chemistry proceedings

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Two symmetrical Squarylium Cyanine Dyes: Synthesis, Photophysics and Antifungal Activity in *Saccharomyces cerevisiae* †

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† Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: https://ecsoc-24.sciforum.net/.

Published: date

Abstract: Two squarylium cyanine dyes were synthesized and characterized by the usual analytical techniques, including UV-Vis-NIR absorption and emission spectroscopy. Their antifungal activity was evaluated, through the obtention of MIC values, using yeasts of the species *Saccharomyces cerevisiae* as a biological model.

Keywords: squarylium cyanine dyes; antifungal activity; NIR fluorescence dyes; fluorescent probes; fluorochromophores

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1. Introduction

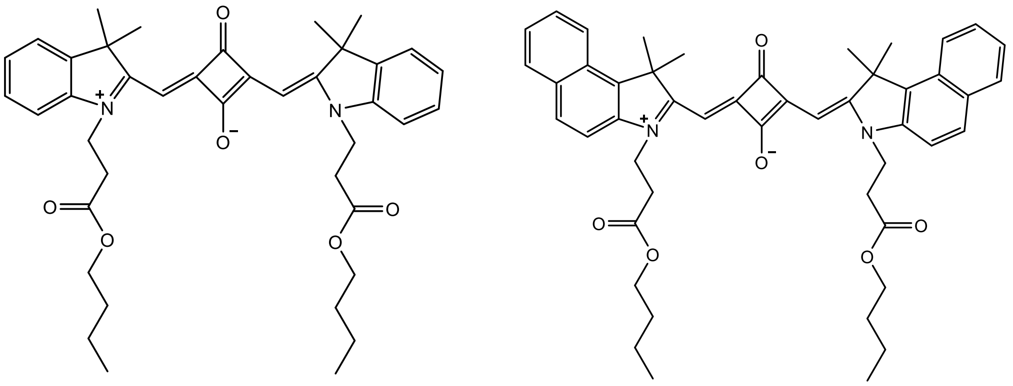
Squaraine cyanine dyes are compounds 1,3-disubstituted with resonance stabilized zwitterionic structure, which presents a central four-membered ring derivative of squaric acid (3,4-dihydroxy-1,2-dioxocyclobut-3-ene) and two electron donor groups in the end of the polymethine chain [1–4]. This class of compounds presents good photochemical stability, narrow and intense absorption, and emission bands in the visible to near infrared region, high molar extinction coefficient and good photoconductivity [5–8]. In the last few years, the interest in these polymethine dyes is increasing, especially because of their technological and biological applications, mainly as organic solar cell sensitizers [9–11], as markers of several biomolecules [12–14] and as photosensitizers (PS) for photodynamic therapy (PDT) [15–17]. Depending on the application intended, changes in their structures can be made in order to improve the required properties.

Considering all these facts, two symmetrical squaraine cyanine dyes derived from indolenine and benzo indolenine possessing ester groups in the chains attached to nitrogen atoms were synthetized. The structural elucidation of dyes, as well as of their precursors, was performed using the usual analytical techniques. Fundamental photophysics studies in ethanol, in addition to determination of antifungal activity of the dyes were carried.

2. Results and Discussion

2.1. Synthesis of Dyes **1** and **2**

Condensation reaction between 1-(2-carboxyethyl)-2,3,3-trimethyl-3*H*-indol-1-ium or 3-(2-carboxyethyl)-1,1,2-trimethyl-1*H*-benzo[*e*]indol-3-ium, as quaternary ammonium salts, and 3,4-dihydroxycyclobut-3-ene-1,2-dione, using *n*-butanol and toluene at reflux using a Dean-Stark system gave squarylium cyanine dyes **1** and **2**, respectively (Figure 1). The blue solids isolated with yields of 17% (**1**) and 59% (**2**) were characterized by standard spectroscopic methods.



1 **2**

Figure 1. Chemical structures of the synthetized and tested squarylium cyanine dyes.

