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# Synthesis of naphtho[2,3-*a*]phenoxazinium chlorides: Structure–activity relationships of these heterocycles and benzo[*a*]phenoxazinium chlorides as new antimicrobials

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**Abstract**—Synthesised functionalised naphtho[2,3-*a*]phenoxazinium chlorides revealed great fluorescence with maximum emission wavelengths between 630 and 676 nm, in ethanol and water at physiological pH. Naphtho[2,3-*a*]phenoxazines, as well as a series of benzo[*a*]phenoxazines, were evaluated against *Saccharomyces cerevisiae*, in a broth microdilution assay. This family of compounds exhibited antifungal activity depending both on the substituents of the heterocycle nucleus as well as on its size. The best activities were obtained for four-ring systems, and particularly for 5,9-diaminobenzo[*a*]phenoxazines with R = Me, $R^1$ = H and $R^2$ = Et. As for $R^3$ substitution, the greatest efficiency was obtained for $R^3 = (CH_2)_3Cl$, with a MIC of 3.75 μM. The linkage of different amino acids to the functional group of the 5-amino position of diaminobenzo[*a*]phenoxazinium moiety resulted in compounds with diverse antimicrobial efficiencies, depending on the polar character of the amino acid, on its linkage position and on the size of the alkyl chain linker.


## 1. Introduction

Oxazine dyes have been a subject of much spectroscopic research due to their great use as a tunable laser dye in the range 600–900 nm. Alternatively, they are also standards for fluorescence studies and are of significant value as biological probes.[1,2] Applications of these long-wavelength fluorophores, namely Nile Blue and its derivatives, include the covalent labelling of model carboxylic acids,[3] amino acids,[4] proteins,[5,6] peptides and DNA.[7]

On the other hand, the use of benzo[*a*]phenoxazines in non-covalent labelling is more common.[8] These heterocycles are valuable for staining nucleic acids in a variety of contexts, including in solutions, in electrophoretic gels or other matrices, in blotting experiments and in assays employing intact, live cells.[9] Furthermore, different modifications of the phenoxazine moiety can yield compounds that directly bind to proteins, mainly through hydrophobic interaction, and which have been developed either for the use in monitoring protein conformation alterations or for therapeutic purposes.[10–13]

Although a large number of studies have focused on the photophysical properties, molecules possessing the phenoxazine counterparts have also assumed increasing importance in life sciences, due to their antiproliferative properties with potential applications both as antitumour and as antimicrobial agents.[14–20] These compounds can exert their antiproliferative activity through the DNA intercalative binding of their large planar polycycles; the stabilization of this binding arises both from the hydrogen bonds and the π–π stacking interactions.[17,21] In addition, the phenoxazine ring system can produce free radical intermediates leading to oxidative stress.[16] Some phenoxazine derivatives have also been shown to display apoptotic activity against different cell lines, both in a caspase-dependent and independent manner.[14,22]

*Keywords*: Naphtho[2,3-*a*]phenoxazinium salts; Benzo[*a*]phenoxazines; Antifungal activity; Long-wavelength fluorescent dyes; Functionalised probes.
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In spite of the interest of the abovementioned phenoxazine dyes, the synthesis and application of compounds starting with 1-aminoanthracene or its derivatives is scarce in the literature.[9]

Considering these facts, in addition to our interest in the synthesis and application of heterocycle fluorescent reagents,[4,23,24] we have synthesised new naphtho[2,3-*a*]phenoxazine derivatives which possess an extended aromatic system. Photophysical studies were carried out in ethanol and water at physiological pH and the results were compared with those of benzo[*a*]phenoxazine analogues.

Using *Saccharomyces cerevisiae* as a model organism, we analysed the antifungal activity of the naphtho[2,3-*a*]phenoxazinium chlorides obtained in the present work. The MIC values were then compared with those of a series of benzo[*a*]phenoxazinium chloride analogues, exploring the effect of alterations in the extent of the aromatic system, the substitution of both the amino groups and the 10-position of the benzo[*a*]phenoxazinium nucleus, as well as the linkage to amino acids.

## 2. Chemistry

Naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**e** were prepared by condensation of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **2** with 1-aminoanthracene or its *N*-substituted-derivatives **3b**–**e** in the presence of hydrochloric acid, refluxed in ethanol (Scheme 1). The required nitrosophenol **2** was synthesised using the usual procedure involving treatment of the corresponding 3-ethylamino-4-methylphenol with sodium nitrite in an acid solution.[25] Compounds **3b**–**e** were prepared by alkylation of 1-aminoanthracene with the appropriate bromo-reagent. After dry chromatography purification (**3b**, **3d** and **3e**; **3c** was not purified), these compounds were obtained as oils (21%, **3b**; 53%, **3d**; 50%, **3e**) and were characterised by high resolution mass spectrometry, IR and NMR ($^1$H and $^{13}$C) spectroscopy.

The IR spectra showed strong stretching vibration bands for the hydroxyl and the amino groups at 3434 (**3d**) and 3398 cm$^{-1}$ (**3e**). In the $^1$H and $^{13}$C NMR, relevant signals were assigned to the *N*-alkyl substituents in the regions of 1.15–3.99 ppm and 11.70–69.06 ppm, respectively.

Preparation of chloropropylamino derivative **1f** was achieved by reaction of compound **1d** with thionyl chloride, at room temperature. After purification by dry chromatography or successive washes with solvent (**1e**), cationic dyes **1a**–**f** were isolated as solid materials in moderate to excellent yields (Table 1) and were fully characterised by the usual analytical techniques.

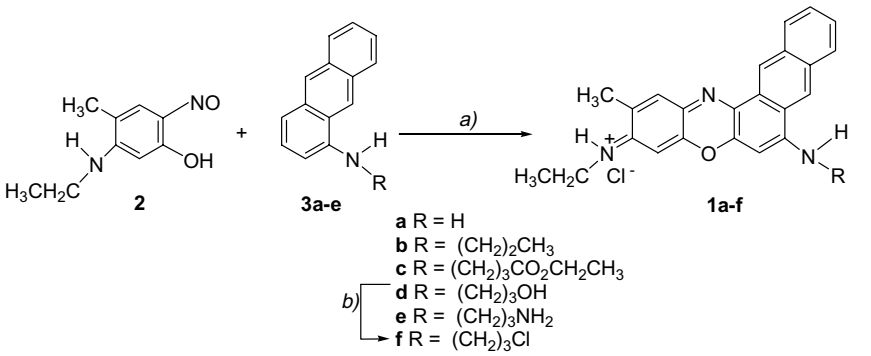
The IR spectra bands at 1724, 3406 and 3197–3446 cm$^{-1}$ were assigned to the ester (**1c**), the hydroxyl (**1d**) and the amino (**1a** and **1e**) groups, respectively. The $^{13}$C NMR of compound **1c** showed a signal at $\delta$ 174.77 ppm which also confirmed the presence of the carbonyl group of the ester function.

