48 (s, 1H, 8-H), 7.73–7.84 (m, 4H, 4 × Ar–H), 7.87–7.95 (m, 2H, 12-H and 1 × Ar–H), 8.10 (t, J = 7.6 Hz, 1H, 11-H), 8.27 (d, J = 8.4 Hz, 1H, 13-H), 8.89 (d, J = 7.2 Hz, 1H, 10-H) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 20.26 (C-1), 20.60 (C-6), 21.66 (C-2), 28.53 (C-7), 51.92 (C-3), 52.41 (C-5), 96.70 (C-15), 106.54 (Ar–C), 119.29 (Ar–C), 123.76 (Ar–C), 124.30 (C-13), 125.24 (C-10), 128.62 (Ar–C), 129.10 (Ar–C), 129.92 (2xAr-C), 130.27 (C-8), 132.14 (C-11), 132.64 (Ar–C, C-12), 133.27 (2 × Ar–C), 144.97 (2xAr-C), 152.16 (Ar–C), 152.65 (Ar–C), 159.18 (C-14), 160.62 (Ar–C) ppm. HRMS: *m/z* (ESI): Found $[M+1]^+$: 454.1691; $C_{28}H_{24}ClN_3O$ requires $[M+1]^+$: 454.1688.

2.4.3. N-(2,3,6,7-Tetrahydro-1H-benzo[a]quinolizino[1,9-hi]phenoxazin-14(5H)-ylidene)propan-1-aminium chloride 4c

The product of the reaction of **1** (0.115 g, 0.525 mmol) in ethanol (1 mL) and concentrated hydrochloric acid (0.014 mL) with *N*-propylnaphthalen-1-amine **2c** (0.048 g, 0.262 mmol) (reflux time 17 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **4c** as blue green solid (0.047 g, yield 20%). Mp 223–225 °C. R_f 0.35 (dichloromethane/methanol, 9.5:0.5 vol). FTIR (KBr 1%): ν_{max} 3438, 3000, 1642, 1468, 1285, 1207, 1150, 1090, 1030, 774 cm^{-1}. ^1H NMR δ_H (CD_3OD, 400 MHz): 1.14 (t, J = 7.6 Hz, 3H $NHCH_2CH_2CH_3$), 1.90 (sext, J = 7.2 Hz, 2H, $NHCH_2CH_2CH_3$), 2.04–2.16 (m, 2H, 6-H and 2-H), 2.90–2.99 (m, 2H, 7-H and 1-H), 3.58–3.66 (m, 4H, 3-H, 5-H and $NHCH_2CH_2CH_3$), 6.82 (s, 1H, 15-H), 7.40 (s, 1H, 8-H), 7.77 (td, J = 7.2 and 1.6 Hz, 1H, 12-H), 7.86 (td, J = 7.6 and 1.2 Hz, 1H, 11-H), 8.28 (d, J = 8.0 Hz, 1H, 13-H), 8.79 (d, J = 7.6 Hz, 1H, 10-H) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 11.80 ($NHCH_2CH_2CH_3$), 20.31 (C-1), 20.57 (C-6), 21.64 (C-2), 22.99 ($NHCH_2CH_2CH_3$), 28.56 (C-7), 47.09 ($NHCH_2CH_2CH_3$), 51.94 (C-3), 52.43 (C-5), 93.48 (C-15), 106.72 (Ar–C), 123.42 (C-13), 124.29 (Ar–C), 125.18 (C-10), 128.68 (Ar–C), 130.04 (C-12), 130.13 (C-8), 131.88 (Ar–C), 132.14 (C-11), 132.54 (Ar–C), 133.31 (Ar–C), 144.89 (Ar–C), 152.20 (Ar–C), 152.53 (Ar–C), 157.40 (C-14) ppm. HRMS: *m/z* (ESI): Found $[M+1]^+$: 420.1855; $C_{25}H_{26}ClN_3O$ requires $[M+1]^+$: 420.1845.

2.4.4. 3-Chloro-N-(2,3,6,7-tetrahydro-1H-benzo[a]quinolizino[1,9-hi] phenoxazin-14(5H)-ylidene)propan-1-aminium chloride 4d

The product of the reaction of **1** (0.335 g, 0.153 mmol) in ethanol (1 mL) and concentrated hydrochloric acid (0.040 mL) with *N*-(3-chloropropyl)naphthalen-1-amine **2d** (0.167 g, 0.076 mmol) (reflux time 24 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **4d** as blue green solid (0.017 g, yield 5%). Mp 209.3–210.8 °C. R_f = 0.60 (dichloromethane/methanol, 9:1 vol). IR (KBr 1%): ν_{max} = 3428, 3223, 3027, 2961, 2929, 1707, 1640, 1588, 1543, 1472, 1438, 1385, 1358, 1314, 1282, 1217, 1176, 1136, 1099, 1047, 893, 780 cm^{-1}. ^1H NMR δ_H (CD_3OD, 400 MHz), 2.05–2.14 (m, 4H, H-2 and H-6), 2.33 (quint, J = 6.4 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 2.83–2.92 (m, 2H, H-1), 2.95 (t, J = 6.0 Hz, 2H, H-7), 3.60–3.69 (m, 4H, H-3 and H-5), 3.78 (t, J = 6.8 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 3.83 (t, J = 6.4 Hz, 2H, $NHCH_2CH_2CH_2Cl$), 6.73 (s, 1H, H-15), 7.33 (s, 1H, H-8), 7.71 (t, J = 7.2 Hz, 1H, H-12), 7.82 (t, J = 7.6 Hz, 1H, H-11), 8.21 (d, J = 8.0 Hz, 1H, H-13), 8.68 (d, J = 7.6 Hz, 1H, H-10) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 20.21 (C-1), 20.46 (C-6), 21.56 (C-2), 28.53 (C-7), 32.47 ($NH_2CH_2CH_2CH_2Cl$), 42.71 ($NHCH_2CH_2CH_2Cl$), 43.30 ($NHCH_2CH_2CH_2Cl$), 52.06 (C-3), 52.56 (C-5), 93.28 (C-15), 106.75 (Ar–C), 123.44 (C-13), 124.01 (Ar–C), 124.97 (C-10), 129.22 (Ar–C), 129.89 (C-12), 130.14 (C-8), 131.24 (Ar–C), 132.02 (C-11), 132.26 (Ar–C), 133.76 (Ar–C), 144.78 (Ar–C), 151.73 (Ar–C), 152.82 (Ar–C), 156.92 (C-14) ppm. HRMS: *m/z* (EI): Found $[M+1]^+$: 454.3900; $C_{25}H_{25}Cl_2N_3O$ requires $[M+1]^+$: 454.3905.

