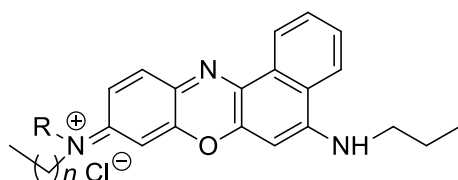


## GRAPHICAL ABSTRACT

### Synthesis and photophysical studies of new benzo[*a*]phenoxazininium chlorides as potential antifungal agents

M. Inês P. S. Leitão <sup>a,b</sup>, B. Rama Raju <sup>a,c</sup>, Sarala Naik <sup>a,c</sup>, Paulo J. G. Coutinho <sup>c</sup>, Maria João Sousa <sup>b</sup>  
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R	<i>n</i>	MIC (μM)
H	1	12.5
CH <sub>2</sub> CH <sub>3</sub>	1	6.25
(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	2	1.56

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# Synthesis and photophysical studies of new benzo[*a*]phenoxazinium chlorides as antifungal agents

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**Abstract:** A set of four new benzo[*a*]phenoxazinium chlorides possessing ethyl, propyl, decyl and tetradecyl groups at the 9-amino function of the heterocycle along with a propyl group at the 5-amino position was efficiently synthesised. These compounds displayed fluorescence with maximum emission wavelengths of 673 and 685 nm, in anhydrous ethanol and water. All the benzo[*a*]phenoxazines were evaluated against the yeast *Saccharomyces cerevisiae* in a broth microdilution assay. It was found that their antifungal activity depended on the variation in the lengths of the aliphatic chains. The highest MIC activity of 1.56  $\mu$ M was obtained for compound **7** comprising a di-alkylated propyl substituent at 9-amino position and a propyl chain at the 5-amino position of the heterocycle core.

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**Keywords:** benzo[*a*]phenoxazinium chloride; antimicrobial drugs; *Saccharomyces cerevisiae*; Nile Blue derivatives; NIR probes.

## Introduction

Small organic molecules function as fluorescent probes for monitoring and quantification in chemical and bioanalytical sciences,<sup>1,2</sup> being chromophores with emission in red or NIR possessing good photochemical stability and water solubility of special interest for *in vivo* and *in vitro* studies.<sup>3,4</sup> Benzo[*a*]phenoxazine dyes display good photostability, high molar absorption, strong fluorescence in the NIR region and modest Stokes shifts (20-60 nm) indicating the potential choice of these dyes as fluorophores for biological applications.<sup>5</sup> Furthermore, non-covalent bonding is a common feature of benzo[*a*]phenoxazinium dyes that enables their use for staining purposes in gel electrophoresis and study of their interactions (intercalative and/or groove binding) with DNA.<sup>6</sup>

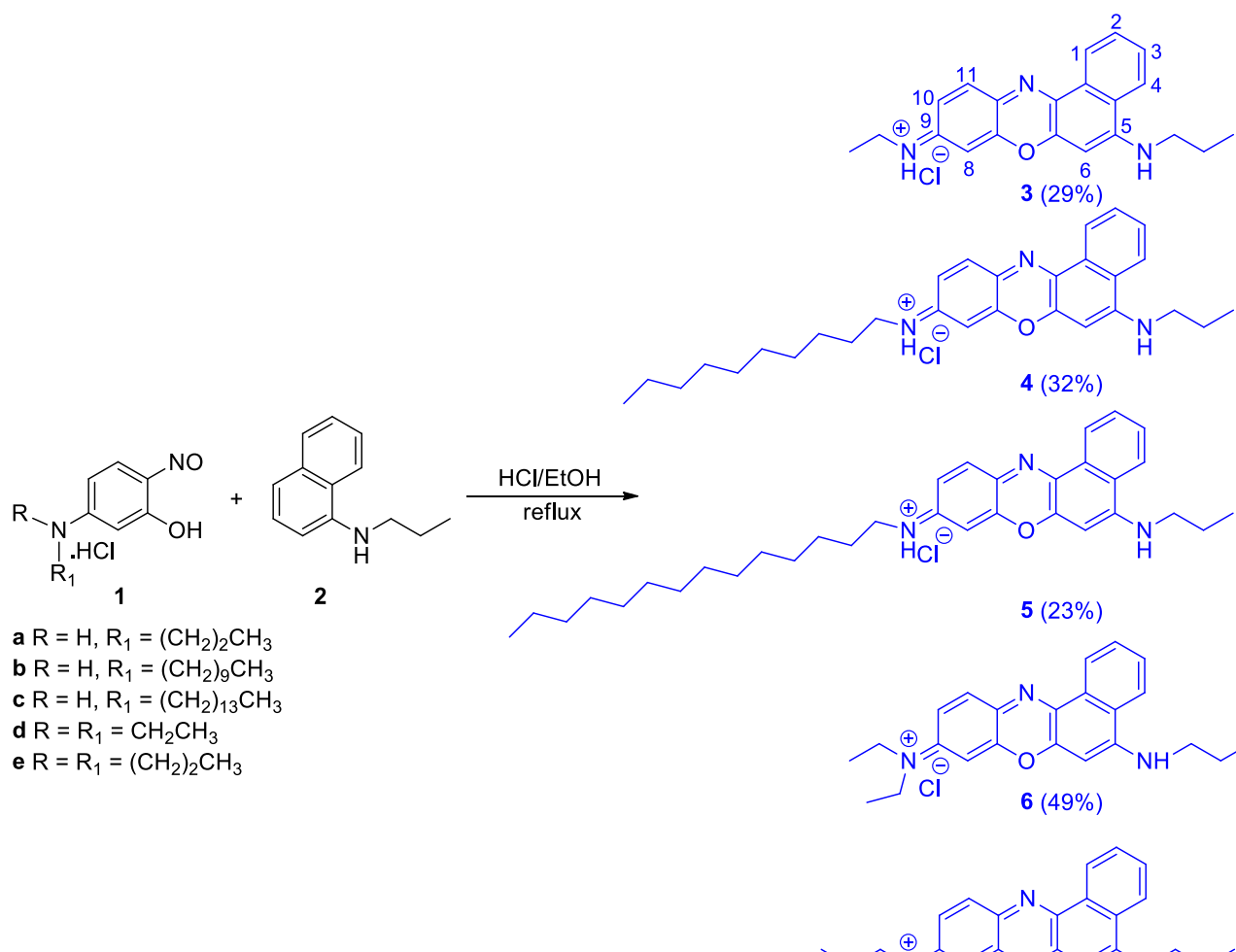
These heterocycles has also been reported as lysosome trackers,<sup>7</sup> sensors for reversible pH measurements and NIR live bioimaging purposes.<sup>8</sup>