Electronic absorption spectra of $10^{-6}$ M solutions of naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**f** in degassed absolute ethanol were measured (Table 1). The longest wavelength of maximum absorption ($\lambda_{max}$) of all compounds was located between 615 and 647 nm. When compared to **1a** (R = H), compounds **1b**–**f** absorbed at longer wavelengths; the bathochromic shift was 25–32 nm. This fact showed the importance of a substituent at the amine group in the 7-position of the polycyclic system in the absorbance properties of these compounds.

**Table 1.** Synthesis and visible data of naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**f**, in ethanol and water (pH 7.4)

| Compound | Yield (%) | $\lambda_{max}$ [nm] ($\varepsilon$) (ethanol) | $\lambda_{max}$ [nm] ($\varepsilon$) (water, pH 7.4) |
|---|---|---|---|
| **1a** | 98 | 615 (62,112) | 615 (46,296) |
| **1b** | 43 | 642 (26,100) | 601 (22,000) |
| **1c** | 16[a] | 647 (19,595) | —[b] |
| **1d** | 61 | 640 (35,000) | 640 (35,000) |
| **1e** | 98 | 645 (14,781) | —[b] |
| **1f** | 50 | 645 (43,300) | —[b] |

[a] Overall yield of the two steps.
[b] Compound not soluble in water (pH 7.4).




**Scheme 1.** Synthesis of naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**f**. Reagents and conditions: (a) H$^+$/ethanol, reflux; (b) $SOCl_2$, dichloromethane/chloroform 2:1, rt.

Comparison of $\lambda_{max}$ of the naphtho[2,3-*a*]phenoxazinium chlorides **1a** and **1c**–**f** synthesised with that of the benzo[*a*]phenoxazinium chloride analogues in ethanol[4,26] showed that fluorophores **1c**–**f** presented the highest values ($\lambda_{max}$ of compound **1a** was slightly lower than that of the benzo[*a*]phenoxazinium analogue, 615/620 nm), the red shift being 25–32 nm. These results seem to be related to the increase of the electronic conjugation resulting from the addition of another aromatic ring to the polycyclic benzo[*a*]phenoxazine nucleus.

The absorption properties of heterocycles **1a**, **1b** and **1d** were also measured in water at physiological pH (pH 7.4, adjusted with HCl and NaOH) (**1c**, **1e** and **1f** were not soluble). Comparison of $\lambda_{max}$ values in ethanol and water showed both no variation (**1a** and **1d**) or a blue shift of 41 nm (**1b**). Figure 1 shows the normalised absorption spectra of compounds **1a**, **1c** and **1d**, in ethanol.

Studies of the fluorescent properties of compounds **1a**–**f** were also carried out in ethanol and water (pH 7.4) (**1a**, **1b** and **1d**). Summarised data are presented in Table 2. The fluorescence quantum yields ($\Phi_F$) were calculated using Oxazine 1 as a standard ($\Phi_F = 0.11$ in ethanol).[27] For the determination of the relative quantum yields, Oxazine 1 was excited at the wavelengths of excitation of each one of the compounds tested. Emission maxima ($\lambda_{em}$) for all compounds in ethanol and water varied from 630 to 676 nm. When compared to **1a** (R = H), compounds **1b**–**f** emitted at longer wavelengths (except compound **1d**, in water), the bathochromic shift was 37–44 nm in both solvents, which might be related to the presence of the *N*-substituents, which is the case of absorbance.

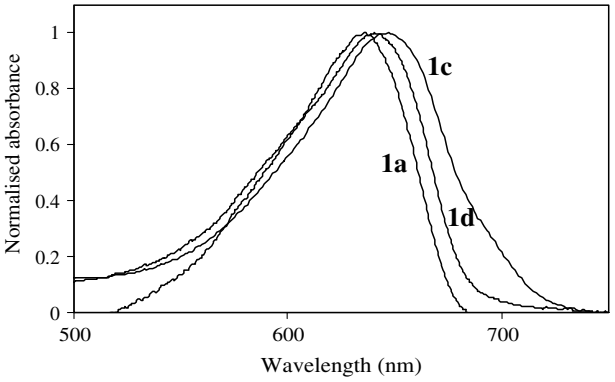



**Figure 1.** Normalised absorbance spectra of compounds **1a**, **1c** and **1d**, in ethanol.

**Table 2.** Fluorescence data of naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**f**, in ethanol and water (pH 7.4)

| Compound | Ethanol | | | | Water (pH 7.4) | | | |
|---|---|---|---|---|---|---|---|---|
| | $\lambda_{exc}$ | $\lambda_{em}$ | $\Phi_F$ | Stokes's shift | $\lambda_{exc}$ | $\lambda_{em}$ | $\Phi_F$ | Stokes's shift |
| **1a** | 590 | 634 | 0.34 | 19 | 590 | 632 | 0.47 | 17 |
| **1b** | 590 | 671 | 0.29 | 29 | 590 | 676 | 0.10 | 34 |
| **1c** | 585 | 674 | 0.20 | 27 | —[a] | — | — | — |
| **1d** | 600 | 672 | 0.37 | 32 | 600 | 630 | 0.10 | 10 |
| **1e** | 575 | 674 | 0.24 | 29 | —[a] | — | — | — |
| **1f** | 590 | 671 | 0.26 | 26 | —[a] | — | — | — |

[a] Compounds not soluble in water (pH 7.4).

Comparison of $\lambda_{em}$ of synthesised naphtho[2,3-*a*]phenoxazinium chlorides **1a**, **1c**–**f** with that of the benzo[*a*]phenoxazinium chloride analogues in ethanol[4,26] showed that the naphtho[2,3-*a*]phenoxazines presented the highest values (except in the case of compound **1a**, which was slightly lower than the benzo[*a*]phenoxazinium analogue, 634/637 nm), the red shift being from 26 to 30 nm. These results seem to be related to an increase in the electronic conjugation resulting from the addition of another aromatic ring in the polycyclic benzo[*a*]phenoxazine nucleus, as in the case of absorbance variation.

All compounds exhibit high levels of fluorescence in ethanol and water (pH 7.4), with quantum yields between 0.10 (**1b** and **1d**, in water) and 0.47 (**1a**, in water) and showed low Stokes's shifts (the highest 32 nm for **1d**, in ethanol). Figure 2 shows the normalised fluorescence spectra of compounds **1a**, **1c** and **1d**, in ethanol.