2.4.5. 3-Hydroxy-N-(2,3,6,7-tetrahydro-1H-benzo[a]quinolizino[1,9-hi] phenoxazin-14(5H)-ylidene)propan-1-aminium chloride 4e

The product of the reaction of **1** (0.115 g, 0.525 mmol) in ethanol (1 mL) and concentrated hydrochloric acid (0.014 mL) with 3-(naphthalen-1-ylamino)propan-1-ol **2e** (0.053 g, 0.262 mmol) (reflux time 18 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **4e** as green blue solid (0.051 g, yield 22%). Mp > 300 °C. R_f = 0.48 (dichloromethane/methanol, 9:1 vol). IR (KBr 1%): ν_{max} = 3450, 2932, 1640, 1588, 1553, 1531, 1471, 1440, 1386, 1355, 1317, 1284, 1217, 1177, 1139, 1101, 781 cm^{-1}. ^1H NMR δ_H (CD_3OD, 400 MHz), 2.04–2.14 (m, 6H, 2-H, 6-H and $NHCH_2CH_2CH_2OH$), 2.88 (t, J = 6.4 Hz, 2H, 1-H), 2.95 (t, J = 6.0 Hz, 2H, 7-H), 3.60–3.67 (m, 4H, 3-H and 5-H), 3.75 (t, J = 7.2 Hz, 2H, $NHCH_2CH_2CH_2OH$), 3.82 (t, J = 6.0 Hz, 2H, $NHCH_2CH_2CH_2OH$), 6.77 (s, 1H, 15-H), 7.34 (s, 1H, 8-H), 7.72 (t, J = 7.2 Hz, 1H, 12-H), 7.83 (t, J = 7,2 Hz, 1H, 11-H), 8.21 (d, J = 8.4 Hz, 1H, 13-H), 8.71 (d, J = 7.6 Hz, 1H, 10-H) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 20.27 (C-1), 20.53 (C-6), 21.62 (C-2), 28.56 (C-7), 32.17 ($NH_2CH_2CH_2CH_2OH$), 42.94 ($NHCH_2CH_2CH_2OH$), 51.97 (C-3), 52.47 (C-5), 60.54 ($NHCH_2CH_2CH_2OH$), 93.35 (C-15), 106.69 (Ar–C), 123.40 (C-13), 124.13 (Ar–C), 125.10 (C-10), 128.77 (Ar–C), 129.96 (C-12), 130.11 (C-8), 131.65 (Ar–C), 132.08 (C-11), 132.36 (Ar–C), 133.33 (Ar–C), 144.77 (Ar–C), 152.0 (Ar–C), 152.56 (Ar–C), 157.15 (C-14) ppm. HRMS: *m/z* (ESI): Found $[M+1]^+$: 436.1785; $C_{25}H_{26}ClN_3O_2$ requires $[M+1]^+$: 436.1794.

2.4.6. 3-Carboxy-N-(2,3,6,7-tetrahydro-1H-benzo[a]quinolizino[1,9-hi] phenoxazin-14(5H)-ylidene)propan-1-aminium chloride 4f

The product of the reaction of **1** (0.254 g, 1.0 mmol) in ethanol (2 mL) and concentrated hydrochloric acid (0.027 mL) with 4-(naphthalen-1-ylamino)butanoic acid **2f** (0.115 g, 0.50 mmol) (reflux time 17 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.5:0.5, to give compound **4f** as green blue solid (0.88 g, yield 19%). Mp 154.6–156.8 °C. R_f = 0.42 (dichloromethane/methanol, 9:1 vol). IR (KBr 1%): ν_{max} = 2923, 2853, 1731, 1721, 1637, 1589, 1545, 1499, 1435, 1375, 1334, 1322, 1290, 1230, 1182, 1162, 1146, 1127, 1100, 1054, 1001, 918, 807, 753 cm^{-1}. ^1H NMR δ_H (CD_3OD, 400 MHz), 2.0–2.16 (m, 6H, 2-H, 6-H and $NHCH_2CH_2CH_2CO_2H$), 2.59 (t, J = 7.2 Hz, 2H, $NHCH_2CH_2CH_2CO_2H$), 2.75 (t, J = 5.6 Hz, 2H, 1-H), 2.88 (t, J = 6.0 Hz, 2H, 7-H), 3.55–3.64 (m, 6H, 3-H, 5-H, and $NHCH_2CH_2CH_2CO_2H$), 6.63 (s, 1H, 15-H), 6.94 (s, 1H, 8-H), 7.65 (t, J = 7.2 Hz, 1H, 12-H), 7.77 (t, J = 7.6 Hz, 1-H, 11-H), 8.06–8.16 (m, 1H, 13-H), 8.57 (d, J = 8.0 Hz, 1H, 10-H) ppm. ^{13}C NMR δ_C (CD_3OD, 100.6 MHz), 20.17 (C-1), 20.57 (C-2), 21.54 (C-6), 24.70 ($NHCH_2CH_2CH_2CO_2H$), 28.49 (C-7), 31.84 ($NHCH_2CH_2CH_2CO_2H$), 44.58 ($NHCH_2CH_2CH_2CO_2H$), 51.97 (C-3), 52.35 (C-5), 93.44 (C-15), 106.70 (Ar–C), 123.32 (C-13), 124.94 (Ar–C), 125.43 (C-10), 128.90 (Ar–C), 130.06 (C-12), 131.17 (C-8), 131.97 (Ar–C), 132.10 (C-11), 133.12 (Ar–C), 133.38 (Ar–C), 144.54 (Ar–C), 151.60 (Ar–C), 152.59 (Ar–C), 157.10 (C-14), 175.91 (C=O) ppm. HRMS: *m/z* (ESI): Found $[M+1]^+$: 464.1745; $C_{26}H_{26}ClN_3O_3$requires $[M+1]^+$: 464.1743.