In addition, benzo[*a*]phenoxazine derivatives are important as antifungals,<sup>9</sup> antimalarials<sup>10</sup> and also function as photosensitizers in photodynamic therapy (PDT) for treatment of *Candida albicans* biofilms.<sup>11</sup> Compounds possessing amino groups as their ring substituents can also be functionalized according to various target analytes.<sup>12</sup> In our earlier communication, it was reported that naphtho[2,3-*a*]phenoxazinium and benzo[*a*]phenoxazinium chlorides exhibited antifungal activity that depended on the substituents of the heterocycle.<sup>9,13</sup> In continuation of our research towards fluorescent heterocycles,<sup>14-16</sup> the present communication describes the synthesis, characterization, and preliminary biological studies of a new class of benzo[*a*]phenoxazinium chlorides which possess different length of aliphatic alkyl chains.

Photophysical studies were carried out in anhydrous ethanol and aqueous medium. The antifungal activity of these compounds was determined by using the yeast *Saccharomyces cerevisiae* as a model organism. Comparing the MIC values of all the analogues revealed that the compounds with a di-substitution at 9-amino position and a propyl group at 5-amino position of the benzo[*a*]phenoxazines exhibited the best activities.

## Results and discussion

The 3-(alkylamino)phenols were obtained by the reaction of 3-aminophenol (**S-1**) with appropriate iodo or bromo alkyl halides such as iodoethane, 1-bromopropane, 1-bromododecane and 1-bromotetradecane in ethanol under reflux conditions (Scheme S1). The reaction afforded two products, among which the mono *N*-alkylated product was isolated as the major fraction, namely 3-(ethylamino)phenol (**S-2a**), 3-(diethylamino)phenol (**S-2b**), 3-(propylamino)phenol (**S-3a**), 3-(dipropylamino)phenol (**S-3b**), 3-(decylamino)phenol (**S-4a**), 3-(didecylamino)phenol (**S-4b**), 3-(tetradecylamino)phenol (**S-5a**) and 3-(ditetradecylamino)phenol (**S-5b**) in good yields.<sup>20</sup> Nitrosophenol precursors **1a-e** were obtained by the nitrosation of the required *N*-alkylated derivative (**S-3a**, **S-4a**, **S-5a**, **S-2b** and **S-3b**, respectively) in an acidic solution of sodium nitrite under ice cold conditions (Scheme S2).<sup>20,21</sup> The other reactant *N*-propylnaphthalen-1-amine **2** was prepared by the *N*-alkylation of naphthalen-1-amine with bromopropane.<sup>22</sup> Benzo[*a*]phenoxazinium chlorides **3-7** were synthesised by the condensation of *N*-alkylated nitroso derivatives **1a-e** with *N*-propylnaphthalen-1-amine **2** in the presence of hydrochloric acid, refluxed in ethanol.<sup>23</sup>



**Scheme 1.** Synthesis of benzo[*a*]phenoxazininium chlorides **3-7**.

After column chromatography purification pure compounds were acquired as blue solids and characterized by high resolution mass spectrometry, IR and NMR (<sup>1</sup>H and <sup>13</sup>C) spectroscopies. <sup>1</sup>H NMR spectra of compounds **3-7** showed triplets ( $\delta$  0.87-1.38 ppm) for the terminal methyl groups of aliphatic chains at 5- and 9-positions. The methylene group adjacent to methyl exhibits quartet, multiplet or broad singlet ( $\delta$  1.75-1.98 ppm) and the methylene group directly attached to the nitrogen atom shows triplet, multiplet or broad singlet ( $\delta$  3.06-3.79 ppm). The aromatic protons 11-H ( $\delta$  7.34-7.84 ppm), 4-H ( $\delta$  8.31-8.65 ppm) and 1-H ( $\delta$  8.79-8.89 ppm) appeared as doublets or multiplets. <sup>13</sup>C NMR spectra of these compounds showed signals of the aliphatic carbons from the methyl group ( $\delta$  11.45-14.08 ppm) and methylene carbons ( $\delta$  21.81-54.56 ppm) of the substituents of 5- and 9-positions. The aromatic signals such as C-11 ( $\delta$  131.75-134.10 ppm), C-4 ( $\delta$  123.34-123.86 ppm) and C-1 ( $\delta$  125.29-125.59 ppm) were shown in the spectra.