## 3. Biological activity

Antimicrobial activity of the synthesised fluorophores was asseded using a broth microdilution method for the antifungal susceptibility testing of yeasts (NCCLS M27-A). The minimum inhibitory concentrations of growth (MIC) obtained for *S. cerevisiae* W303-1B, used as a reference organism, are shown in Scheme 2. The activity of compounds **1a**–**f** was influenced by $R^3$ substitution, although a broad variation in MIC values was not found. In general, the substitution of the amino group increased activity, the only exception being compound **1e** (entry 5) where the presence of the amine function in $R^3$ was related to the lowest antimicrobial activity. Compounds **1b**–**d** (entries 2–4) and **1f** (entry 6) all shared a MIC of 15 μM.

In order to extend structure–activity relationship (SAR) analysis, we also evaluated the antimicrobial activity of a large series of compounds containing the

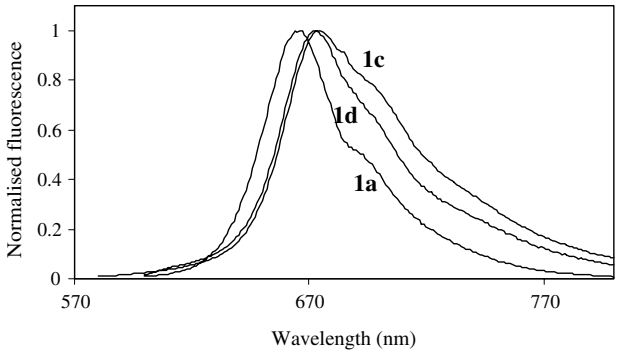
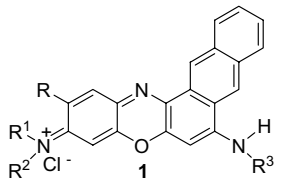



**Figure 2.** Normalised fluorescence spectra of compounds **1a**, **1c** and **1d**, in ethanol.

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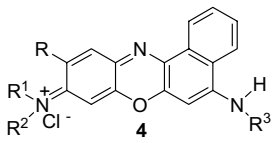



4

| Entry | Compound | R | $R^1$ | $R^2$ | $R^3$ | MIC[a] | log *P* |
|---|---|---|---|---|---|---|---|
| 1 | **1a** | Me | H | Et | H | 30 | 2.67 |
| 2 | **1b** | Me | H | Et | $(CH_2)_2Me$ | 15 | 3.93 |
| 3 | **1c** | Me | H | Et | $(CH_2)_3CO_2Et$ | 15 | 3.52 |
| 4 | **1d** | Me | H | Et | $(CH_2)_3OH$ | 15 | 2.69 |
| 5 | **1e** | Me | H | Et | $(CH_2)_3NH_2$ | 60 | 2.12 |
| 6 | **1f** | Me | H | Et | $(CH_2)_3Cl$ | 15 | 3.92 |
| 7 | **4a** | Me | H | Et | H | 15 | 1.51 |
| 8 | **4b** | Me | H | Et | $(CH_2)_3CO_2Et$ | 7.5 | 2.36 |
| 9 | **4c** | Me | H | Et | $(CH_2)_3OH$ | 60 | 1.53 |
| 10 | **4d** | Me | H | Et | $(CH_2)_3NH_2$ | 30[26] | 0.96 |
| 11 | **4e** | Me | H | Et | $(CH_2)_3Cl$ | 3.75 | 2.77 |
| 12 | **4f** | Me | H | Et | $(CH_2)_3CO_2H$ | 120 | 1.68 |
| 13 | **4g** | Me | H | Et | $(CH_2)_2CO_2Et$ | 15 | 2.09 |
| 14 | **4h** | Me | H | Et | $(CH_2)_2CO_2Me$ | 15 | 1.71 |
| 15 | **4i** | Me | H | Et | $(CH_2)_2OH$ | 30 | 1.26 |
| 16 | **4j** | Me | H | Et | $(CH_2)_2NH_2$ | 120 | 0.69 |
| 17 | **4l** | Me | H | Et | $(CH_2)_2CO_2H$ | 15 | 1.41 |
| 18 | **4m** | Me | H | Et | Ph | >120 | 4.02 |
| 19 | **4n** | H | Et | Et | H | 60 | 0.70 |
| 20 | **4o** | H | Et | Et | $(CH_2)_3OH$ | 120 | 0.71 |
| 21 | **4p** | H | Et | Et | $(CH_2)_2CO_2Me$ | 15 | 0.89 |
| 22 | **4q** | H | Et | Et | $(CH_2)_2CO_2H$ | 120 | 0.59 |
| 23 | **4r** | H | Me | Me | H | >120 | -0.06 |
| 24 | **4s** | H | Me | Me | $(CH_2)_2CO_2Et$ | 15 | 0.52 |
| 25 | **4t** | H | Me | Me | $(CH_2)_2CO_2Me$ | >120 | 0.14 |
| 26 | **4u** | H | Me | Me | $(CH_2)_3OH$ | 120 | -0.04 |
| 27 | **4v** | H | Me | Me | $(CH_2)_2CO_2H$ | >120 | -0.17 |
| 28 | **4x** | H | Me | Me | Ph | >120 | 2.45 |
| 29 | **4z** | H | H | $(CH_2)_5Me$ | Ph | >120 | 5.72 |

[a] Minimal Inhibitory Concentration of growth (μM).

**Scheme 2.** Activity against *Saccharomyces cerevisiae* W303-1B and log *P* values of naphtho[2,3-*a*]phenoxazinium chlorides **1a**–**f** and benzo[*a*]phenoxazinium chlorides **4a**–**z**.

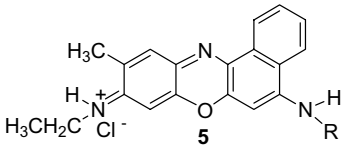
5,9-diaminobenzo[*a*]phenoxazinium moiety, which had been previously obtained.[4,24,28]

The decrease in the aromatic ring system, by replacing fused naphthalene with a benzene ring in the phenoxazine skeleton, generally increased the activity of the compounds (compare compounds **1a/4a**, entries 1/7; **1c/4b**, entries 3/8; **1e/4d**, entries 5/10 and **1f/4e**, entries 6/11). On the other hand, decreasing the alkyl chain size in the $R^3$ position either increased or decreased the efficiency of the compounds depending on the functional group of $R^3$ (for the increase, compare **4c/4i**, entries 9/15 and **4f/4l**, entries 12/17; for the decrease, compare **4b/4g**, entries 8/13 and **4d/4j**, entries 10/16). Alterations