The ^{1}H NMR spectrum of compound **1** showed aromatic protons from the heterocyclic system at δ 7.08–7.35 ppm and the spectrum of compound **2** at δ 7.42–8.20 ppm. The methine protons appeared at δ 5.93 ppm for compound **1** and at δ 6.00 ppm for compound **2** as a singlet of two protons. As expected, the ^{1}H NMR spectra of synthesized dyes just presented a sole methine protons signal, which is in accordance with the symmetrical character of these molecules. Methylene protons of the presented compounds appeared as sextets, quintets, triplets, and broad singlets at δ 1.28–1.31 ppm $(N(CH_2)_2COOCH_2CH_2C\underline{H}_2CH_3)$, δ 1.52–1.54 ppm $(N(CH_2)_2COOCH_2C\underline{H}_2CH_2CH_3)$, δ 2.82–2.90 ppm $(NCH_2C\underline{H}_2COO(CH_2)_3CH_3)$, δ 4.05 ppm $(NC\underline{H}_2CH_2COO(CH_2)_3CH_3)$ and δ 4.34–4.47 ppm $(N(CH_2)_2COOC\underline{H}_2CH_2CH_2CH_3)$. The terminal methyl groups of chain linked to the nitrogen atom emerged at δ 0.84–0.89 ppm $(N(CH_2)_2COO(CH_2)_3C\underline{H}_3)$, while the methyl groups of heterocyclic rings arise at δ 1.78–2.07 ppm $(C(C\underline{H}_3)_2)$.

The ^{13}C NMR spectra exhibited aromatic carbons in the form of 4 signals at δ 109.68–127.99 ppm, for compound **1** and five signals at δ 110.31–129.84 ppm for compound **2**. Methyl groups of the molecules are presented at δ 13.70–13.78 ppm $(C(\underline{C}H_3)_2)$ and δ 26.95–27.25 ppm $(N(CH_2)_2COO(CH_2)_3\underline{C}H_3)$. Methine groups arise at δ 86.67–86.93 $(\underline{C}H{=}C)$ ppm and methylene groups of both compounds present very similar chemical shifts, emerging at δ 19.12–19.17 ppm $(N(CH_2)_2COOCH_2CH_2\underline{C}H_2CH_3)$, δ 30.55–30.57 $(N(CH_2)_2COOCH_2\underline{C}H_2CH_2CH_3)$ ppm, δ 31.75–32.01 ppm $(NCH_2\underline{C}H_2COO(CH_2)_3CH_3)$, δ 39.42–39.52 ppm $(N\underline{C}H_2CH_2COO(CH_2)_3CH_3)$, and δ 65.25–65.26 ppm $(N(CH_2)_2COO\underline{C}H_2CH_2CH_2CH_3)$.

2.2. Fundamental Photophysics of Dyes **1** and **2**

The absorption and fluorescence properties of the synthetized squaraine cyanine dyes 1 and 2 were evaluated in ethanol. The relative fluorescence quantum yields were determined using zinc phthalocyanine as a reference ($\Phi_F = 0.17$ in DMF) [18] and using an excitation wavelength of 580 nm. All the results are showed in Table 1.

Table 1. Fundamental photophysical data of synthesized dyes **1** and **2** in ethanol.

Dye	Ethanol				
	λ_{abs} (nm)	ε (M^{-1} cm^{-1})	λ_{em} (nm)	Φ_F (%)	$\Delta\lambda$ (nm)
1	631	385,684	639	80.7	8
2	663	324,591	672	25.8	9

Compound **2** showed maximum absorption wavelength (λ_{abs}) and maximum emission wavelength (λ_{em}) superior to compound **1**, which is in line with expectations, due to the introduction of another fused aromatic ring in the heterocyclic base. Regarding the molar extinction coefficient (ε), the highest value is attributed to compound **1** (385,684 M^{-1} cm^{-1}), as well as the highest fluorescence quantum yield (Φ_F) value (81%).

The normalized absorption and emission spectra of compounds **1** and **2** in ethanol are shown in Figures 2 and 3, respectively.