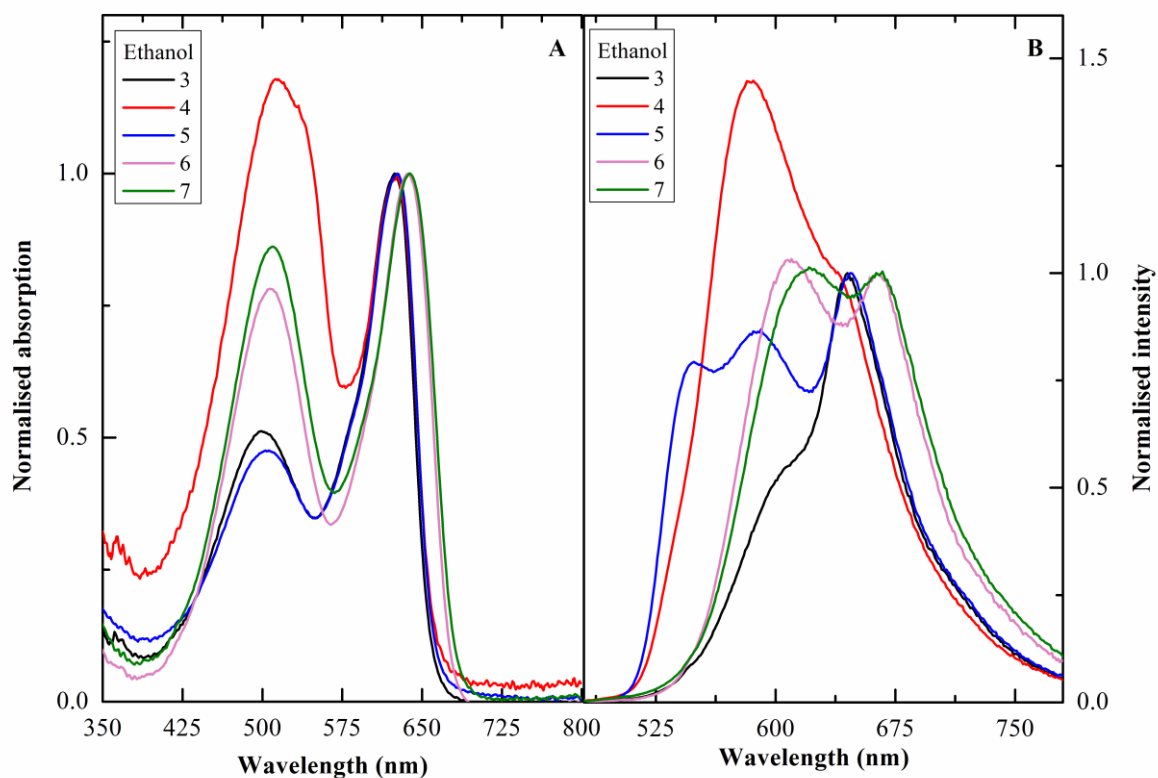
Electronic absorption and emission spectra of  $4 \times 10^{-6}$  M solutions in acidic anhydrous ethanol and water were measured for the synthesised benzo[*a*]phenoxazinium chlorides (Table 1). The relative fluorescence quantum yields ( $\Phi_F$ ) were determined using Oxazine 1 as a standard ( $\Phi_F = 0.11$  in ethanol)<sup>24,25</sup> at 575 nm excitation. In acidified ethanol and water, the absorption maxima ( $\lambda_{\text{abs}}$ ) for compounds **3-7** lie in the range of 568-645 nm, and molar extinction coefficients ( $\epsilon$ ) between 24225 and 96350  $\text{M}^{-1}\text{cm}^{-1}$ . The emission maxima ( $\lambda_{\text{em}}$ ) was found to be in the range of 633-685 nm at excitation of 575 nm with moderate to high Stokes shifts ( $\Delta\lambda$ , 10-65 nm).

**Table 1.** Photophysical data of compounds **3-7** in anhydrous ethanol and aqueous solution ( $C = 4 \times 10^{-6}$  M) acidified with trifluoroacetic acid (TFA).

Cpd	Anhydrous ethanol acidified with TFA					Water acidified with TFA				
	$\lambda_{\text{abs}}^{\text{a}}$	$\epsilon^{\text{b}}$	$\lambda_{\text{em}}^{\text{a}}$	$\Delta\lambda^{\text{a}}$	$\Phi_F$	$\lambda_{\text{abs}}^{\text{a}}$	$\epsilon^{\text{b}}$	$\lambda_{\text{em}}^{\text{a}}$	$\Delta\lambda^{\text{a}}$	$\Phi_F$
<b>3</b>	623	69250	645	22	0.28	617	35600	652	35	0.17
<b>4</b>	624	37025	647	23	0.53	625	31650	635	10	0.003
<b>5</b>	627	74350	649	22	0.51	568	24225	633	65	0.007
<b>6</b>	637	96350	670	33	0.11	642	36325	681	39	0.05
<b>7</b>	639	93125	673	34	0.28	645	40075	685	40	0.04

<sup>a</sup>Unit: nm; <sup>b</sup>Unit:  $\text{M}^{-1}\text{cm}^{-1}$

It can be seen that compounds **4** and **5** upon elongation of the lateral chain at 9-amino position almost do not alter the wavelengths of absorption and fluorescence maxima in anhydrous ethanol and water media. However, comparing the fluorophore **3** with **6**, the latter shows greatest values of  $\lambda_{\text{em}}$  (670 nm) in anhydrous ethanol and  $\lambda_{\text{em}}$  (681 nm) in water, which could be mainly due to the di-alkylation at 9-amino position of the benzo[*a*]phenoxazinium dye.<sup>13,14</sup> The same behaviour was observed for compound **7**. Similarly, compounds **4** and **5** possessing decyl and tetradecyl groups at 9-amino position showed the highest fluorescence efficiency in ethanolic media, while in water the compound **3** with an ethyl group at 9-amino position is the one with highest quantum yield. This was expected to be related to the higher hydrophobicity of compounds **4** and **5**.