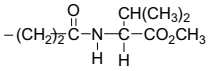
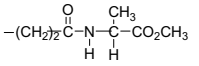
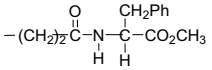
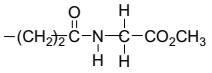
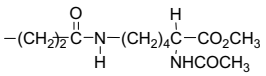
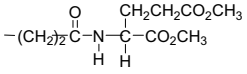
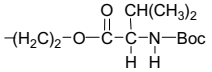
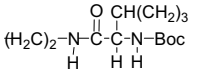
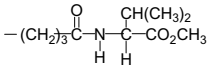
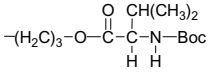
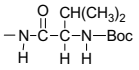
in R, $R^1$ and $R^2$ also influenced antifungal activity, in general the combination R = Me, $R^1$ = H and $R^2$ = Et was associated with the best activity, which decreased for R = H, $R^1$ = $R^2$ = Et and for R = H, $R^1$ = $R^2$ = Me (compare, for example, compounds **4a/4n/4r**, entries 7/19/23 or **4c/4o/4u**, entries 9/20/26). A phenyl substituent at $R^3$ (**4m**, **4x** and **4z**, entries 18, 28 and 29) completely abolished antifungal activity and was independent from the different combinations of R, $R^1$ and $R^2$.

The effect of coupling 5,9-diaminobenzo[*a*]phenoxazinium chlorides to α-amino acids was also tested (Scheme 3). The linkage of the amino group of six different amino acids to the carboxylic acid of $R^3$ from **4l** produced compounds whose MIC varied from 15 to 120 μM. These can be ordered as follows, according to their activity: phenylalanine (**5c**) > alanine (**5b**) = valine (**5a**) > glycine (**5d**) = lysine (**5e**) = glutamate (**5f**). These results seemed to indicate that the non-polar character of the amino acid was associated with a better activity. Consequently, we used one of the apolar amino acids, valine, bonded at its C-terminus or at the amino function to benzo[*a*]-phenoxazine through an ester or amide linkage, respectively, and varied the size of the alkyl chain linker (compounds **5g**–**5l**, entries 7–11). Size increase of the alkyl chain link as well as its removal decreased the activity of the compounds (compare compounds **5a/5i**, entries 1/9; **5g/5j/5l**, entries 7/10/11). On the other hand, binding of the amino acid with its carboxylic group, rather than with its amino group, increased the activity of the compounds with $-NH(CH_2)_2$ (compare **5a/5g/5h**, entries 1/7/8) and decreased its value with $-NH(CH_2)_3$ (compare **5i/5j**, entries 9/10).

For the two compounds showing the best antifungal activities, **4b** and **4e** (Scheme 2, entries 8 and 11), MIC values were also determined varying the initial cell densities in the assays (Table 3). Four different initial



**5**

| Entry | Compound | R | MIC[a] | log *P* |
|---|---|---|---|---|
| 1 | **5a** |  | 30 | 2.24 |
| 2 | **5b** |  | 30 | 1.46 |
| 3 | **5c** |  | 15 | 2.71 |
| 4 | **5d** |  | 120 | 1.13 |
| 5 | **5e** |  | 120 | 1.31 |
| 6 | **5f** |  | 120 | 1.21 |
| 7 | **5g** |  | 15 | 5.48 |
| 8 | **5h** |  | 15 | 4.72 |
| 9 | **5i** |  | 60 | 2.51 |
| 10 | **5j** |  | >120 | 5.75 |
| 11 | **5l** |  | 60 | 4.94 |

[a] Minimal Inhibitory Concentration of growth (μM). Boc = *N-tert*-butyloxycarbonyl.

**Scheme 3.** Activity against *Saccharomyces cerevisiae* W303-1B and log *P* values of benzo[*a*]phenoxazinium chlorides **5a**–**l**.

**Table 3.** Activity against *Saccharomyces cerevisiae* W303-1B of compounds **4b** and **4e**, measured with different initial cell densities

| Cell concentration (cell/mL) | MIC[a] | |
|---|---|---|
| | **4b** | **4e** |
| $0.5 \times 10^3$ | 7.5 | 3.75 |
| $0.5 \times 10^4$ | 7.5 | 7.5 |
| $0.5 \times 10^5$ | 7.5 | 7.5 |
| $0.5 \times 10^6$ | 30 | 15 |

[a] Minimal inhibitory concentration of growth (μM).

concentrations with 10-fold increases, in a range from $0.5 \times 10^3$ to $0.5 \times 10^6$ cells/mL, were used. Although higher MIC values were found for the higher cell concentrations, there was not a direct correlation between these parameters, and effective drug/cell ratios decreased with increasing cell concentrations. For both compounds, a 1000-fold increase in cell concentration resulted only in a 4-fold increase in the MIC value. However, the variation pattern was different for the two compounds, showing for compound **4e** an almost log correlation. The results suggest that the antifungal activity of these compounds may be exerted through different mechanisms.

Considering that the octanol–water partition coefficient, expressed as log *P*, is one physical parameter often linked with biological effects, it was predicted for all compounds under study and correlated with MIC values (Schemes 2 and 3).[30,31] The results showed that there was not a specific correlation between the lipophilicity and the antimicrobial activity for the naphtho[2,3-*a*]phenoxazine (**1a**–**f**) and benzo[*a*]phenoxazine derivatives tested (**4a**–**z** and **5a**–**l**), the same MIC values being observed for compounds exhibiting a wide variation in log *P*. For example, with a MIC value of 15 μM, compounds **1f**, **4s**, **5c** and **5g** have log *P* of 3.92, 0.52, 2.71 and 5.48, respectively.

## 4. Conclusion

In this work, several naphtho[2,3-*a*]phenoxazinium chlorides containing different *N*-substituents were synthesised in low to excellent yields. These compounds with absorption in the range of 601–647 nm were highly fluorescent, revealing maximum emission wavelengths from 630 to 676 nm in ethanol and water at physiological pH. Their photophysical properties, as well as the cationic structure and the presence of functional groups (except in compound **1b**; the ester group of **1c** could be hydrolysed to the corresponding carboxylic acid), strongly suggested that these polycyclic heterocycles are promising fluorescent probes for biological applications, either by non-covalent or covalent bonds.

Growth inhibition assays showed that the naphtho[2,3-*a*]phenoxazinium and 5,9-diaminobenzo[*a*]phenoxazinium chlorides studied in this work present significant antifungal activity, emerging as good candidates for further studies. Regarding SAR analysis, we concluded that the extent of the aromatic system influenced activity, the best results being obtained for the four-ring compounds. As for substitution of the 5,9-diaminobenzo[*a*]phenoxazine nucleus, the most efficient compounds were obtained when R = Me, $R^1$ = H and $R^2$ = Et, and particularly for $R^3 = (CH_2)_3Cl$, with an MIC of 3.75 μM. The high efficiency of the halogenated compound could be related to its capacity to increase ROS production and subsequently oxidative damage as has been reported in previous work where Nile Blue was used as a lead compound.[29] The linkage of different α-amino acids to *N*-functionalised substituents at the 5-position of 5,9-diaminobenzo[*a*]phenoxazinium moiety resulted in compounds with diverse antimicrobial efficiencies, which depended on the amino acid, on its linkage position and on the size of the alkyl linker. Phenylalanine and valine residues yielded the most active compounds (**5c**, **5g** and **5h**) with MIC values of 15 μM. A specific correlation between partition coefficients, expressed as log *P*, and the antifungal activities (MIC values) was not found for the compounds under study.