2.4.7. 9,10,11,13,14,15-Hexahydro-6H-naphtho[2,3-a]quinolizino[1,9-hi]phenoxazin-6-iminium chloride 5

The product of the reaction of **1** (0.115 g, 0.525 mmol) in ethanol

(1 mL) and concentrated hydrochloric acid (0.014 mL) with anthracen-1-amine **3** (0.051 g, 0.262 mmol) (reflux time 18 h), was chromatographed with dichloromethane and dichloromethane/methanol 9.0:1.0, to obtain the compound **5** as green blue solid (0.047 g, yield 21%). Mp 225–227 °C. R_f = 0.53 (dichloromethane/methanol, 9:1 vol). FTIR (KBr 1%): ν_{max} 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}. ^{1}H NMR (CD_3OD, 400 MHz): δ_H 1.92–2.05 (m, 4 H, 10-H and 14-H), 2.58 (t, J = 6.8 Hz, 2 H, 9-H), 2.80 (t, J = 6.4 Hz, 2 H, 15-H), 3.42 (t, J = 6.0 Hz, 2 H, 11-H), 3.48–3.54 (m, 2 H, 13-H), 6.49 (s, 1 H, 7-H), 7.08 (s, 1 H, 16-H), 7.60–7.70 (m, 2 H, 2-H and 3-H), 8.0 (d, J = 8.0 Hz, 1 H, 1-H), 8.04 (d, J = 7.2 Hz, 1 H, 4-H), 8.69 (s, 1 H, 5-H), 8.91 (s, 1 H, 18-H) ppm. ^{13}C NMR (CD_3OD, 100.6 MHz): δ_C 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, 1 × Ar–C), 128.0 (1 × Ar–C), 128.70 (C-3), 128.88 (C-1), 129.82 (C-4), 130.10 (2 × Ar–C), 130.33 (Ar–C), 131.22 (2 × Ar–C), 133.47 (Ar–C), 135.23 (Ar–C), 151.00 (Ar–C), 156.00 (C-6) ppm. HRMS: m/z (ESI): Found $[M+1]^+$: 428.1521; $C_{26}H_{22}ClN_3O$ requires $[M+1]^+$: 428.1531.

2.5. Photophyscial measurements

Electronic absorption and fluorescence spectra of solutions of fluorophores **4a-f** and **5** in absolute ethanol and water were measured. Ethanol was dried by the use of molecular sieves. Ethanol was either acidified or basified by the addition of small quantities of trifluoroacetic acid (TFA) or tetraethylammonium hydroxide (TEAH) solution 25% in methanol, respectively.

Absorption spectra (200–800 nm) were recorded on a Shimadzu UV-3101PC UV/Vis/NIR spectrophotometer. Fluorescence measurements were performed using a Spex Fluorolog 2 spectrofluorometer, equipped with double monochromators in both excitation and emission. Spectra were corrected for the instrumental response of the system.

Fluorescence quantum yields (Φ) were determined using the standard method (Equation (1), taking into account the effect of sample or reference absorption slightly above 0.1 [38,39], with Oxazine 1 in ethanol as reference, Φ_r = 0.11 [40]:

$$\Phi_s = \frac{(1 - 10^{-A_r})\ F_s\ n_s^2}{(1 - 10^{-A_s})\ F_r\ n_r^2}\ \Phi_r \quad (1)$$

where A is the absorbance at the excitation wavelength, F the integrated emission area and n the refraction index of the solvents used. Subscripts (r) and (s) denotes the reference and sample compounds.

2.6. Biological activity assays

Minimum Inhibitory Concentrations of growth (MIC) were assessed using a broth microdilution method for antifungal susceptibility testing of yeasts (NCCLS M27-A). The yeast *Saccharomyces cerevisiae* PYCC 4072 was used as a model organism. Briefly, cells were cultivated in 96-microwell plates in RPMI 1640 medium, buffered to pH 7.0 with 0.165 M morpholenepropanesulfonic acid (MOPS) buffer (Sigma). Initial cell concentration was 0.5 × 10^3 cells/mL. Growth was assessed by measuring the absorbance at 640 nm in a microplate photometer (Molecular Devices SpectraMax Plus) after 48 h of incubation at 30 °C. MIC values were considered as the lowest concentration of drug that resulted in an inhibition of growth > 80%. Stock solutions of the compounds were prepared in DMSO and a final dilution was carried out in an RPMI 1640 medium (Sigma, St. Louis, Mo.). Each drug concentration (from 400 μM to bellow the MIC value, using a two-fold dilution scheme) was tested in triplicate and in at least two independent experiments.