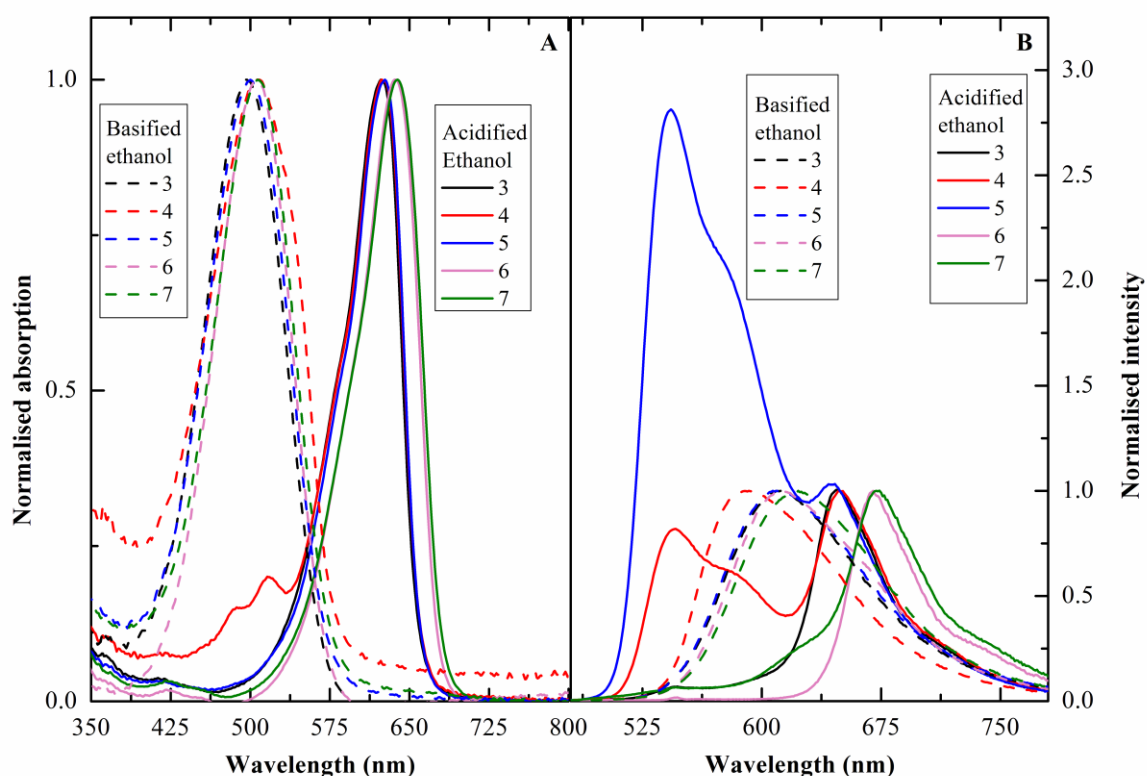


**Figure 1.** Normalised absorption and emission spectra of compounds **3-7** in anhydrous ethanol.

Figure 1 shows the absorption and emission spectra at 470 nm excitation of the compounds **3-7** in anhydrous ethanol. It can be seen in the absorption spectra (panel A) that benzo[*a*]phenoxazinium chlorides exhibited both the acidic ( $\text{BzH}^+$ ) and neutral form (Bz) in ethanol media, corresponding to the second and first bands of the absorption spectrum, as previously observed with similar type of compounds.<sup>16,26,27</sup> With an exception of compound **4**, the studied dyes show a higher fraction of acidic form in ethanolic solution. It has been shown previously that the fluorescence maximum of neutral form occurs near 600 nm while the acid form occurs in the range 640-680 nm and the quantum yield of the neutral form is 10-fold lower than the one corresponding to the acid form.<sup>26,27</sup> In dry ethanol medium, at 470 nm excitation, the basic form is mostly excited with a small fraction of acidic form depending on the 9-amino position. Nevertheless, the acid form emission has comparable fluorescence intensity due to its higher quantum yield. The neutral form emission is slightly blue shifted for compounds **4** and **5** appearing at 580 nm. In addition, another band appears at 540 nm for compounds **4** and **5** as seen in Figure 1