As a whole, the results are compatible with a DNA binding mechanism, as proposed for other compounds of this class, since we observed that antimicrobial activity depended both on the size of the aromatic system and on the size and character of the functional groups bounded, suggesting steric interference and group interactions. However, further studies are required in order to clarify this aspect.

## 5. Experimental

### 5.1. General

All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. TLC analyses were carried out on precoated silica plates, 0.25 mm thick (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light. Dry chromatography on silica gel was carried out on Merck Kieselgel (230–240 mesh). IR spectra were determined on a Perkin-Elmer FTIR-1600 using KBr discs, Nujol or neat samples. UV/visible spectra were run on a Hitachi U-2000 spectrophotometer. $^1$H NMR spectra were recorded on a Varian 300 spectrometer in $CDCl_3$ or $CD_3OD$ solution at 300 MHz at 25 °C. All chemical shifts are given in ppm using $\delta_H Me_4Si = 0$ ppm as reference and *J* values are given in Hz. $^{13}$C NMR spectra were run on the same instrument at 75.4 MHz using the solvent peak as the internal reference. 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 HMBC and HMQC techniques. Mass spectrometry analyses were performed at the 'C.A.C.T.I.—Unidad de Espectrometria de Masas' of the University of Vigo, Spain, on a Hewlett Packard 5989 A spectrometer for low resolution spectra and an Autospec M spectrometer for high resolution mass spectra. Fluorescence spectra were collected using a Spex Fluorolog 1680 Spectrometer.

### 5.2. General method for the synthesis of compounds (1a–e)

To a cold solution (ice bath) of 5-ethylamino-4-methyl-2-nitrosophenol hydrochloride **2** in ethanol (2 mL), compounds **3a**–**e** and concentrated hydrochloric acid ($5.0 \times 10^{-2}$ mL) were added. The mixture was refluxed during the time given below and monitored by TLC (chloroform or dichloromethane/methanol). After evaporation of the solvent and dry chromatography on silica gel or successive washes with a solvent (**1e**), compounds **1a**–**e** were obtained as blue solids.

**5.2.1. *N*-(7-Amino-2-methyl-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene)ethanaminium chloride (1a).** The product of the reaction of **2** (0.209 g, $1.16 \times 10^{-3}$ mol) with **3a** (0.165 g, $8.54 \times 10^{-4}$ mol) (reflux time, 1 h) was purified by chromatography ($CH_2Cl_2$/MeOH, 5.6:0.4) to give compound **1a** (0.302 g, 98%). Mp above 300 °C. TLC $R_f$ 0.62 ($CHCl_3$/MeOH, 6:1). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.20 (broad s, 3H, $NHCH_2CH_3$), 2.0 (broad s, 3H, $CH_3$), 3.20 (broad s, 2H, $NHCH_2CH_3$), 6.30 (broad s, 1H, 4-H), 7.0 (broad s, 1H, 6-H), 7.53 (broad s, 3H, 1-H, 6-H naphthyl and 7-H naphthyl), 7.75 (broad s, 3H, 5-H naphthyl, 8-H naphthyl and 1-H naphthyl), 8.57 (broad s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 14.20 ($NHCH_2CH_3$), 17.73 ($CH_3$), 39.50 ($NHCH_2CH_3$), 94.22 (4-C), 97.91 (6-C), 122.81 (Ar-C), 124.68 (Ar-C), 125.85 (Ar-C), 126.92 (4-C naphthyl), 128.07 (1-C naphthyl), 128.92 (7-C or 6-C naphthyl), 129.77 (5-C and 8-C naphthyl), 131.84 (6-C or 7-C naphthyl), 131.93 (1-C), 134.86 (Ar-C), 136.45 (Ar-C), 138.74 (Ar-C), 140.14 (Ar-C), 147.54 (Ar-C), 153.61 (3-C), 154.78 (7-C), 163.0 (Ar-C). IR (KBr 1%, $cm^{-1}$): $\nu_{max}$ 3446, 2923, 2853, 1653, 1636, 1558, 1541, 1522, 1507, 1489, 1473, 1457, 1398, 1301, 1261, 1154, 1014. HRMS (FAB) Calcd for $C_{23}H_{20}N_3O$ [$M^+$]: 354.1606. Found: 354.1605.

**5.2.2. *N*-(2-Methyl-7-propylamino-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene)ethanaminium chloride (1b).** The product of the reaction of **2** (0.008 g, 4.26 $10^{-5}$ mol) with **3b** (0.010 g, $4.26 \times 10^{-5}$ mol) (reflux time, 5 h) was purified by chromatography ($CHCl_3$/MeOH, 5.5:0.5) to give compound **1b** (0.072 g, 43%). Mp above 300 °C. TLC $R_f$ 0.30 ($CHCl_3$/MeOH, 5.5:0.5). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.17 (t, $J$ = 6.6 Hz, 3H, $NHCH_2CH_3$), 1.34–1.46 (m, 2H, $NHCH_2CH_2CH_2$), 1.94 (broad s, 2H, $NHCH_2CH_2CH_2$), 2.32 (s, 3H, $CH_3$), 3.38–3.50 (m, 2H, $NHCH_2CH_3$), 3.62–3.75 (m, 2H, $NHCH_2CH_2CH_2$), 6.70 (s, 1H, 4-H), 6.85 (s, 1H, 6-H), 7.60 (s, 1H, 1-H), 7.75 (t, $J$ = 7.2 Hz, 2H, 6-H naphthyl and 7-H naphthyl), 8.06–8.20 (m, 2H, 5-H naphthyl and 8-H naphthyl), 8.88 (s, 1H, 1-H naphthyl), 9.27 (s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 14.19 ($NHCH_2CH_3$), 17.71 ($CH_3$), 30.76 ($NHCH_2CH_2CH_2$ and $NHCH_2CH_2CH_2$), 39.53 ($NHCH_2CH_3$), 47.57 ($NHCH_2CH_2CH_2$), 94.31 (6-C), 94.50 (4-C), 121.0 (Ar-C), 125.10 (4-C naphthyl), 125.58 (1-C naphthyl), 127.65 (2× Ar-C), 129.32 (7-C naphthyl or 6-C naphthyl), 129.87 (5-C naphthyl and 8-C naphthyl), 130.35 (6-C naphthyl or 7-C naphthyl), 130.69 (Ar-C), 132.22 (C-1), 134.05 (2× Ar-C), 135.76 (Ar-C), 148.58 (Ar-C), 154.83 (Ar-C), 155.41 (Ar-C), 160.03 (Ar-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3420, 2954, 2924, 2854, 1653, 1647, 1636, 1558, 1457, 1541, 1521, 1507, 1489, 1376, 1289, 1124, 1016. HRMS (FAB) Calcd for $C_{26}H_{26}N_3O$ [$M^+$]: 396.2076. Found: 396.2076.

