

GRAPHICAL ABSTRACT

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

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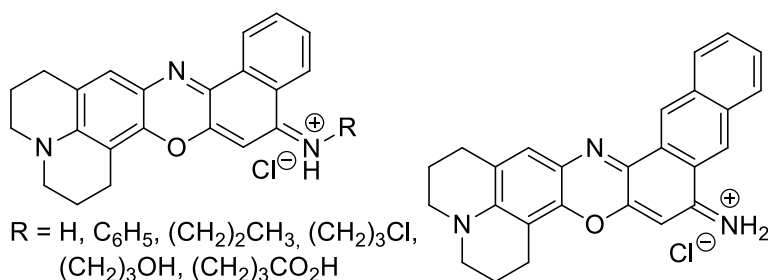
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Ethanol: $\lambda_{\text{abs}} = 531 - 667 \text{ nm}$; $\lambda_{\text{em}} = 640 - 712 \text{ nm}$; $\varepsilon > 2.90 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$; $\Phi = 0.07 - 0.31$

Water: $\lambda_{\text{abs}} = 661 - 668 \text{ nm}$; $\lambda_{\text{em}} = 685 - 694 \text{ nm}$; $\varepsilon > 2.29 \times 10^4 \text{ M}^{-1} \text{cm}^{-1}$; $\Phi = 0.02 - 0.04$

MIC - 0.78 μM (best value)

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HIGHLIGHTS

- New NIR fluorescent dyes of quinolizino[1,9-*hi*]phenoxazines were synthesised.
- The new dyes show absorbance and emission maxima up to 675 and 712 nm, respectively.
- A bathochromic shift of 33 nm occurs in λ_{em} for dye possessing the anthracene ring system.
- The fluorochromophores displayed interesting antifungal activity.
- The best candidate for further developments presents a MIC value of 0.78 μ M.

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 μ M, 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.

Keywords: Nile Blue; Julolidine dyes; Phenoxazinium dyes; NIR probes; Antimicrobial drugs; *Saccharomyces cerevisiae*.

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 condensation of nitroso derivative of 8-hydroxyjulolidine with naphthalen-1-amine derivatives and anthracen-1-amine, respectively. The introduction of the julolidine nucleus 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 possess 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 assessed 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 60F₂₅₄), 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 ¹H and 100.6 MHz for ¹³C using the solvent peak as internal reference at 25 °C. All chemical shifts are given in ppm using δ_{H} Me₄Si = 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 ^1H 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. ^1H 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): ^1H NMR (CDCl_3 , 400 MHz): δ_{H} 6.81 (dd, $J = 6.8$ and 1.6 Hz, 2 H, 2-H, 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-1*H*-benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-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} . $^1\text{H NMR } \delta_H$ (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. $^{13}\text{C NMR } \delta_C$ (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-1*H*-benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-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 (CD₃OD, 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 (CD₃OD, 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 (2×Ar-C), 130.27 (C-8), 132.14 (C-11), 132.64 (Ar-C, C-12), 133.27 (2×Ar-C), 144.97 (2×Ar-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-1*H*-benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-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 $\text{NHCH}_2\text{CH}_2\text{CH}_3$), 1.90 (sext, $J = 7.2$ Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_3$), 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 $\text{NHCH}_2\text{CH}_2\text{CH}_3$), 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 ($\text{NHCH}_2\text{CH}_2\text{CH}_3$), 20.31 (C-1), 20.57 (C-6), 21.64 (C-2), 22.99 ($\text{NHCH}_2\text{CH}_2\text{CH}_3$), 28.56 (C-7), 47.09 ($\text{NHCH}_2\text{CH}_2\text{CH}_3$), 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 $[\text{M}+1]^+$: 420.1855; $\text{C}_{25}\text{H}_{26}\text{ClN}_3\text{O}$ requires $[\text{M}+1]^+$: 420.1845.