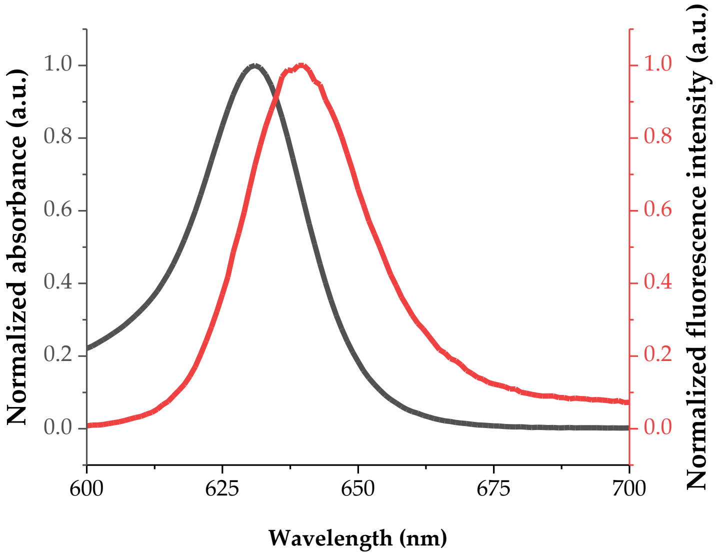


Figure 2. Normalized absorption (dark line) and fluorescence spectra (red line) of squaraine cyanine dye **1** in ethanol.

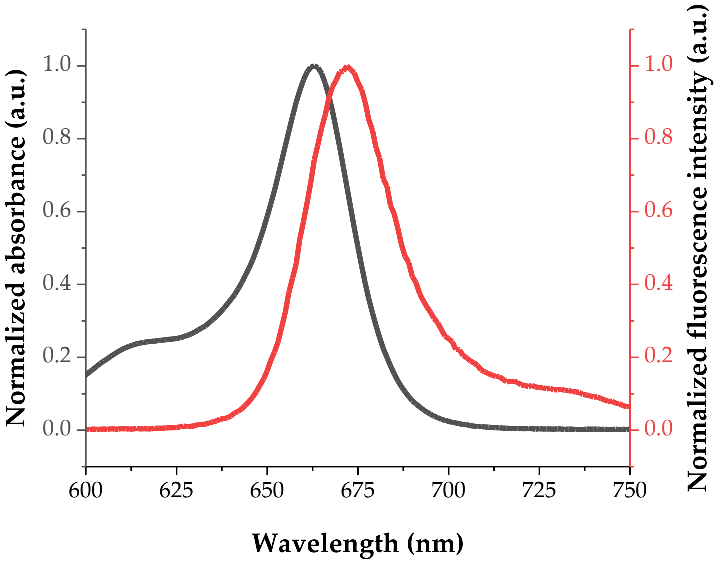


Figure 3. Normalized absorption (dark line) and fluorescence spectra (red line) of squaraine cyanine dye **2** in ethanol.

2.3. Antifungal Activity of Dyes **1** *and* **2**

The antifungal activity of squaraine cyanine dyes **1** and **2** against yeasts of the species *Saccharomyces cerevisiae* was determined and presented through the minimum inhibitory concentration (MIC) value. The value of Log P was also determined and corresponds to an estimated measure of the compounds hydrophobicity by calculating the partition between membranes and aqueous media (Table 2).

Table 2. MIC values obtained for dyes **1** and **2** in the antifungal activity tests against *Saccharomyces cerevisiae*.

Dye	MIC (µM)	Log P
1	>100	1.70
2	50	4.02

Compound **1** has a MIC value superior than 100 µM, while compound **2** displays better activity with MIC of 50 µM. These results indicate that the presence of the naphthalene instead of a benzene ring in the heterocyclic base seems to improve antifungal activity. Compounds with smaller Log P values are more soluble in water while compounds with higher values have greater affinity for membrane systems. Compounds **2** were found to have a higher Log P value which will be related to their greater antifungal activity.