**5.2.3. *N*-[7-(4-Ethoxy-4-oxobutylamino)-2-methyl-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene] ethanaminium chloride (1c).** The product of the reaction of **2** (0.279 g, $1.55 \times 10^{-3}$ mol) with **3c** (0.436 g, $1.55 \times 10^{-3}$ mol) (reflux time, 16 h) was purified by chromatography ($CHCl_3$/MeOH, 5.6:0.4) to give compound **1c** (0.118 g, 16%). Mp above 300 °C. TLC $R_f$ 0.46 ($CHCl_3$/MeOH, 6:1). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.20–1.40 (m, 6H, $NHCH_2CH_3$ and $OCH_2CH_3$), 2.10 (broad s, 5H, $NHCH_2CH_2CH_2$ and $CH_3$), 2.56 (t, $J$ = 6.6 Hz, 2H, $NHCH_2CH_2CH_2$), 3.20–3.40 (m, 2H, $NHCH_2CH_3$), 3.35–3.45 (m, 2H, $NHCH_2CH_2CH_2$), 4.19 and 4.23 (q, $J$ = 6.9 Hz, 2H, $OCH_2CH_3$), 6.02 (s, 1H, 4-H), 6.30 (s, 1H, 6-H), 6.92 (s, 1H, 1-H), 7.40–7.60 (m, 2H, 6-H naphthyl and 7-H naphthyl), 7.72 (d, $J$ = 7.2 Hz, 2H, 5-H naphthyl and 8-H naphthyl), 8.31 (s, 1H, 1-H naphthyl), 8.46 (s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz) $\delta$ 14.15 ($NHCH_2CH_3$), 14.67 ($OCH_2CH_3$), 17.83 ($CH_3$), 24.71 ($NHCH_2CH_2CH_2$), 31.91 ($NHCH_2CH_2CH_2$), 39.57 ($NHCH_2CH_3$), 44.86 ($NHCH_2CH_2CH_2$), 61.93 ($OCH_2CH_3$), 93.67 (6-C), 94.12 (4-C), 121.36 (Ar-C), 124.69 (4-C naphthyl), 124.88 (1-C naphthyl), 127.26 (Ar-C), 127.57 (Ar-C), 128.90 (7-C naphthyl or 6-C naphthyl), 129.53 (Ar-C), 130.03 (5-C naphthyl and 8-C naphthyl), 130.14 (6-C naphthyl or 7-C naphthyl), 131.66 (1-C), 133.32 (Ar-C), 134.19 (Ar-C), 134.51 (Ar-C), 147.55 (Ar-C), 153.35 (3-C), 155.06 (Ar-C), 158.75 (Ar-C), 174.77 (CO). IR (KBr 1%, $cm^{-1}$): $\nu_{max}$ 3422, 2956, 2925, 2854, 1724, 1638, 1585, 1549, 1512, 1467, 1450, 1376, 1354, 1297, 1262, 1210, 1165, 1141, 1085, 1016. HRMS (FAB) Calcd for $C_{29}H_{30}N_3O_3$ [$M^+$]: 468.2287. Found: 468.2292.

**5.2.4. *N*-[7-(3-Hydroxypropylamino)-2-methyl-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene]ethanaminium chloride (1d).** The product of the reaction of **2** (0.082 g, $4.54 \times 10^{-4}$ mol) with **3d** (0.114 g, $4.54 \times 10^{-4}$ mol) (reflux time, 4 h and 30 min), was purified by chromatography ($CH_2Cl_2$/MeOH, 5.5:0.5) to give compound **1d** (0.114 g, 61%). Mp above 300 °C. TLC $R_f$ 0.68 ($CH_2Cl_2$/MeOH, 6:1). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.20–1.40 (m, 3H, $NHCH_2CH_3$), 2.10–2.20 (m, 2H, $NHCH_2CH_2CH_2$), 2.31 (broad s, 2H, $NHCH_2CH_2CH_2$), 2.36 (s, 3H, $CH_3$), 3.50–3.60 (m, 2H, $NHCH_2CH_3$), 3.85 (broad s, 2H, $NHCH_2CH_2CH_2$), 6.71 (broad s, 1H, 4-H), 6.91 (broad s, 1H, 6-H), 7.63 (s, 1H, 1-H), 7.73 (broad s, 2H, 6-H naphthyl and 7-H naphthyl), 8.16 (broad s, 2H, 5-H naphthyl and 8-H naphthyl), 8.80 (broad s, 1H, 1-H naphthyl), 9.28 (broad s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 14.66 ($NHCH_2CH_3$), 18.33 ($CH_3$), 30.21 ($NHCH_2CH_2CH_2$), 32.75 ($NHCH_2CH_2CH_2$), 40.06 ($NHCH_2CH_3$), 43.95 ($NHCH_2CH_2CH_2$), 94.27 (4-C), 94.67 (6-C), 122.00 (Ar-C), 125.28 (Ar-C), 125.45 (Ar-C), 127.83 (4-C naphthyl), 127.94 (1-C naphthyl), 129.45 (7-C naphthyl or 6-C naphthyl), 130.08 (5-C naphthyl and 8-C naphthyl), 130.61 (6-C naphthyl or 7-C naphthyl), 132.19 (1-C), 133.87 (Ar-C), 134.95 (Ar-C), 135.06 (Ar-C), 135.49 (Ar-C), 148.08 (Ar-C), 154.0

(3-C), 155.50 (2-C), 159.42 (Ar-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3406, 2954, 2924, 2854, 1658, 1638, 1549, 1463, 1455, 1377, 1278, 1124, 1016. HRMS (FAB) Calcd for $C_{26}H_{26}N_3O_2$ [$M^+$]: 412.2025. Found: 412.2021.