3. Results and discussion

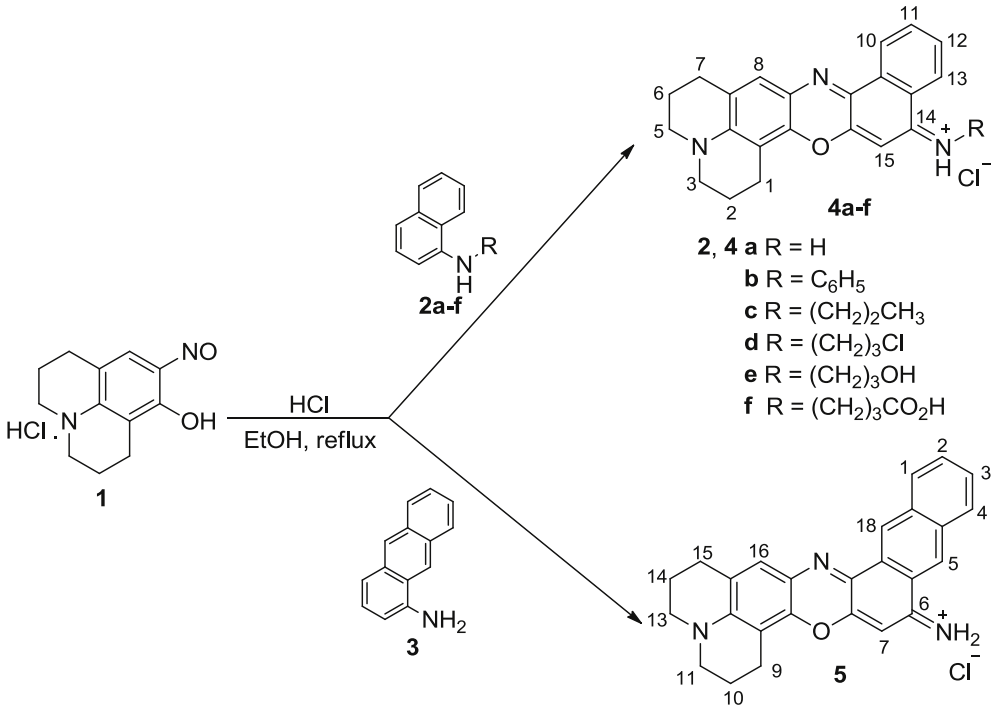
3.1. Synthesis of quinolizino[1,9-hi]phenoxazin-6-iminium chlorides 4a-f and 5

The synthesis of phenoxazinium chlorides **4a-f** and **5** was started with the preparation of required precursors such as nitroso derivative **1** and *N*-alkylated napthalen-1-amines **2b-f** (**2a** and **3** are commercial reagents). 9-Nitroso-1,2,3,5,6,7-hexahydropyrido [3,2,1-*ij*]quinolin-8-ol **1** was obtained by nitrosation of 1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol (common name 8-hydroxyjulolidine) with sodium nitrite in acid solution under ice cold conditions [41]. *N*-Phenylnaphthalen-1-amine **2b** was obtained as a solid in good yield by the alkylation of naphthalen-1-amine with chlorobenzene in ethanol under reflux conditions. The other precursors namely *N*-propylnaphthalen-1-amine **2c**, *N*-(3-chloropropyl)naphthalen-1-amine **2d**, 3-(naphthalen-1-ylamino)propan-1-ol **2e** and 4-(naphthalen-1-ylamino)butanoic acid **2f** were obtained in accordance with the earlier reported procedure [26,28].

The reaction of 9-nitroso-1,2,3,5,6,7-hexahydropyrido[3,2,1-*ij*]quinolin-8-ol **1** with naphthalen-1-amine **2a** and its derivatives **2b-f** or anthracen-1-amine **3**, in an acidic medium afforded the corresponding phenoxazinium chlorides **4a-f** and **5**, respectively. Thus, reaction between nitroso derivative of 8-hydroxyjulolidine **1** with precursors **2a-f** and **3** in ethanol, in the presence of concentrated hydrochloric acid, and after silica gel column chromatography purification gave the phenoxazinium chlorides **4a-f** and **5**, possessing at the free lateral amine the hydrogen atom, phenyl, alkyl along with the functionalities such as chloro, hydroxyl and carboxyl. All these compounds were obtained as green blue solids and were fully characterized by high resolution mass spectrometry, IR and NMR (^{1}H and ^{13}C) spectroscopy (Scheme 1).

The ^{1}H NMR spectra (**4a-f** and **5**) showed the signals of aliphatic protons from the methylenic groups of 1-H and 7-H (for **4a-f**) or 9-H and 15-H (for **5**) as triplets or multiples (**4b**, **4c**) (δ 2.58–3.0 ppm), 2-H and 6-H (for **4a-f**) or 10-H and 14-H (for **5**) as multiplets (δ 1.92–2.18 ppm), and methylene protons close to the nitrogen atom 3-H and 5-H (for **4a-f**) or 11-H and 13-H (for **5**) appeared as multiplets (δ 3.42–3.69 ppm). Similarly, for compounds **4a-f** the methylenic groups of substituents at 14-position, directly linked to the nitrogen atom $NHCH_2$ appeared as a multiplet or a triplet (**4d**, **5e**) (δ 3.55–3.78 ppm), as well as groups close to the same atom, $NHCH_2\mathit{CH_2}$, showed as multiplets, sextet (**4c**) or quintet (**4d**) (δ 1.90–2.14 ppm). The terminal methyl group exhibited a triplet (δ 1.14 ppm) and methylene protons adjacent to chloro, hydroxyl and carboxylic functionalities (**4d-f**) showed triplets (δ 2.59–3.83 ppm). In addition, spectra showed the aromatic protons of the polycyclic system, in particular, 8-H (δ 6.94–7.47 ppm) and 15-H (for **4a-f**), as well as H-7 (δ 6.49 ppm) and H-16 (δ 7.08 ppm) (for **5**) (δ 6.49–6.85 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 (for **4a-f**) or C-9 and C-15 (for **5**) (δ 20.07–21.61 ppm), C-2 and C-6 (for **4a-f**) or C-10 and C-14 (for **5**) (δ 20.09 to 21.66) and close to the nitrogen atom C-3 and C-5 (for **4a-f**) or C-11 and C-13 (for **5**) (δ 51.43–52.56 ppm). The groups of substituents at the 14-position, directly linked to the nitrogen atom $NHCH_2$ (**4c-e**) (δ 42.71–47.09 ppm), as well as the groups close to the same atom, $NHCH_2\mathit{CH_2}$, (δ 22.99–44.58 ppm). In addition, there was the presence of carbons of the methyl group (**4c**, δ 11.80 ppm) and the carbon proximity to chloro, hydroxyl and carboxylic functionalities (**4d-f**, δ 31.84–60.54 ppm). Spectra showed the aromatic carbons, in particular C-8 (for **4a-f**) or C-16 (for **5**) (δ 124.86–131.17 ppm), and C-15 (for **4a-f**) or C-7 (for **5**) (δ 93.28–96.77 ppm). The IR spectrum of benzophenoxazine **4e** showed the bands of the hydroxyl group (3450 cm^{-1}) and also, as in the remaining phenoxazines, strong bands are showed of the C=N bond (1641-1573 cm^{-1}) due to the fused oxazine ring.