2.4.4. 3-Chloro-*N*-(2,3,6,7-tetrahydro-1*H*-benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-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 %): $\nu_{\max} = 3428, 3223, 3027, 2961, 2929, 1707, 1640, 1588, 1543, 1472, 1438, 1385, 1358, 1314, 1282, 1217, 1176, 1136, 1099, 1047, 893, 780 \text{ cm}^{-1}$. ^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, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 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, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 3.83 (t, $J = 6.4$ Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 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 ($\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 42.71 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 43.30 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 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 $[\text{M}+1]^+$: 454.3900; $\text{C}_{25}\text{H}_{25}\text{Cl}_2\text{N}_3\text{O}$ requires $[\text{M}+1]^+$: 454.3905.

2.4.5. 3-Hydroxy-*N*-(2,3,6,7-tetrahydro-1*H*-benzo[*a*]quinolizino[1,9-*hi*]phenoxazin-14(5*H*)-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%): $\nu_{\text{max}} = 3450, 2932, 1640, 1588, 1553, 1531, 1471, 1440, 1386, 1355, 1317, 1284, 1217, 1177, 1139, 1101, 781 \text{ cm}^{-1}$. ^1H NMR δ_{H} (CD_3OD , 400 MHz), 2.04-2.14 (m, 6H, 2-H, 6-H and $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH}$), 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, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH}$), 3.82 (t, $J = 6.0$ Hz, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH}$), 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 ($\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 42.94 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH}$), 51.97 (C-3), 52.47 (C-5), 60.54 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{OH}$), 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 %): $\nu_{max} = 2923, 2853, 1731, 1721, 1637, 1589, 1545, 1499, 1435, 1375, 1334, 1322, 1290, 1230, 1182, 1162, 1146, 1127, 1100, 1054, 1001, 918, 807, 753 \text{ cm}^{-1}$. $^1\text{H NMR } \delta_H$ (CD_3OD , 400 MHz), 2.0-2.16 (m, 6H, 2-H, 6-H and $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.59 (t, $J = 7.2 \text{ Hz}$, 2H, $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 2.75 (t, $J = 5.6 \text{ Hz}$, 2H, 1-H), 2.88 (t, $J = 6.0 \text{ Hz}$, 2H, 7-H), 3.55-3.64 (m, 6H, 3-H, 5-H, and $\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 6.63 (s, 1H, 15-H), 6.94 (s, 1H, 8-H), 7.65 (t, $J = 7.2 \text{ Hz}$, 1H, 12-H), 7.77 (t, $J = 7.6 \text{ Hz}$, 1-H, 11-H), 8.06-8.16 (m, 1H, 13-H), 8.57 (d, $J = 8.0 \text{ Hz}$, 1H, 10-H) ppm. $^{13}\text{C NMR } \delta_C$ (CD_3OD , 100.6 MHz), 20.17 (C-1), 20.57 (C-2), 21.54 (C-6), 24.70 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 28.49 (C-7), 31.84 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 44.58 ($\text{NHCH}_2\text{CH}_2\text{CH}_2\text{CO}_2\text{H}$), 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} . ^1H 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 $[\text{M}+1]^+$: 428.1521; $\text{C}_{26}\text{H}_{22}\text{ClN}_3\text{O}$ requires $[\text{M}+1]^+$: 428.1531.

2.4. Photophysical 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, $\Phi_r = 0.11$ [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 refractive index of the solvents used. Subscripts (r) and (s) denotes the reference and sample compounds.