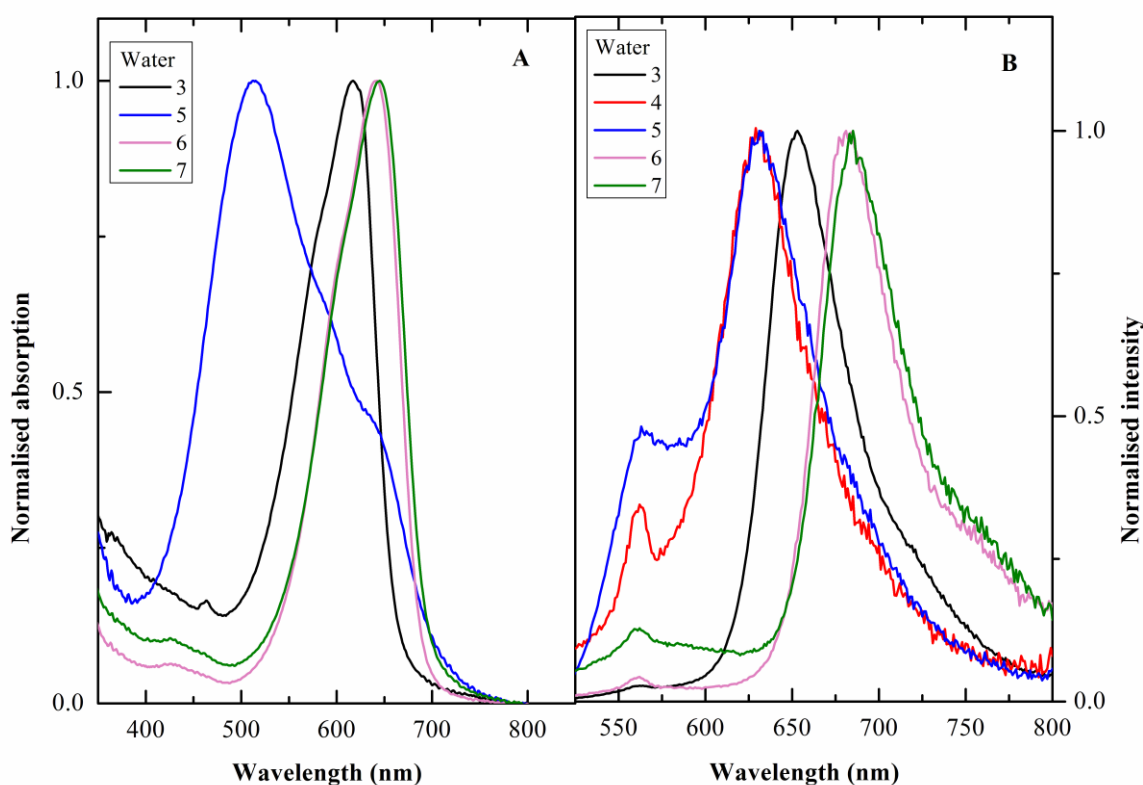
(B). This band has been proposed and supported by *ab initio* calculations as arising from tautomers with localized positive charge and a slight loss of resonance among the  $\pi$ -electron system.<sup>16</sup>

Figure 2 shows the absorption spectra of compounds **3-7** under acidic and basic conditions in anhydrous ethanol. It can be seen that displacement of acid-base equilibrium can be done by the addition of small amounts of acid (TFA) or base (triethylammonium hydroxide, TEAH) to the benzo[*a*]phenoxazine solutions. Upon addition of acid to the ethanolic solution of the fluorophore, except for compound **4**, it was possible to completely shift the equilibrium towards the acidic form as shown in Figure 2 (A). Similarly, compound **4** shows an additional absorption band around 500 nm indicating a high percentage of tautomerization. The emission spectra at 470 nm excitation show bands around 645-673 nm that correspond to the acidic form emission and correspond to the higher quantum yields as observed for similar type of compounds.<sup>16,26,27</sup> The peaks around 540 nm observed for compounds **4** and **5** significantly increased indicating a higher fraction of tautomeric form, which was obtained upon acidification.



**Figure 2.** Normalised absorption and emission spectra of compounds **3-7** in anhydrous ethanol under acidic and basic conditions.

Figure 3 shows the absorption and emission spectra at 470 nm excitation of the compounds **3-7** in aqueous media. Compound **5** shows an enormous hypsochromic shift of  $\sim 100$  nm in  $\lambda_{\text{abs}}$  in the comparison with other dyes. Comparing with Figure 2, it seems that a long chain in the 9-amino position promotes the presence of the neutral form in aqueous media. In the emission spectra, except compound **3**, all the other fluorophores exhibited a small bump around 560 nm showing small amounts of tautomer emission Figure 3(B). When excited at 470 nm, compounds **3**, **6** and **7** only show emission on the acid form while compounds **4** and **5** mainly show neutral form emission red-shifted in comparison to ethanol media (see Figures 1B and 2B).



**Figure 3.** Normalised absorption and emission spectra of compounds **3-7** in water.

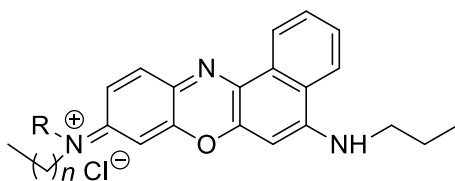
The potential antifungal activity of the synthesised fluorophores **3-7** was investigated using the yeast *Saccharomyces cerevisiae* PYCC 4072 as a model organism and a broth microdilution method for antifungal activity testing.<sup>28,29</sup> The minimum inhibitory concentration of growth (MIC) and log *P* values, which are theoretically predicted<sup>30</sup> are shown in Table 2. Compounds with lower log *P* values are hydrophilic, showing higher tendency towards the intracellular environment which is aqueous in nature. In contrast, these molecules possess lower affinity for the cell membranes. As



it can be seen from the data presented (Table 2), there is a general correlation between the hydrophilicity of the compounds and their antifungal properties. However, this relation is not strict since compound **6** has the lowest log *P* value (1.446) and MIC value of 6.25 μM, which is two-fold higher than that of compound **3** with a log *P* of 1.888. Compound **5** with the highest log *P* value and being highly lipophilic showed virtually no activity.