Scheme 1. Synthesis of quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides **4a-f** and **5**.

3.2. Photophysical studies of quinolizino[1,9-hi]phenoxazin-6-iminium chlorides 4a-f and 5

Fundamental photophysical studies of quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides **4a-f** and **5** were carried out in dry ethanol, and water.

Previous work showed that in proton-accepting solvents the photophysical behaviour of benzo[*a*]phenoxazinim chlorides is determined by acid-base equilibria, mainly at the 5-amino position [5,42]. The main features of absorption spectra corresponded to an acidic form (AH^+) around 650 nm and a ~100 nm blue shifted neutral form (A) [43]. Basic form fluorescence was broad and centered at around 600 nm while the acid form (AH^+) showed a band centered near 660 nm with a higher fluorescence quantum yield that reached 0.4 when the 9-amino position was mono-alkylated and varied between 0.1 and 0.2 when it was di-alkylated [5,42,43]. At 470 nm the basic form was mostly excited while at higher excitation wavelengths the acid form was the main molecular form. Figs. 1 and 2 show the absorption and fluorescence of compounds **4a-f** and **5** either in ethanolic or aqueous based media.

Fluorescence and absorption spectra in ethanol either basified with TEAH (Fig. 1A) or acidified with TFA (Fig. 1B) are consistent with the above general characteristics. However, the acid-base behaviour is different from what was reported for similar compounds but without the julolidine moiety [44,45], given that the same amount of TEA not completely displaced the equilibrium to the basic form and consequently emission corresponding to acid form is still observable (Fig. 1). Using the same amount of TFA as above the equilibrium is nearly completely shifted towards the acid form as very little emission from the basic form is observed when exciting at 470 nm (Fig. S1). Yet, the absorption spectra of the acid form are broader (Fig. 1B).

In water, the absorption spectra of these type of compounds usually evidences the presence of non-fluorescent H-aggregates of the acid form through a ~40 nm blue shifted shoulder [43]. Through the dimerization equilibria, the relative amount of that shoulder depends on dye concentration [43]. For the studied julolidine fused compounds **4a-f** and **5** the spectra at 4×10^{-6} M (Fig. 2B) are similar to the ones obtained for compounds without the julolidine moiety at 5×10^{-5}M [43]. This clearly indicates the much higher tendency for aggregation of the

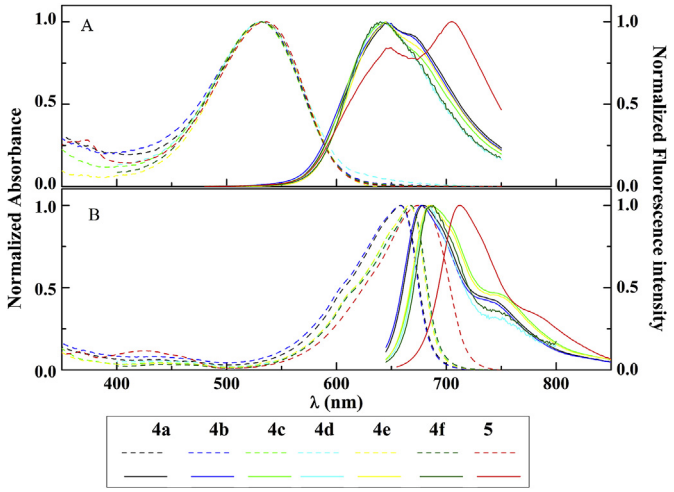


Fig. 1. Absorption and emission spectra of compounds **4a-f** and **5** at 4 μM concentration in either basified (panel **A**) or acidified (panel **B**) dried ethanol. Emission of basic form at 470 nm excitation and emission of acid form at 640 nm excitation.

synthesised quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides.

Table 1 shows absorption (λ_{abs}) and emission (λ_{em}) maxima, Stokes shifts ($\Delta\lambda$) and fluorescence quantum yields (Φ) for the acid/basic form in dried ethanol and for the acid form in water.

Through stiffening of the 9-amino position, it was expected that fluorescence quantum yields in acidified ethanol will be higher than the values previously obtained for benzophenoxazinium chlorides without a fused julolidine moiety and di-alkylated compounds (between 0.1 and 0.2) [5,42,43]. In fact, improvement in fluorescence quantum yields 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 is possible to conclude that the 5-amino position (6- or 14-positions in **5** and **4a-f**, respectively) is the main pathway of excited state non-radiative deactivation. Regarding the basic form, the usual fluorescence

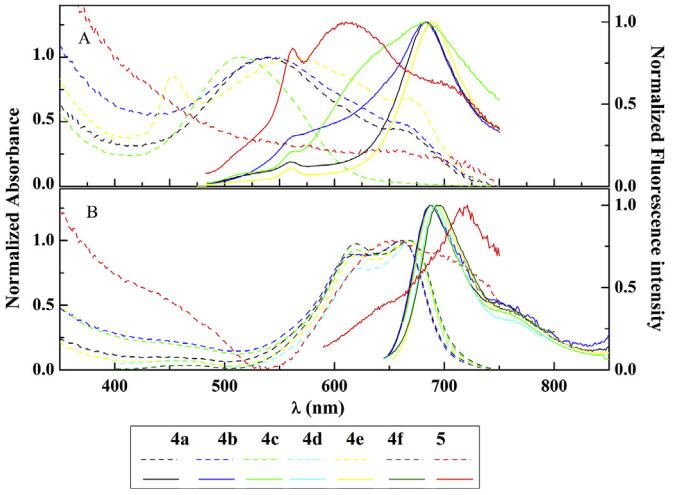


Fig. 2. Absorption and emission spectra of compounds **4a-f** and **5** at 4 μM concentration in either basified (panel **A**) or acidified (panel **B**) water. Emission of basic form at 470 nm excitation and emission of acid form at 640 or 575 nm excitation.

quantum yields are in the range 0.01–0.03 [7,43]. It is seen that the expected improvement from juolidine stiffening of 9-amino position is clearly observed as the quantum yield is above 0.1 for almost all compounds reaching 0.31 for **4b**. The slightly lower quantum yield of basic form of compound **4f** is probably related with an interaction of the COOH group with the secondary amine in 14-position.