2.6. Biological activity assays

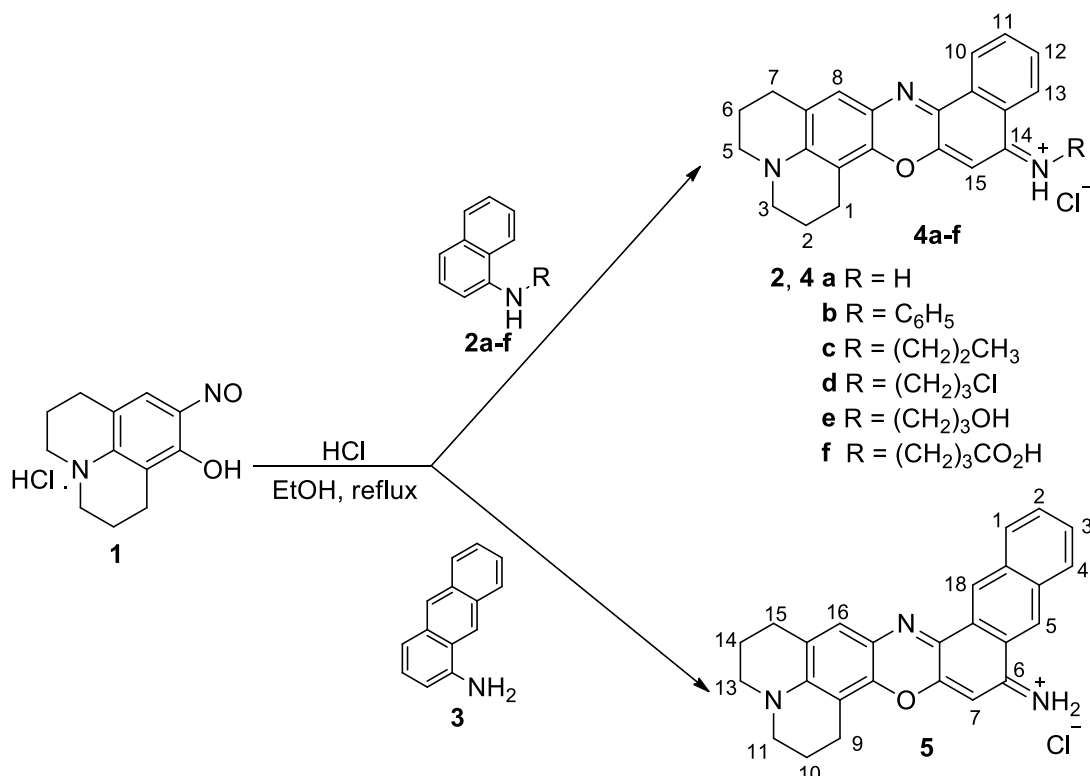
Minimum Inhibitory Concentrations of growth (MIC) were assessed using a broth microdilution method for antifungal susceptibility testing of yeasts (NCCLS M27-A). The yeast *Saccharomyces cerevisiae* PYCC 4072 was used as a model organism. Briefly, cells were cultivated in 96-microwell plates in RPMI 1640 medium, buffered to pH 7.0 with 0.165 M morpholene propanesulfonic 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 μ M to below 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 naphthalen-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 (¹H and ¹³C) spectroscopy (Scheme 1).



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

The ¹H 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 to 3.0 ppm), 2-H and 6-H (for **4a-f**) or 10-H and 14-H (for **5**) as multiplets (δ 1.92 to 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 to 3.69 ppm). Similarly, for compounds **4a-f** the methylenic groups of substituents at 14-position, directly linked to the nitrogen atom NHCH₂ appeared as a multiplet or a triplet (**4d**, **5e**) (δ 3.55 to 3.78 ppm), as well as groups close to the same atom, NHCH₂CH₂, showed as multiplets, sextet (**4c**) or quintet (**4d**) (δ 1.90 to 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 to 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 to 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 to 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 to 52.56 ppm). The groups of substituents at the 14-position, directly linked to the nitrogen atom NHCH_2 (**4c-e**) (δ 42.71 to 47.09 ppm), as well as the groups close to the same atom, NHCH_2CH_2 , (δ 22.99 to 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 to 60.54 ppm). Spectra showed the aromatic carbons, in particular C-8 (for **4a-f**) or C-16 (for **5**) (δ 124.86 to 131.17 ppm), and C-15 (for **4a-f**) or C-7 (for **5**) (δ 93.28 to 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 ($1641\text{-}1573\text{ cm}^{-1}$) due to the fused oxazine ring.

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. Figures 1 and 2 show the absorption and fluorescence of compounds **4a-f** and **5** either in ethanolic or aqueous based media.