The antifungal activity of the benzo[*a*]phenoxazinium dyes was evaluated with a propylamino group as substituent at 5-position and varying the length and number of alkyl chain at the amine function of the 9-position. The study showed that the inhibitory properties of the compounds are dependent on both these changes at the 9-amino position. For instance, compounds **4** and **5**, with one alkyl chain of 10 and 14 carbon atoms, respectively, showed reduced antifungal activities probably due to their low water solubility. The introduction of a second ethyl group at 9-amino position in compound **3** resulted in **6** with a better antifungal activity and, moreover, the presence of *N*-propyl groups instead of *N*-ethyl resulted in a 4-times increase of activity, 1.56 μM being the lowest MIC value observed. The increase in activity with increase of the alkyl chain size of the substituents at 9-amino position was also observed by replacing a di-methyl by a di-ethyl groups in benzo[*a*]phenoxazines with different substituents or even no substituent at 5-amino position.<sup>29</sup> In the present communication we obtained one benzo[*a*]phenoxazine with even higher activity by further increasing the length of the 9-amino substituents by one carbon. Hence, the results indicate there is an optimal medium length for these substituents and that compound **7** possessing a di-propyl group at 9-amino position and the propylamino at the 5-position of the polycyclic ring is the best candidate for antifungal activity and for further development of compounds with improved activity.

**Table 2.** Activity against *Saccharomyces cerevisiae* PYCC 4072 and log *P* values of the benzo[*a*]phenoxazinium chlorides **3-7**.



Compound			MIC <sup>a,b</sup>	log <i>P</i>
	R	<i>n</i>		
<b>3</b>	H	1	12.5	1.888
<b>4</b>	H	9	25	6.484
<b>5</b>	H	13	>200 <sup>c</sup>	8.408
<b>6</b>	CH <sub>2</sub> CH <sub>3</sub>	1	6.25	1.446
<b>7</b>	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	2	1.56	2.954

<sup>a</sup>Minimal inhibitory concentration of growth. <sup>b</sup>Experiments were performed in triplicate and at least two independent experiments were conducted. <sup>c</sup>Insoluble at the highest concentration tested – 400 μM (1% DMSO).

## Acknowledgments

Thanks are due to the *Fundação para a Ciência e Tecnologia* (FCT, Portugal) for financial support to the NMR portuguese 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 CFUM [PEst-C/FIS/UI0607/2011 (F-COMP-01-0124-FEDER-022711)] and CQ/UM [PEst-C/QUI/UI0686/2011 (FCOMP-01-0124-FEDER-022716)]. A post-doctoral grant to B. R. Raju (SFRH/BPD/62881/2009) is also acknowledged to *FCT*, *POPH-QREN*, *FSE*.

This work was supported by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through the COMPETE2020 - *Programa Operacional Competitividade e Internacionalização* (POCI).

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20. *Typical procedure for preparation of nitroso precursors Ia-e (described for Ie)*: To an ice-cold solution of 3-(dipropylamino)phenol **S-3b** (0.193 g, 1 mmol) in ethanol (2 mL), 2M hydrochloric acid (0.4 mL) was added and stirred during 5 min. The solution of sodium nitrite (0.083 g, 1.2 mmol) in water (0.3 mL) was then added dropwise within an interval of 10-15 min. The resulting

mixture was stirred for 3 h and monitored by TLC (dichloromethane/methanol, 9.5:0.5). After evaporation of the reaction, 2-nitroso-5-(dipropylamino)phenol hydrochloride **1e** was obtained as green solid (0.258 g), and was used in the following step without any purification.

3-(Dipropylamino)phenol **S-3b** resulted from the reaction of a suspension of 3-aminophenol (2.0 g, 18.3 mmol) in ethanol (8 mL) and 1-bromopropane (2.50 mL, 27.5 mmol), at reflux temperature during 4h 30 min, followed by TLC (dichloromethane/methanol 9.5:0.5). After solvent evaporation and chromatography with dichloromethane and dichloromethane/methanol, mixtures of increasing polarity, as the eluent, 3-(dipropylamino)phenol **S-3b** was obtained as a brown solid (0.715 g, 21%). Mp = 99.7-100.3 °C. TLC (dichloromethane):  $R_f$  = 0.22. FTIR (KBr 1%):  $\nu_{\max}$  3208, 3188, 3053, 2966, 2880, 2711, 2672, 2636, 2508, 1613, 1508, 1488, 1471, 1456, 1431, 1417  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.84 (6 H, t  $J$  = 7.6 Hz,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 1.59 (4 H, br s,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 3.30 (4 H, t  $J$  8.0 Hz,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 6.87 (br s, 2 H, 4-H, 6-H), 7.05 (1 H, br s, 2-H), 7.17 (1 H, t  $J$  8.0 Hz, 5-H) ppm.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100.6 MHz):  $\delta$  10.85 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 18.86 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 58.17 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 107.61 (C-2), 113.60 (C-4, C-6), 130.63 (C-5), 137.52 (C-3), 158.22 (C-1) ppm. HRMS:  $m/z$  (TOF EI): calcd for  $\text{C}_{12}\text{H}_{19}\text{NO}$  [ $\text{M}^+$ ] 193.1467; found 193.1474.