Comparing compounds **5** and **4a** significant red shifts were observed both in absorption (17 nm) and emission (22 nm). 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 acidified aqueous media. This is easily understandable by the fact that H-aggregates are non-fluorescent and they are very prominent for the studied compounds (Fig. 2B). In basified aqueous media (Fig. 2A) there is a significant dispersive background in the absorption spectra. This indicates the low solubility of the neutral molecular forms in aqueous media resulting in the formation of crystallites that act as light scattering centres. This leads to huge decrease in fluorescence efficiency so that Raman peaks are now observable. It is interesting to note that although in basic pH, the emission is dominated by the small fraction of protonated molecular form that still remains.

Table 2
Activity against *Saccharomyces cerevisiae* PYCC 4072 and log *P* values of quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides **4a-f** and **5**.

Compound	MIC[a]	log *P*
4a	25	2.85
4b	> 400	4.05
4c	6.25	5.60
4d	0.78	5.47
4e	400	4.42
4f	12.5	2.14
5	12.5	4.02

[a] Experiments were performed in triplicate and at least two independent experiments were conducted.

3.3. Biological activity of quinolizino[1,9-hi]phenoxazin-6-iminium chlorides 4a-f and 5

The potential antifungal activity of the synthesised dyes **4a-f** and **5** was investigated using the yeast *Saccharomyces cerevisiae* PYCC 4072 as a model organism and a broth microdilution method for antifungal activity testing [17,18]. The minimum inhibitory concentration of growth (MIC) and log *P* values, which were theoretically predicted [46] are showed in Table 2.

The results showed that all tested dyes exhibited considerable antiproliferative activity against the yeast *S. cerevisiae*, with MIC values between 0.78 and 25 μM (with exception of **4b**, that has no activity and **4e**, MIC 400 μM). This is especially relevant if we consider the MIC values for fluconazole and miconazole, two reference antifungal compounds, which were 50 and 100 μM, respectively[47]. The calculated log *P* of the dyes, ranged from 2.85 to 5.60, but the differences in this values did not correlate with the MIC values for the compounds.

Our reference compound, **4a**, exhibits a MIC value of 25 μM, and a notable increment (four-times) in its biological activity was observed by the introduction of a propyl group at 14-position of the polycyclic aromatic system (**4c**). Substitution of the methyl group (**4c**) by a chlorine atom, using a chloropropyl group (**4d**) results in the largest increase of activity, 0.78 μM being the lowest MIC value observed. In contrast, the presence of a terminal hydroxyl group, namely the hydroxylpropyl group drastically decreases the MIC value, making the activity of compound **4e** residual (MIC value 400 μM). On the other hand, the carboxylic acid derivative **4f** showed a significant activity, doubling the eficacy of compound **4a** (MIC value 12.5 μM). These achievements suggest the presence of terminal chloride group at 14-position is favourable for the antiproliferative activity, and thus supporting the results previously published by our group [17].

Table 1
Yield and photophysical studies of quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides **4a-f** and **5** in dry ethanol, water and after the addition of either TFA or TEAH.

Cpd	Yield (%)	Dry ethanol + TFA|TEAH				Water + TFA			
		λ_{abs} (nm) ε ($10^4 M^{-1}cm^{-1}$)	λ_{em}(nm)[a]	Δ λ(nm)	Φ[a]	λ_{abs} (nm) ε ($10^4 M^{-1}cm^{-1}$)	λ_{em} (nm)	Δ (nm)	Φ
4a	19	658|532 7.66|3.55	679|645	21|113	0.18|0.27	662 2.54	687	25	0.024
4b	20	658|532 6.08|2.90	677|645	19|113	0.19|0.31	661 2.29	685	24	0.026
4c	21	667|531 10.4|4.34	687|645	20|114	0.22|0.10	667 3.82	694	27	0.027
4d	5	667|530 –	685|640	18|110	0.19|0.10	670 –	694	24	0.040
4e	22	666|531 8.73|3.49	684|645	18|114	0.24|0.16	668 3.60	694	26	0.032
4f	19	667|532 –	688|640	21|108	0.15|0.069	668 –	693	25	0.017
5	21	675|536 12.4|6.54	712|650	37|114	0.17|0.28	663 –	–	–	–

[a] Emission spectra and quantum yield determination were obtained at 575 nm or 470 nm excitation when, TFA or TEAH, respectively, were used.

Furthermore, the addition of a third fused benzene ring in the polycyclic system, compound **5**, also increases the activity to the double in relation to compound **4a**.

4. Conclusion

New quinolizino[1,9-*hi*]phenoxazin-6-iminium chlorides, namely six benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-iminium chlorides **4a-f** with the 14-amine position unsubstituted, and bearing phenyl, propyl, 3-chloropropyl, 3-hydroxypropyl, and 3-carboxypropyl, as well as a naphtho[2,3-*a*]quinolizino[1,9-*hi*]phenoxazin-6-iminium chloride **5**, were synthesised. The photophysics of the acid and basic forms were studied in dried ethanolic media, by adding either an acid or a 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.

