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Proceeding Paper

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

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Abstract: Two squarylium cyanine dyes were synthesized and characterized by the usual analytical techniques, including Vis-NIR absorption and emission spectroscopy. Their antifungal activity was evaluated, through the obtention of minimum inhibitory concentration (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

1. Introduction

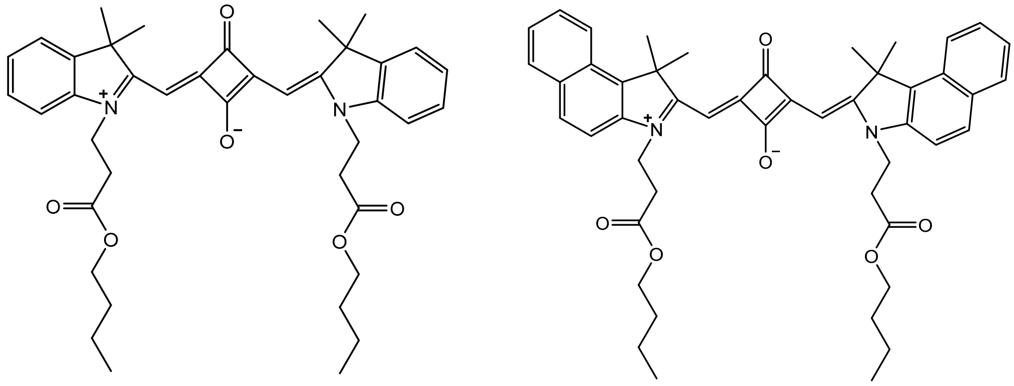
Squarylium cyanine dyes are 1,3-disubstituted compounds, owning a resonance stabilized zwitterionic structure, which presents a central four-membered ring derivative of squaric acid (3,4-dihydroxycyclobut-3-en-1,2-dione) and two electron donor groups at the end of the polymethine chain [1–4]. This class of compounds presents good photochemical stability, narrow and intense absorption, emission bands in the visible to near infrared region, a high molar extinction coefficient and good photoconductivity [5–8]. In the last few years, interest in these polymethine dyes has been increasing, specially 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 benz[*e*]indole possessing ester groups in the chains attached to nitrogen atoms were synthetized. The structural elucidation of the dyes, as well as of their precursors, was performed using the usual analytical techniques. Fundamental photophysics studies in ethanol, in addition to determination of the antifungal activity of the dyes were carried out.

2. Results and Discussion

2.1. Synthesis of squarylium cyanine dyes 1 and 2

Condensation reaction between 1-(2-carboxyethyl)-2,3,3-trimethylindol-1-ium bromide or 1-(2-carboxyethyl)-2,3,3-trimethylbenzo[*e*]indol-1-ium bromide, as quaternary ammonium salts, and 3,4-dihydroxycyclobut-3-ene-1,2-dione, using *n*-butanol and toluene at reflux in 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. It should be noted that during the condensation reaction mentioned above, the esterification of the carboxylic groups of the *N*-alkyl chain of the quaternary salts occurred, as can be confirmed by analysis of ^{1}H and ^{13}C NMR spectra of both dyes.



1 **2**

Figure 1. Chemical structures of the synthetized and tested squarylium cyanine dyes **1** and **2**

The ^{1}H NMR spectrum of the cyanine **1** showed aromatic protons from the heterocyclic system at δ 7.08–7.35 ppm and the spectrum of cyanine **2** at δ 7.42–8.20 ppm. The methine protons appeared at δ 5.93 ppm for **1** and at δ 6.00 ppm for **2** as a singlet of two protons. As expected, the ^{1}H NMR spectra of synthesized dyes **1** and **2** presented only a sole methine proton 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 ($N(CH_2)_2COOCH_2CH_2C\mathit{H}_2CH_3$), δ 1.52–1.54 ppm ($N(CH_2)_2COOCH_2C\mathit{H}_2CH_2CH_3$), δ 2.82–2.90 ppm ($NCH_2C\mathit{H}_2COO(CH_2)_3CH_3$), δ 4.05 ppm ($NC\mathit{H}_2CH_2COO(CH_2)_3CH_3$) and δ 4.34–4.47 ppm ($N(CH_2)_2COOC\mathit{H}_2CH_2CH_2CH_3$). The terminal methyl groups of chain linked to the nitrogen atom emerged at δ 0.84–0.89 ppm ($N(CH_2)_2COO(CH_2)_3C\mathit{H}_3$), while the methyl groups of heterocyclic rings arise at δ 1.78 and 2.07 ppm ($C(C\mathit{H}_3)_2$).