In the same reaction, 3-(propylamino)phenol **S-3a** was also isolated as brown liquid (1.410 g, 51%), TLC (dichloromethane):  $R_f$  = 0.17. FTIR (KBr 1%):  $\nu_{\max}$  3250, 2642, 2500, 2415, 1619, 1577, 1487, 1426  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.05 (3 H, t  $J$  7.2 Hz,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 1.75-1.86 (2 H, m,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 3.35-3.46 (2 H, m,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 6.92-7.01 (3 H, m, 4-H, 6-H, 2-H), 7.35-7.42 (1 H, m, 5-H) ppm.  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100.6 MHz):  $\delta$  11.14 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 20.37 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 54.75 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 110.58 (C-2), 114.09 (C-6), 117.63 (C-4), 132.03 (C-5), 137.52 (C-3), 160.19 (C-1). HRMS:  $m/z$  (TOF EI): calcd for  $\text{C}_9\text{H}_{13}\text{NO}$  [ $\text{M}^+$ ] 151.0997; found 151.1003.

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23. *General procedure for synthesis of benzo[a]phenoxazines 3-7 (described for 7)*: To a cold solution (ice bath) of 2-nitroso-5-(dipropylamino)phenol hydrochloride **1e** (0.258 g, 1.0 mmol) in ethanol (2 mL), *N*-propylnaphthalen-1-amine **2** (0.185 g, 1.0 mmol) and concentrated hydrochloride acid (0.027 mL) were added. The reaction mixture was refluxed for 12 h and monitored by TLC (dichloromethane/methanol 9.5:0.5). After evaporation of the solvent and column chromatography purification 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 **7** was obtained as a blue solid (0.307 g, 73%).  $R_f = 0.19$  (chloroform/methanol, 9.4:0.6). Mp = 240-242 °C. FTIR (KBr 1%):  $\nu_{\max}$  3392, 3175, 3051, 2967, 2931, 2872, 1640, 1588, 1547, 1495, 1452, 1435  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  1.08 (6 H, t  $J$  7.6 Hz,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 1.14 (3 H, t  $J$  7.2 Hz,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 1.75-1.85 (4 H, m,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 1.86-1.96 (2 H, m,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 3.61 (4 H, t  $J$  8.0 Hz,  $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 3.67 (2 H, t  $J$  7.2 Hz,  $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 6.81 (1 H, d  $J$  2.0 Hz, 8-H), 6.88 (1 H, s, 6-H), 7.17-7.27 (1 H, m, 10-H), 7.73-7.83 (2 H, m, 11-H, 3-H), 7.88 (1 H, t  $J$  7.6 Hz, 2-H), 8.31 (1 H, d  $J$  8.0 Hz, 4-H), 8.79 (1 H, d  $J$  8.0 Hz, 1-H) ppm.  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100.6 MHz):  $\delta$  11.45 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 11.77 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 21.81 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 23.08 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 47.54 ( $\text{NHCH}_2\text{CH}_2\text{CH}_3$ ), 54.56 ( $\text{N}(\text{CH}_2\text{CH}_2\text{CH}_3)_2$ ), 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. HRMS:  $m/z$  (FAB): calcd. for  $\text{C}_{25}\text{H}_{30}\text{N}_3\text{O}$  [ $\text{M}^+$ ] 388.23774; found 388.23834.

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25. 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.

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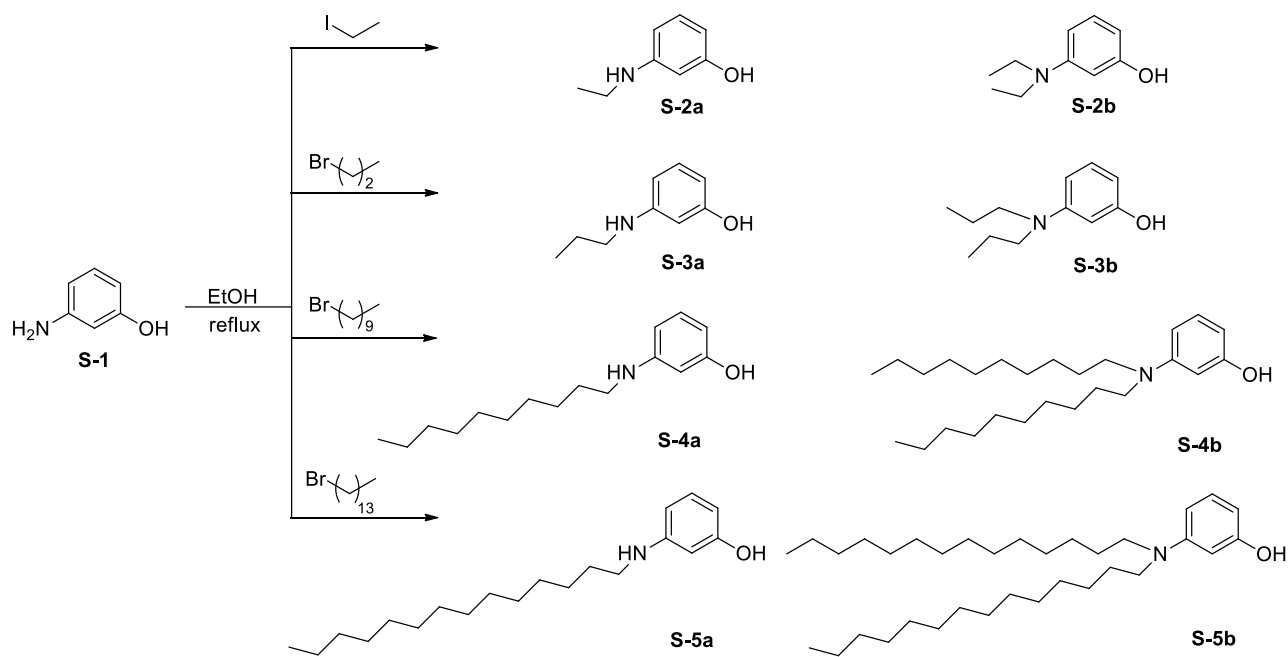
28. Minimum Inhibitory Concentrations of growth (MIC) were assessed using a broth microdilution method for antifungal susceptibility testing of yeasts (NCCLS M27-A). The yeast *Saccharomyces cerevisiae* PYCC 4072 was used as a model organism. Briefly, cells were cultivated in 96-microwell plates in RPMI 1640 medium, buffered to pH 7.0 with 0.165 M morpholenepropanesulfonic acid (MOPS) buffer (Sigma). Initial cell concentration was  $0.5 \times 10^3$  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 a 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  $\mu\text{M}$  to bellow the