**5.2.5. *N*-[7-(3-Aminopropylamino)-2-methyl-3*H*-naphtho [2,3-*a*]phenoxazin-3-ylidene]ethanaminium chloride (1e).** The product of the reaction of **2** (0.122 g, $6.80 \times 10^{-4}$ mol) with **3e** (0.170 g, $6.80 \times 10^{-4}$ mol) (reflux time, 9 h) was successively washed with chloroform and mixtures of increasing polarity of chloroform and methanol to give compound **1e** (0.273 g, 98%). Mp above 300 °C. $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.30–1.50 (m, 3H, $NHCH_2CH_3$), 2.12–2.20 (m, 2H, $NHCH_2CH_2CH_2$), 2.35 (broad s, 2H, $NHCH_2CH_2CH_2$), 2.36 (s, 3H, $CH_3$), 3.56–3.66 (m, 2H, $NHCH_2CH_3$), 3.90 (broad s, 2H, $NHCH_2CH_2CH_2$), 6.76 (broad s, 1H, 4-H), 6.95 (broad s, 1H, 6-H), 7.67 (s, 1H, 1-H), 7.80 (broad s, 2H, 6-H naphthyl and 7-H naphthyl), 8.20 (broad s, 2H, 5-H naphthyl and 8-H naphthyl), 8.86 (broad s, 1H, 1-H naphthyl), 9.38 (broad s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 14.70 ($NHCH_2CH_3$), 18.40 ($CH_3$), 30.31 ($NHCH_2CH_2CH_2$), 32.85 ($NHCH_2CH_2CH_2$), 40.20 ($NHCH_2CH_3$), 44.0 ($NHCH_2CH_2CH_2$), 95.30 (4-C), 96.70 (6-C), 124.00 (Ar-C), 126.38 (Ar-C), 126.50 (Ar-C), 128.50 (4-C naphthyl), 128.60 (1-C naphthyl), 130.0 (7-C naphthyl or 6-C naphthyl), 131.08 (5-C naphthyl and 8-C naphthyl), 132.60 (6-C naphthyl or 7-C naphthyl), 133.20 (1-C), 134.0 (Ar-C), 135.90 (Ar-C), 136.10 (Ar-C), 137.60 (Ar-C), 149.00 (Ar-C), 155.0 (3-C), 156.0 (2-C), 160.20 (Ar-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3396, 3197, 2927, 1633, 1604, 1581, 1548, 1514, 1465, 1451, 1403, 1289, 1262, 1211, 1165 1143, 1015. HRMS (FAB) Calcd for $C_{26}H_{27}N_4O$ [$M^+$]: 411.2185. Found: 411.2188.

**5.2.6. *N*-[7-(3-Chloropropylamino)-2-methyl-3*H*-naphtho[2,3-*a*]phenoxazin-3-ylidene]ethanaminium chloride (1f).** Thionyl chloride ($3.0 \times 10^{-2}$ mL, $4.12 \times 10^{-4}$ mol) was added to a solution of compound **1d** (0.020 g, $4.85 \times 10^{-5}$ mol) in dichloromethane/chloroform, 2:1 (3.0 mL) and the reaction mixture was stirred at room temperature for 74 h. The solvent was removed under reduced pressure and the crude mixture was purified by dry chromatography ($CHCl_3$/MeOH, 5.7:0.3). Compound **1f** was obtained as a blue solid (0.011 g, 50%). Mp above 300 °C. TLC $R_f$ 0.32 ($CH_2Cl_2$/MeOH, 5.5:0.5). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 1.20–1.40 (m, 5H, $NHCH_2CH_3$ and $NHCH_2CH_2CH_2$), 2.23 (s, 3H, $CH_3$), 2.34 (broad s, 2H, $NHCH_2CH_2CH_2$), 3.30–3.40 (m, 2H, $NHCH_2CH_3$), 3.81–3.90 (m, 2H, $NHCH_2CH_2CH_2$), 6.45 (s, 1H, 4-H), 6.59 (s, 1H, 6-H), 7.34 (s, 1H, 1-H), 7.66 (t, $J$ = 9.0 Hz, 2H, 6-H naphthyl and 7-H naphthyl), 7.90–8.04 (m, 2H, 5-H naphthyl and 8-H naphthyl), 8.63 (s, 1H, 1-H naphthyl), 8.90 (s, 1H, 4-H naphthyl). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 14.18 ($NHCH_2CH_3$), 17.81 ($CH_3$), 30.77 ($NHCH_2CH_2CH_2$), 32.49 ($NHCH_2CH_2CH_2$), 39.63 ($NHCH_2CH_3$), 43.23 ($NHCH_2CH_2CH_2$), 93.85 (6-C), 94.40 (4-C), 121.96 (Ar-C), 125.12 (4-C naphthyl and 1-C naphthyl), 127.92 (Ar-C), 128.02 (Ar-C), 129.17 (7-C naphthyl or 6-C naphthyl), 129.72 (5-C naphthyl or 8-C naphthyl), 130.28 (6-C naphthyl or 7-C naphthyl and 5-C naphthyl or 8-C naphthyl), 130.85 (Ar-C), 132.07 (1-C), 133.71 (2× Ar-C), 134.93 (Ar-C), 148.27 (Ar-C), 154.10 (3-C), 155.54 (7-C), 159.40 (Ar-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3412, 2954, 2925, 2854, 1638, 1583, 1547, 1463, 1377, 1281, 1208, 1163, 1139, 1014. HRMS (FAB): Calcd for $C_{26}H_{25}N_3O^{35}Cl$ [$M^+$]: 430.1686. Found: 430.1693. Calcd for $C_{26}H_{25}N_3O^{37}Cl$ [$M^+$]: 432.1657. Found: 432.1665.

### 5.3. Synthesis of compounds (3b–e)

**5.3.1. *N*-Propylanthracen-1-amine (3b).** To a solution of 1-aminoanthracene (0.300 g, $1.55 \times 10^{-3}$ mol) in ethanol (2 mL), 1-bromopropane (0.30 mL, $3.26 \times 10^{-3}$ mol) was added and the resulting mixture was refluxed for 14 h and monitored by TLC ($CH_2Cl_2$). The solvent was removed under reduced pressure and purified by dry chromatography (*n*-hexane/$CHCl_3$, 8.5:1.5) to produce compound **3b** as a greenish oil (0.077 g, 21%). TLC $R_f$ 0.55 (*n*-hexane/$CHCl_3$, 4:3). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 1.15 (t, $J$ = 7.5 Hz, 3H, $CH_3$), 1.80–2.0 (m, 2H, $NHCH_2CH_2CH_3$), 3.34 (t, $J$ = 7.2 Hz, 2H, $NHCH_2CH_2CH_3$), 6.58 (d, $J$ = 6.9 Hz, 1H, 4-H), 7.32–7.50 (m, 4H, 3-H, 2-H, 7-H and 8-H), 7.94–8.60 (m, 2H, 6-H and 9-H), 8.39 (s, 1H, 5-H or 10-H), 8.39 (s, 1H, 10-H or 5-H). $^{13}$C NMR ($CDCl_3$, 75.4 MHz): $\delta$ 11.70 ($NHCH_2CH_2CH_3$), 21.90 ($NHCH_2CH_2CH_2$), 47.68 ($NHCH_2CH_2CH_2$), 109.70 (4-C), 118.95 (2-C and 5-C), 123.66 (4a-C), 125.25 (8-C), 125.64 (7-C), 125.71 (3-C), 126.74 (10-C), 127.72 (6-C), 128.48 (9-C), 131.07 (5a-C), 131.57 (9a-C), 132.41 (10a-C), 140.65 (1-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3425, 2954, 2924, 2854, 1569, 1552, 1462, 1377, 1308, 1154, 1042. HRMS (EI) Calcd for $C_{17}H_{17}N$ [$M^+$]: 235.1361. Found: 235.1363.