The ^{13}C NMR spectra exhibited aromatic carbons in the form of four signals at δ 109.68–127.99 ppm, for cyanine **1** and six signals at δ 110.31–129.84 ppm for cyanine **2**. Methyl groups of the molecules are presented at δ 13.70 and 13.78 ppm ($N(CH_2)_2COO(CH_2)_3CH_3$) and δ 26.95 and 27.25 ppm ($C(CH_3)_2$). Methine groups arise at δ 86.93 and 86.67 (CH=C) ppm and methylene groups of both compounds present very similar chemical shifts, emerging at δ 19.12 and 19.17 ppm ($N(CH_2)_2COOCH_2CH_2CH_2CH_3$), δ 30.55 and 30.57 ($N(CH_2)_2COOCH_2CH_2CH_2CH_3$) ppm, δ 31.75 and 32.01 ppm ($NCH_2CH_2COO(CH_2)_3CH_3$), δ 39.42 and 39.52 ppm ($NCH_2CH_2COO(CH_2)_3CH_3$), and δ 65.25 and 65.26 ppm ($N(CH_2)_2COOCH_2CH_2CH_2CH_3$).

2.2. Fundamental Photophysics of Dyes 1 and 2

The absorption and fluorescence properties of the synthetized squarylium cyanine dyes **1** and **2** were evaluated in ethanol. The relative fluorescence quantum yields were determined using zinc phthalocyanine as a reference (Φ_F = 0.17 in dimethylformamide) [18] and using an excitation wavelength of 580 nm. All the results are shown 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	81	8
2	663	324,591	672	26	9

Cyanine **2** showed maximum absorption wavelength (λ_{abs}) and maximum emission wavelength (λ_{em}) superior to cyanine **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 cyanines **1** and **2** in ethanol are shown in Figures 2 and 3, respectively.

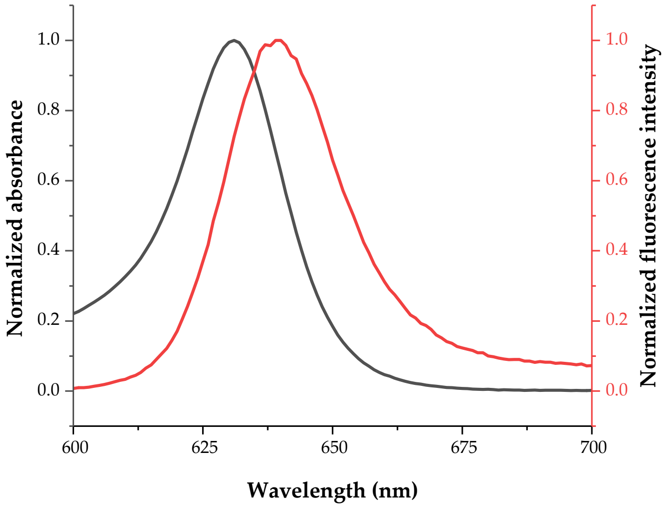


Figure 2. Normalized absorption (dark line) and fluorescence spectra (red line) of squarylium cyanine dye **1** in ethanol.

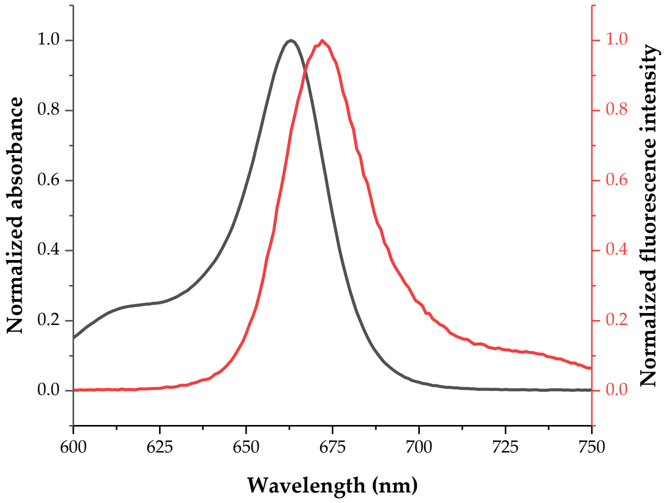


Figure 3. Normalized absorption (dark line) and fluorescence spectra (red line) of squarylium cyanine dye **2** in ethanol.

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

The antifungal activity of squarylium 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. Minimum inhibitory concentration (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