MIC value, using a two-fold dilution scheme) was tested in triplicate and in at least two independent experiments.

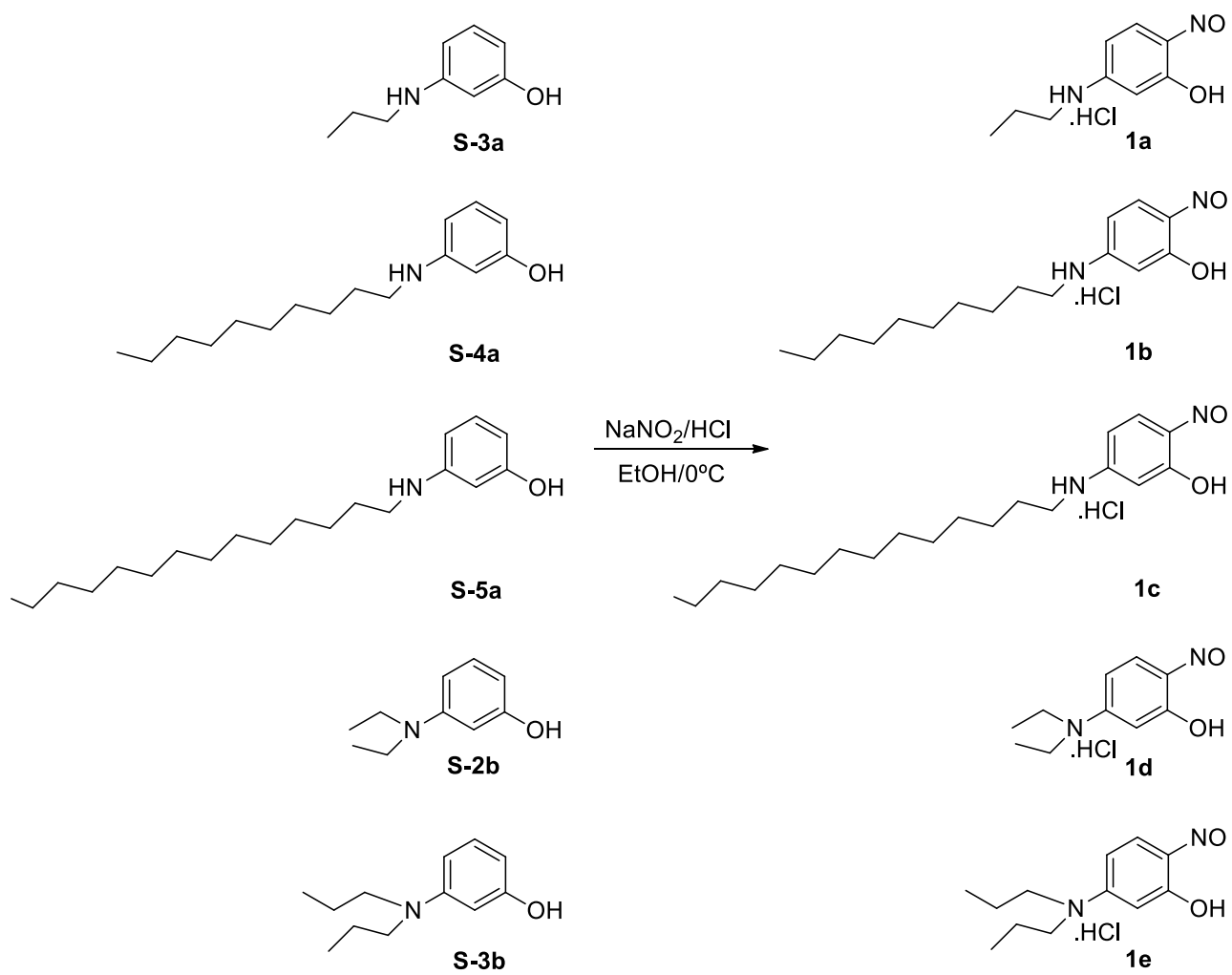
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30. Calculation of molecular properties and drug-likeness-Molinspiration cheminformatics software tools (<http://www.molinspiration.com>).

## Supporting Information



**Scheme S1.** Synthesis of derivatives S-2 to S-5.



**Scheme S2.** Synthesis of nitrosophenols **1a-e**.