The results from the antifungal assays showed that the fusion of julolidine to the phenoxazine moiety led to an increase in the activity of the dyes, which still depended on the substitution at the 14 position of the polycyclic system, being negatively influenced by a hydroxy terminal and positively influenced by the presence of a middle size apolar group (propyl). Furthermore, the extension of the aromatic system, in compound **5**, also had a positive influence in the compounds activity. The best activity found with compound **4d** suggests this compound as a potential interesting molecule for further development.

Declarations of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.107870.

References

[1] Liu T, Ning J, Wang B, Dong B, Li S, Tian X, et al. Activatable near-infrared fluorescent probe for dipeptidyl peptidase IV and its bioimaging applications in living cells and animals. Anal Chem 2018;90:3965–73.
[2] Frade VHJ, Gonçalves MST, Moura CVP. Synthesis of fluorescent water-soluble functionalised benzo[*a*]phenoxazinium salts. Tetrahedron Lett 2006;47:8567–70.
[3] Frade VHJ, Barros SA, Moura JCVP, Gonçalves MST. Fluorescence derivatisation of amino acids in short and long-wavelengths. Tetrahedron Lett 2007;48:3403–7.
[4] Frade VHJ, Barros SA, Moura JCVP, Coutinho PJG, Gonçalves MST. Synthesis of short and long-wavelength functionalised probes: amino acids' labelling and photophysical studies. Tetrahedron 2007;63:12405–18.
[5] Alves CMA, Naik S, Coutinho PJG, Gonçalves MST. Novel long alkyl side chain benzo[*a*]phenoxazinium chlorides: synthesis, photophysical behaviour and DNA interaction. Tetrahedron 2009;65:10441–52.
[6] Alves CMA, Naik S, Coutinho PJG, Gonçalves MST. New long alkyl side-chain benzo[*a*]phenoxazines as micellisation probes. Tetrahedron Lett 2009;50:4470–4.
[7] Raju BR, Carvalho MMT, Leitão MIPS, Coutinho PJG, Gonçalves MST. Synthesis, photophysical characterisation and photostability studies of NIR probes with aliphatic, aromatic and chlorinated terminals in 5- and 9-amino positions of benzo[*a*]phenoxazines. Dyes Pigments 2016;132:204–12.
[8] Raju BR, Gonçalves MST, Coutinho PJG. Fluorescent probes based on side-chain chlorinated benzo[*a*]phenoxazinium chlorides: studies of interaction with DNA. Spectrochim Acta A Mol Biomol Spectrosc 2017;171:1–9.
[9] Martinez V, Henary M. Nile Red and Nile Blue: applications and syntheses of structural analogues. Chem Eur J 2016;22:13764–82.
[10] Yuan L, Lin W, Zheng K, He L, Huang W. Far-red to near infrared analyte-responsive fluorescent probes based on organic fluorophore platforms for fluorescence imaging. Chem Soc Rev 2013;42:622–61.
[11] Mishra SS, Subuddhi U. Spectroscopic investigation of interaction of Nile Blue A, a potent photosensitizer, with bile salts in aqueous medium. J Photochem Photobiol, B 2014;14:67–75.
[12] Lopes M, Alves CT, Raju BR, Gonçalves MST, Coutinho PJG, Henriques M, Belo I. Application of benzo[*a*]phenoxazinium chlorides in antimicrobial photodynamic therapy of *Candida albicans* biofilms. J Photochem Photobiol, B 2014;141:93–9.
[13] Madsen J, Canton I, Warren NJ, Themistou E, Blanazs A, Ustbas B, Tian X, Pearson R, Battaglia G, Lewis AL, Armes SP. Nile Blue-based nanosized pH sensors for simultaneous far-red and near-infrared live bioimaging. J Am Chem Soc 2013;135:14863–70.
[14] Mizukawa Y, Ge J-F, Md AB, Itoh I, Scheurer C, Wittlin S, Brun R, Matsuoka H, Ihara M. Novel synthetic route for antimalarial benzo[*a*]phenoxazine derivative SSJ-183 and two active metabolites. Bioorg Med Chem 2014;22:3749–52.
[15] Thimmaiah KN, Horton JK, Seshadri R, Israel M, Houghton JA, Harwood FC, Houghton PJ. Synthesis and chemical characterization of *N*-substituted phenoxazines directed toward reversing vinca alkaloid resistance in multidrug-resistant cancer cells. J Med Chem 1992;35:3358–64.
[16] Küçükkilinç T, Ozer I. Multi-site inhibition of human plasma cholinesterase by cationic phenoxazine and phenothiazine dyes. Arch Biochem Biophys 2007;461:294–8.
[17] Frade VHJ, Sousa MJ, Moura JCVP, Gonçalves MST. Synthesis of naphtho[2,3-*a*]phenoxazinium chlorides. Structure-activity relationships of these heterocycles and benzo[*a*]phenoxazinium chlorides as new antimicrobials. Bioorg Med Chem 2008;16:3274–82.
[18] Leitão MIPS, Raju BR, Naik S, Coutinho PJG, Sousa MJ, Gonçalves MST. Development of new benzo[*a*]phenoxazinium chlorides for fluorescence imaging and their application as antiproliferative agents. Tetrahedron Lett 2016;57:3936–41.
[19] Xie ZY, Hung LS, Lee ST. High-efficiency red electroluminescence from a narrow recomination zone confined by an organic double heterostructure. Appl Phys Lett 2001;79:1048–50.
[20] Lee KH, Park MH, Kim CS, Kim YK, Yoon SS. Modified 4-(dicyanomethylene)-2-*tert*-butyl-6-(1,1,7,7-tetra-methyljulolidy-19-enyl)-4*H*-pyran-containing red fluorescent emitters for efficient organic light-emitting diodes. Thin Solid Films 2011;520:510–4.
[21] Dall'Agnese C, Komatsu K, Koshika M, Morikawa D, Ching KIM, Mori S. Enhancement of quantum efficiency by co-adsorbing small julolidine dye and bulky triphenylamine dye in dye-sensitized solar cells. J Photochem Photobiol, A 2018;356:403–10.
[22] Pawlowski G. Eur pat appl EP. Chem Abstr 1986;206:270. 1987;107:7084s.
[23] Gompel JV, Schuster GB. Chemiluminescence of organic peroxides: intramolecular electron-exchange luminescence from a secondary perester. J Org Chem 1987;52:1465–8.
[24] Braun HP, Deneke U, Guethlein W, Nagel R. Ger offen DE 3,917,677, 1990. Chem Abstr 1991;115:25542j.
[25] Saito K, Suzuki H, Teruya C, Maki A, Fukasaku N. Jpn kokai tokkyo koho JP 01 75. Chem Abstr 1989;112:470. 1990138918t.
[26] Kurihara T, Matsumoto S, Kaino T, Kanbara H, Kubodera K. Eur pat appl EP 326. Chem Abstr 1989;112:133. 1990;:87901x.
[27] Piloto AM, Hungerford G, Costa SPG, Gonçalves MST. Photoinduced release of neurotransmitter amino acids from novel coumarin fused julolidine ester cages. Eur J Org Chem 2013;34:7715–23.
[28] Vejdělek Z, Protiva M. Potential antidepressants and tranquillizers: synthesis of some 9-(aminoalkoxy)-2,3,6,7-tetrahydro-1*H*,5*H*-benzo[*ij*]quinolizines and 1-(substituted amino)-3-(1-naphthoxy)-2-propanols. Collect Czechoslov Chem Commun 1990;55:1290–6.
[29] Maity D, Manna AK, Karthigeyan D, Kundu TK, Pati SK, Govindaraju T. Visible-near-infrared and fluorescent copper sensors based on julolidine conjugates: selective detection and fluorescence imaging in living cells. Chem Eur J 2011;17:11152–61.
[30] Narayanaswamy N, Govindaraju T. Aldazine-based colorimetric sensors for Cu^{2+} and Fe^{3+}. Sens Actuators B Chem 2012;161:304–10.
[31] Noh JY, Kim S, Hwang IH, Lee GY, Kang J, Kim SH, Min J, Park S, Kim C, Kim J. Solvent-dependent selective fluorescence assay of aluminum and gallium ions using julolidine-based probe. Dyes Pigments 2013;99:1016–21.
[32] Na YJ, Hwang IH, Jo HY, Lee SA, Park GJ, Kim C. Fluorescent chemosensor based-on the combination of julolidine and furan for selective detection of zinc ion. Inorg Chem Commun 2013;35:342–5.
[33] Fares M, Li Y, Liu Y, Miao K, Gao Z, Zhai Y, Zhang X. A molecular rotor-based halotag ligand enables a fluorogenic proteome stress sensor to detect protein misfolding in mildly stressed proteome. Bioconjug Chem 2018;29:215–24.
[34] Kanitz A, Hartmann H. Preparation and characterization of bridged naphthoxazinium salts. Eur J Org Chem 1999;4:923–30.
[35] Firmino ADG, Raju BR, Gonçalves MST. Microwave synthesis of water-soluble 2-,5- and 9-substituted benzo[*a*]phenoxazinium chlorides in comparison with conventional heating. Eur J Org Chem 2013:1506–14.
[36] Raju BR, Sampaio DMF, Silva MM, Coutinho PJG, Gonçalves MST. Ultrasound promoted synthesis of Nile Blue derivatives. Ultrason Sonochem 2014;21:360–6.