**5.3.2. Ethyl 4-(anthracen-1-ylamino)butanoate (3c).** The product of the reaction of 1-aminoanthracene (0.300 g, $1.55 \times 10^{-3}$ mol) with ethyl-4-bromobutyrate (0.23 mL, $1.63 \times 10^{-3}$ mol), according to the procedure described above for the preparation of compound **3b** (reflux time 25 h) was used in the following reaction without any purification (0.476 g, 100%, crude mixture).

**5.3.3. 3-(Anthracen-1-ylamino)propan-1-ol (3d).** The product of the reaction of 1-aminoanthracene (0.300 g, $1.55 \times 10^{-3}$ mol) with 3-bromo-1-propanol (0.147 mL, $1.63 \times 10^{-2}$ mol), according to the procedure described above for the preparation of compound **3b** (reflux time, 20 h) was purified by chromatography ($CH_2Cl_2$ and $CH_2Cl_2$/MeOH, 5.9:0.1) to produce compound **3d** as a brown oil (0.205 g, 53%). TLC $R_f$ 0.42 ($CH_2Cl_2$/MeOH, 5.8:0.2). $^1$H NMR ($CDCl_3$, 300 MHz): $\delta$ 2.14 (t, $J$ = 5.7 Hz, 2H, $NHCH_2CH_2CH_2$), 3.54 (t, $J$ = 6.3 Hz, 2H, $NHCH_2CH_2CH_2$), 3.99 (t, $J$ = 5.7 Hz, 2H, $NHCH_2CH_2CH_2$), 6.58 (d, $J$ = 7.5 Hz, 1H, 4-H), 7.30–7.38 (m, 2H, 3-H and 2-H), 7.40–7.50 (m, 2H, 7-H and 8-H), 7.94–8.04 (m, 2H, 6-H and 9-H), 8.37 (s, 2H, 5-H and 10-H). $^{13}$C NMR ($CDCl_3$, 75.4 MHz) $\delta$ 31.45 ($NHCH_2CH_2CH_2$), 42.52 ($NHCH_2CH_2CH_2$), 62.17 ($NHCH_2CH_2CH_2$), 102.13 (4-C), 117.59 (2-C), 118.65 (5-C), 123.75 (4a-C), 124.97 (8-C), 125.44 (7-C), 126.17 (3-C), 126.53 (10-C), 127.73 (6-C), 128.40 (9-C), 130.82 (5a-C), 131.51 (9a-C), 132.49 (10a-C), 143.27

(1-C). IR (neat, $cm^{-1}$): $\nu_{max}$ 3434, 2956, 2925, 2854, 1642, 1462, 1378, 1121, 1031. HRMS (EI): Calcd for $C_{17}H_{17}NO$ [$M^+$]: 251.1310. Found: 251.1318.

**5.3.4. $N^1$-(Anthracen-1-yl) propane-1,3-diamine (3e).** The product of the reaction of 1-aminoanthracene (0.300 g, $1.55 \times 10^{-3}$ mol) with 3-bromopropylamine hydrobromide (0.357 g, $1.63 \times 10^{-3}$ mol), according to the procedure described above for the preparation of compound **3b** (reflux time, 26 h) was purified by chromatography ($CH_2Cl_2$/MeOH, 5.5:0.5) to produce compound **3e** as a brown oil (0.191 g, 50%). TLC $R_f$ 0.46 ($CH_2Cl_2$/MeOH, 5:1). $^1$H NMR ($CD_3OD$, 300 MHz): $\delta$ 2.14–2.28 (m, 2H, $NHCH_2CH_2CH_2$), 3.20 (t, $J$ = 7.5 Hz, 1H, $NHCH_2CH_2CH_2$), 3.53 (t, $J$ = 6.6 Hz, 2H, $NHCH_2CH_2CH_2$), 6.58 (d, $J$ = 6.6 Hz, 1H, 4-H), 7.30–7.39 (m, 2H, 2-H and 3-H), 7.40–7.50 (m, 2H, 7-H and 8-H), 7.98 (dd, $J$ = 9.3 and 3.9 Hz, 1H, 6-H), 8.07 (dd, $J$ = 9.0 and 3.6 Hz, 1H, 9-H), 8.34 (s, 1H, 5-H or 10-H), 8.70 (s, 1H, 10-H or 5-H). $^{13}$C NMR ($CD_3OD$, 75.4 MHz): $\delta$ 31.40 ($NHCH_2CH_2CH_2$), 39.39 ($NHCH_2CH_2CH_2$), 69.06 ($NHCH_2CH_2CH_2$), 118.16 (4-C and 2-C), 120.80 (5-C), 122.77 (4a-C), 125.80 (8-C), 126.50 (7-C), 127.10 (3-C), 127.23 (10-C), 128.64 (6-C), 129.59 (9-C), 130.52 (5a-C), 132.03 (9a-C), 132.98 (10a-C), 144.56 (1-C). IR (Nujol, $cm^{-1}$): $\nu_{max}$ 3398, 2954, 2923, 2854, 1627, 1504, 1463, 1456, 1377, 1113, 1029, 1005, 911.

### 5.4. Antifungal activity tests

Minimum inhibitory concentrations of growth (MIC) for the different compounds were determined using a broth microdilution method for the antifungal susceptibility testing of yeasts (NCCLS M27-A). The yeast strain *S. cerevisiae* W303-1B (*MATa, ade2, his3, leu2, trp1, ura3*) was used. Cells were incubated at 30 °C in RPMI 1640 medium, buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Sigma) and supplemented with the required amino acids. Initial cell concentration was $0.5 \times 10^3$ cells/mL, or as specified in the text. MIC values were determined visually after 48 h of incubation, as the lowest concentration of drug that resulted in no detectable growth. 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 was tested in triplicate and in two independent experiments.

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