3. Experimental Section

3.1. Typical Procedure for the Synthesis of Dyes **1** *and* **2** *(Illustrated for* **1***)*

1-(2-Carboxyethyl)-2,3,3-trimethyl-3*H*-indol-1-ium (0.400 g, 1.28 mmol) and 3,4-dihydroxy-1,2-dioxocyclobut-3-ene (0.073 g, 0.640 mmol) in a mixture of *n*-butanol/toluene (10 mL, 1:1 *v*/*v*), was stirred for 6 h at reflux in a Dean-Stark apparatus. Cold distilled water was added to the reaction mixture and after separation by decantation, the organic layer was dried with anhydrous $\mathrm{Na_2SO_4}$ and the solvent removed under reduced pressure. After successive recrystallizations from $\mathrm{CH_2Cl_2}$/MeOH/petroleum ether/diethyl ether, (*Z*)-4-((1-(3-butoxy-3-oxopropyl)-3,3-dimethyl-3*H*-indol-1-ium-2-yl)methylene)-2-(((*E*)-1-(3-butoxy-3-oxopropyl)-3,3-dimethylindolin-2-ylidene) methyl)-3-oxocyclobut-1-en-1-olate (**1**) was obtained as a light blue crystals (0.059 g, 17%). ^{1}H NMR (600.10 MHz, $\mathrm{CDCl_3}$) δ: 7.35 (2H, d, *J* = 7.2 Hz, ArH), 7.31 (2H, t, *J* = 7.5 Hz, ArH), 7.14 (2H, t, *J* = 7.2 Hz, ArH), 7.08 (2H, d, *J* = 7.8 Hz, ArH), 5.93 (2H, s, $\mathrm{C\underline{H}{=}C}$), 4.34 (2H, bs, $\mathrm{N(CH_2)_2COOC\underline{H}_2CH_2CH_2CH_3}$), 4.05 (4H, t, *J* = 6.6 Hz, $\mathrm{NC\underline{H}_2CH_2COO(CH_2)_3CH_3}$), 2.82 (4H, t, *J* = 7.5 Hz, $\mathrm{NCH_2C\underline{H}_2COO(CH_2)_3CH_3}$), 1.78 (12H, s, $\mathrm{C(C\underline{H}_3)_2}$), 1.54 (4H, qt, $\mathrm{N(CH_2)_2COOCH_2C\underline{H}_2CH_2CH_3}$), 1.31 (4H, st, $\mathrm{N(CH_2)_2COOCH_2CH_2C\underline{H}_2CH_3}$), 0.89 (6H, t, *J* = 7.5 Hz, $\mathrm{N(CH_2)_2COO(CH_2)_3CH_3}$ ppm. ^{13}C NMR (150.91 MHz, $\mathrm{CDCl_3}$) δ:182.22, 180.93, 170.88, 170.16, 142.08, 127.99 (ArCH), 124.12 (ArCH), 122.48 (ArCH), 109.68 (ArCH), 86.93 (CH=C), 65.25 ($\mathrm{CH_2}$), 49.51 $\mathrm{C(CH_3)_2}$, 39.40 ($\mathrm{CH_2}$), 31.75 ($\mathrm{CH_2}$), 30.57 ($\mathrm{CH_2}$), 27.25 ($\mathrm{C(CH_3)_2}$), 19.17 ($\mathrm{CH_2}$), 13.78 ($\mathrm{CH_3}$) ppm.

3.2. Antifungal Activity Assays

Minimum Inhibitory Concentration of growth for the two compounds was determined using a broth microdilution method for the antifungal susceptibility against *Saccharomyces cerevisiae* yeasts. This assay was prepared in 96-well microplates using a cell concentration of 2.25×10^3 cells/mL, to which compounds were added at previously established concentrations. The stock solutions of compounds were prepared in DMSO and then dissolved in RPMI to obtain the desired concentrations per well. The plates were incubated at 30 °C for 48 h, after which the absorbance at 640 nm was read, using a microplate photometer. The obtained values allowed the determination of MICs, which corresponds to the lowest concentration of compound that causes a growth inhibition of at least 80%, when compared to a control. Five concentrations of each dye were tested, each in triplicate and in three independent assays.

4. Conclusions

Two squarylium cyanine dyes were successfully synthesized and characterized. Photophysical studies in ethanol showed maxima absorption and fluorescence emission wavelengths between 631–672 nm and fluorescence quantum yields of 81% and 26%, being the highest value attributed to the compound derived from 2,3,3-trimethylindolenine (**1**). The two compounds revealed antifungal activity, with dye derived from 1,1,2-trimethyl-1*H*-benzo[*e*]indole (**2**) showing the best result with a MIC value of 50 μM.

To increase the antifungal capacity of the presented dyes, tests are being carried out using an LED irradiance system with an appropriate wavelength. The results have been quite promising, indicating that this type of dyes may be used in PDT.

Acknowledgments: Thanks are also due to Fundação para a Ciência e Tecnologia (FCT) and FEDER (European Fund for Regional Development)-COMPETEQREN-EU for financial support to the research centres CQ/UM (UIDB/00686/2020), CBMA (UID/BIA/04050/2020), CQ/VR (UID/QUI/UI0616/2019) and CICSUBI (POCI-01-0145-FEDER-007491), as well as PhD grants to J.C.C.F. (SFRH/BD/133207/2017) and V.S.D.G. (UMINHO/BD/43/2016).

Conflicts of Interest: The autors declare no conflict of interests.

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