[37] Raju BR, Garcia AMF, Costa ALS, Coutinho PJG, Gonçalves MST. Synthesis of new benzo[*a*]phenoxazinium probes possessing carboxylic ester, hydroxyl and amino functional groups: photophysical studies in dry ethanol and conjugation with CdTe quantum dots. Dyes Pigments 2014:203–13.
[38] Crosby GA, Demas JN. Measurement of photoluminescence quantum yields. Review. J Phys Chem 1971;75:991–1024.
[39] Fery-Forgues S, Lavabre D. Are fluorescence quantum yields so tricky to measure? A demonstration using familiar stationery products. J Chem Educ 1999;76:1260–4.
[40] Sens R, Drexhage KH. Fluorescence quantum yield of oxazine and carbazine laser dyes. J Lumin 1981;24:709–12.
[41] Crossley ML, Turner RJ, Hofmann CM, Dreisbach PF, Parker RP. Chemotherapeutic dyes. II. 5-Arylamino-9-dialkylaminobenzo[*a*]phenoxazines. J Am Chem Soc 1952;74:578–84.
[42] Naik S, Alves CMA, Coutinho PJG, Gonçalves MST. *N*-(Di)icosyl-substituted benzo [*a*]phenoxazinium chlorides: synthesis and evaluation as near-infrared membrane probes. Eur J Org Chem 2011;13:2491–7.
[43] Frade VHJ, Gonçalves MST, Coutinho PJG, Moura JCVP. Synthesis and spectral properties of long-wavelength fluorescent dye. J Photochem Photobiol, A 2007;185:220–30.
[44] Raju BR, Firmino ADG, Costa ALS, Coutinho PJG, Gonçalves MST. Synthesis and photophysical properties of side-chain chlorinated benzo[*a*]phenoxazinium chlorides. Tetrahedron 2013;69:2451–61.
[45] Alves CMA, Naik S, Coutinho PJG, Gonçalves MST. Novel DNA fluorescence probes based on *N*-[5-(11-functionalised-undecylamino)-9*H*-benzo[*a*]phenoxazin-9-ylidene]propan-1-aminium chlorides: synthesis and photophysical studies. Tetrahedron Lett 2011;52:112–6.
[46] Calculation of molecular properties and drug-likeness-Molinspiration cheminformatics software tools (http://www.molinspiration.com).
[47] Bettencourt AP, Castro M, Silva JP, Fernandes F, Coutinho OP, Sousa MJ, Proença MF, Areias FM. Phenolic imidazole derivatives with dual antioxidant/antifungal activity: synthesis and structure-activity relationship. Med Chem 2019;15:341–51.