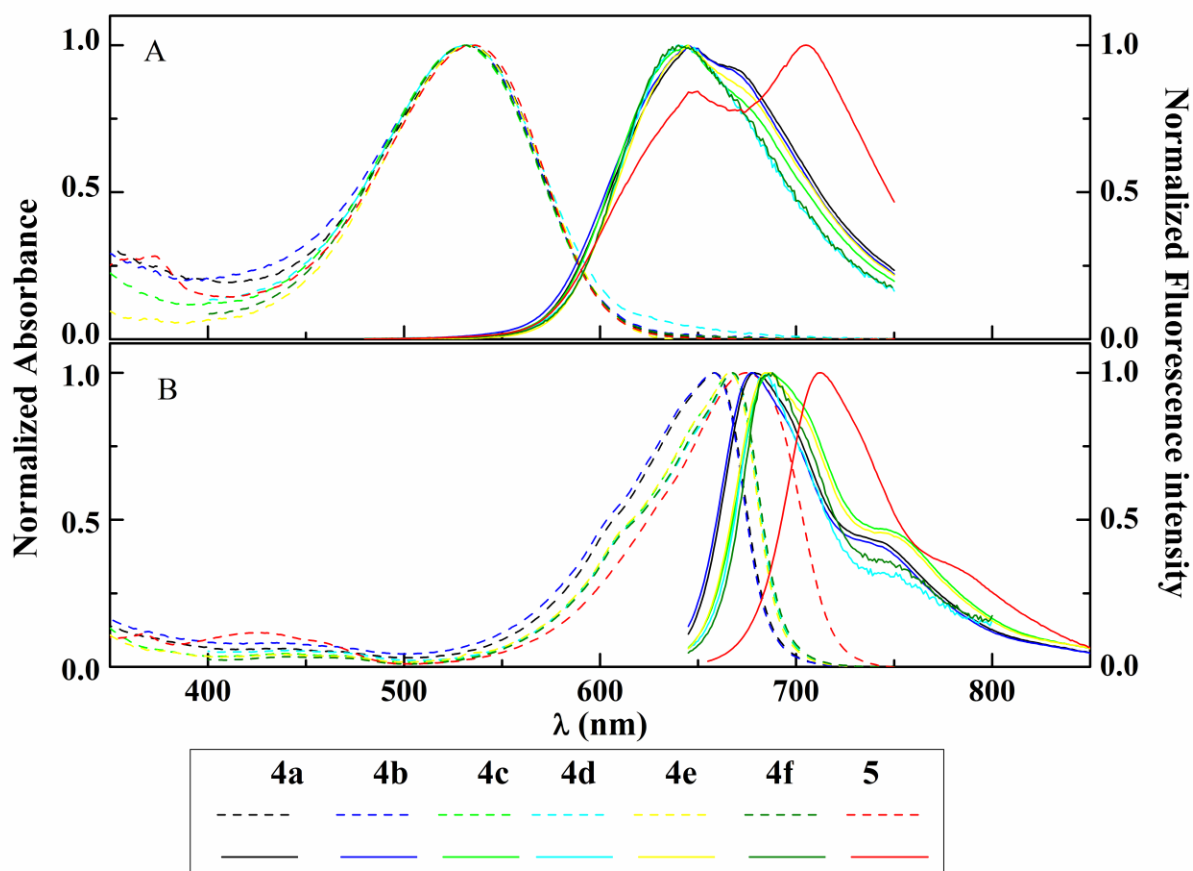


Figure 1. Absorption and emission spectra of compounds **4a-f** and **5** at $4\mu\text{M}$ concentration in either basified (panel **A**) or acidified (panel **B**) dried ethanol. Emission of basic form at 470 nm excitation and emission of acid form at 640 nm excitation.

Fluorescence and absorption spectra in ethanol either basified with TEAH (Figure 1A) or acidified with TFA (Figure 1B) are consistent with the above general characteristics. However, the acid-base behavior

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 (Figure 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 (Figure S1). Yet, the absorption spectra of the acid form are broader (Figure 1B).

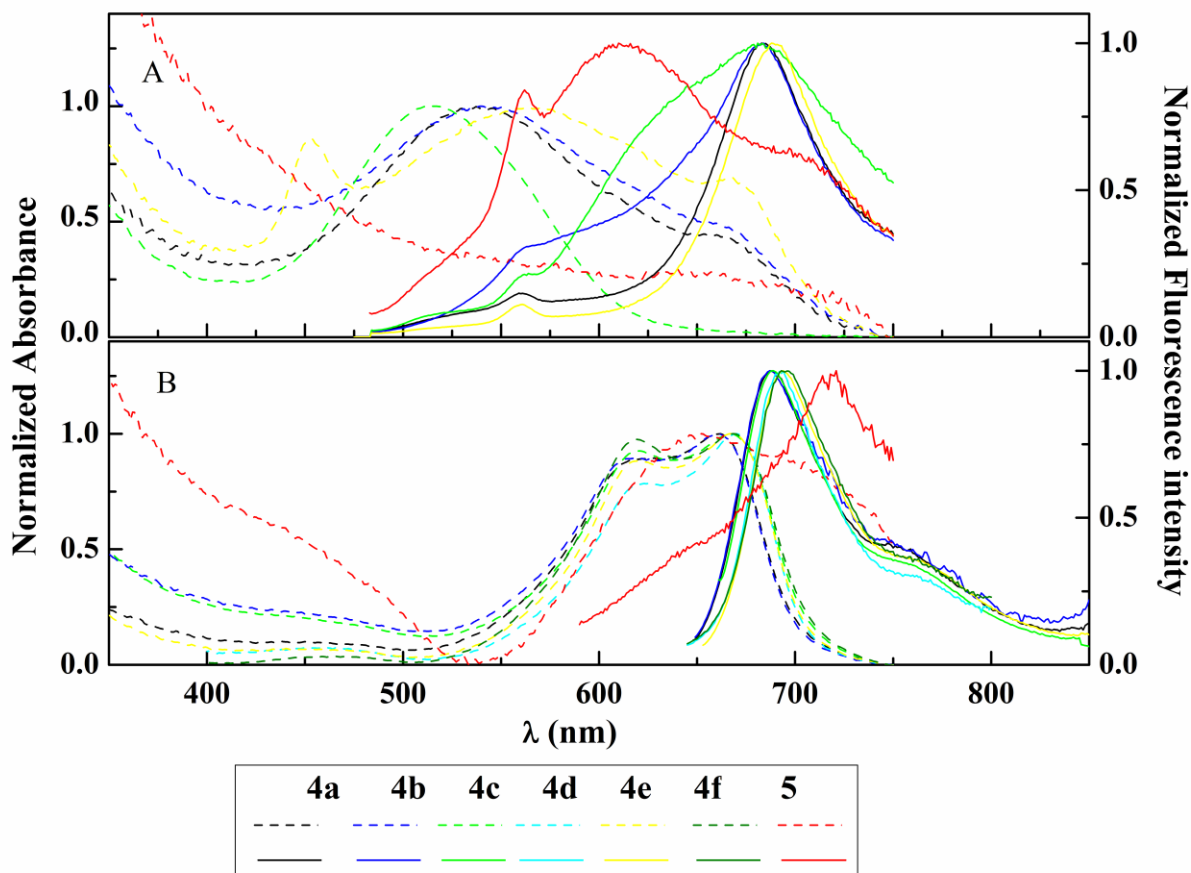


Figure 2. Absorption and emission spectra of compounds **4a-f** and **5** at 4 μ M concentration in either basified (panel **A**) or acidified (panel **B**) water. Emission of basic form at 470 nm excitation and emission of acid form at 640 or 575 nm excitation.

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

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

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) $\varepsilon (10^4 \text{M}^{-1} \text{cm}^{-1})$	$\lambda_{\text{em}}(\text{nm})^{\text{a}}$	Δ (nm)	Φ^{a}	λ_{abs} (nm) $\varepsilon (10^4 \text{M}^{-1} \text{cm}^{-1})$	λ_{em} (nm)	Δ (nm)	Φ
4a	19	658 532	679 645	21 113	0.18 0.27	662	687	25	0.024
		7.66 3.55				2.54			
4b	20	658 532	677 645	19 113	0.19 0.31	661	685	24	0.026
		6.08 2.90				2.29			
4c	21	667 531	687 645	20 114	0.22 0.10	667	694	27	0.027
		10.4 4.34				3.82			
4d	5	667 530	685 640	18 110	0.19 0.10	670	694	24	0.040
		-				-			
4e	22	666 531	684 645	18 114	0.24 0.16	668	694	26	0.032
		8.73 3.49				3.60			
4f	19	667 532	688 640	21 108	0.15 0.06	668	693	25	0.017
		-			9	-			
5	21	675 536	712 650	37 114	0.17 0.28	663	-	-	-
		12.4 6.54				-			

^aEmission spectra and quantum yield determination were obtained at 575 nm or 470 nm excitation when, TFA or TEAH, respectively, were used.

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 nonradiative deactivation. Regarding the basic form, the usual fluorescence quantum yields are in the range 0.01-0.03 [7,43]. It is seen that the expected improvement from julolidine 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 (Figure 2B). In basified aqueous media (Figure 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 centers. 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.

3.3. Biological activity of 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 are theoretically predicted [46] are showed in Table 2.

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 <i>P</i>
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

^aExperiments were performed in triplicate and at least two independent experiments were conducted.

The results showed that all tested dyes exhibited considerable antiproliferative activity against the yeast *S. cerevisiae*, with MIC values between 0.78 and 25 μ M (with exception of **4b**, that has no activity and **4e**, MIC 400 μ M). This is especially relevant if the MIC values for fluconazole and miconazole, two reference antifungal compounds, that were 50 and 100 μ M, respectively, were considered [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 efficacy 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].

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.

Acknowledgments

Thanks are also due to Fundação para a Ciência e Tecnologia (Portugal) for financial support to the Portuguese NMR network (PTNMR, Bruker Avance III 400-Univ. Minho), FCT and FEDER (European Fund for Regional Development)-COMPETE/QREN-EU for financial support to the research centres CQ/UM [Ref. UID/QUI/00686/2013 and UID/ QUI/0686/2016], CF-UM-UP (Ref. UID/FIS/04650/2013 and UID/FIS/04650/2019), and CBMA (Ref. UID/BIA/04050/2019). A post-doctoral grant to B. R. Raju (SFRH/BPD/62881/2009) is also acknowledged to FCT, POPH-QREN, FSE.

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Supplementary material

Emission spectra of compounds **4a-f** at 4 μ M concentration in acidified dried ethanol at 470 nm excitation.

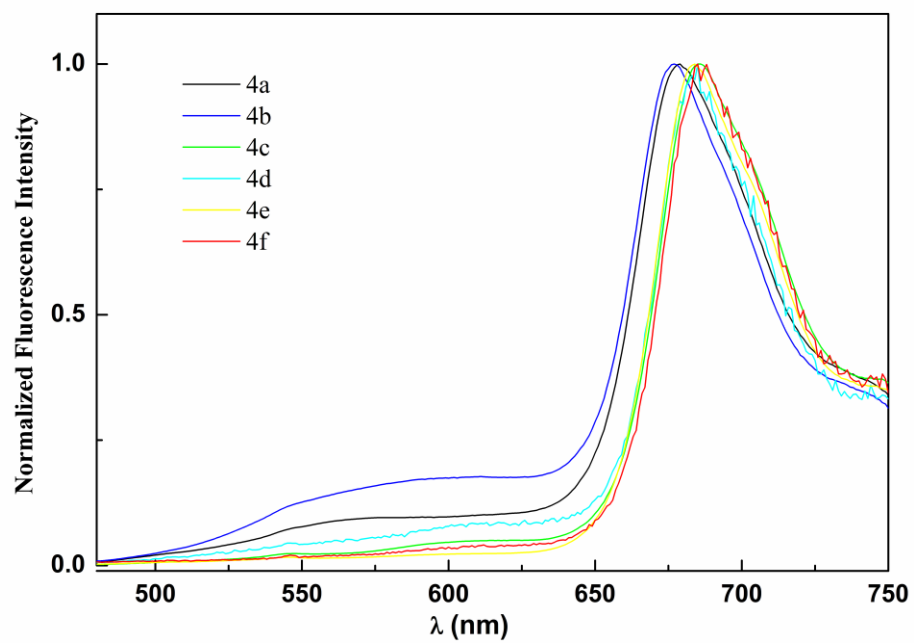


Figure S1. Emission spectra of compounds **4a-f** at 4 μ M concentration in acidified dried ethanol at 470 nm excitation.