Cyanine **1** has a MIC value superior to 100 µM, while cyanine **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. Cyanine **2** was found to have a higher Log P value, which will be related to its 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-trimethylindol-1-ium bromide (0.400 g, 1.28 mmol) and 3,4-dihydroxycyclobut-3-ene-1,2-dione (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 Na_2SO_4 and the solvent removed under reduced pressure. After successive recrystallizations from CH_2Cl_2/ MeOH/ petroleum ether/ diethyl ether, 4-[(1-(2-butoxycarbonylethyl)-3,3-dimethyl-3*H*-indol-1-ium-2-yl)methylene]-2-[(1-(2-butoxycarbonylethyl)-3,3-dimethylindolin-2-ylidene)methyl]-3-oxocyclobut-1-en-1-olate (**1**) was obtained as a light blue solid (0.059 g, 17%). ^1H NMR (600.10 MHz, $CDCl_3$) 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, C*H*=C), 4.34 (2H, bs, $N(CH_2)_2COOC\mathit{H}_2CH_2CH_2CH_3$), 4.05 (4H, t, *J* = 6.6 Hz, $NC\mathit{H}_2CH_2COO(CH_2)_3CH_3$), 2.82 (4H, t, *J* = 7.5 Hz, $NCH_2C\mathit{H}_2COO(CH_2)_3CH_3$), 1.78 (12H, s, $C(CH_3)_2$), 1.54 (4H, qt, *J* = 7.0 Hz $N(CH_2)_2COOCH_2C\mathit{H}_2CH_2CH_3$), 1.31 (4H, st, *J* = 7.4 Hz, $N(CH_2)_2COOCH_2CH_2C\mathit{H}_2CH_3$), 0.89 (6H, t, *J* = 7.5 Hz, $N(CH_2)_2COO(CH_2)_3C\mathit{H}_3$) ppm. ^{13}C NMR (150.91 MHz, $CDCl_3$) 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 (CH_2), 49.51 $\mathit{C}(CH_3)_2$, 39.40 (CH_2), 31.75 (CH_2), 30.57 (CH_2), 27.25 ($C(\mathit{C}H_3)_2$), 19.17 (CH_2), 13.78 (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 dimethyl sulfoxide (DMSO) and then dissolved in RPMI (Roswell Park Memorial Institute) 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 correspond 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 cyanine **1** derived from 2,3,3-trimethylindolenine. Both cyanine dyes revealed antifungal activity, although dye **2** derived from 1,1,2-trimethyl-1*H*-benz[*e*]indole showed 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 as antifungal agents.

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References

1. Reis: L.V.; Serrano, J.P.; Almeida, P.; Santos, P.F. The synthesis and characterization of novel, aza-substituted squarylium cyanine dyes. *Dyes Pigments* **2009**, *81*, 197–202.
2. Yano, S.; Hirohara, S.; Obata, M.; Hagiya, Y.; Ogura, S.; Ikeda, A.; Kataoka, H.; Tanaka, M.; Joh, T. Current states and future views in photodynamic therapy. *J. Photochem. Photobiol. C* **2011**, *12*, 46–67.
3. Sreejith, S.; Carol, P.; Chithra, P.; Ajayaghosh, A. Squaraine dyes: A mine of molecular materials. *J. Mater. Chem.* **2008**, *18*, 264–274.
4. Pisoni, D.S.; Abreu, M.P.; Petzhold, C.L.; Rodembusch, F.S.; Campo, L.F. Synthesis, photophysical study and BSA association of water-insoluble squaraine dyes. *J. Photochem. Photobiol. A* **2013**, *252*, 77–83.
5. Martins, T.D.; Pacheco, M.L.; Boto, R.E.; Almeida, P.; Farinha, J.P.S.; Reis, L.V. Synthesis, characterization and protein-association of dicyanomethylene squaraine dyes. *Dyes Pigments* **2017**, *147*, 120–129.
6. Liu, L.; Chen, J.; Ku, Z.; Li, X.; Han, H. Unsymmetrical squaraine sensitizers containing auxiliary arylamine donor for NIR-harvesting on dye-sensitized solar cell. *Dyes Pigments* **2014**, *106*, 128–135.
7. Park, J.; Viscardi, G.; Barolo, C.; Barbero, N. Near-infrared Sensitization in Dye-sensitized Solar Cells. *Chimia* **2013**, *67*, 129–135.
8. Avirah, R.R.; Jayaram, D.T.; Adarsh, N.; Ramaiah, D. Squaraine dyes in PDT: From basic design to in vivo demonstration. *Org. Biomol. Chem.* **2012**, *10*, 911–920.
9. Al-horaibi, S.A.; Asiri, A.M.; El-Shishtawy, R.M.; Gaikwad, S.T.; Rajbhoj, A.S. Synthesis and characterization of new squaraine dyes with bis-pendent carboxylic groups for dye-sensitized solar cells. *J. Mol. Struct.* **2019**, *1195*, 850–858.
10. Chen, G.; Sasabe, H.; Igarashi, T.; Hong, Z.; Kido, J. Squaraine dyes for organic photovoltaic cells. *J. Mater. Chem. A* **2015**, *3*, 14517–14534.

11. Fan, B.; Maniglio, Y.; Simeunovic, M.; Kuster, S.; Geiger, T.; Hany, R.; Nüesch, F. Squaraine Planar-Heterojunction Solar Cells. *Int. J. Photoenergy* **2009**, *2009*, 581068.
12. Gomes, V.S.D.; Gonçalves, H.M.R.; Boto, R.E.F.; Almeida, P.; Reis, L.V. Barbiturate squaraine dyes as fluorescent probes for serum albumins detection. *J. Photochem. Photobiol. A* **2020**, *400*, 112710.
13. Saikiran, M.; Sato, D.; Pandey, S.S.; Kato, T. Photophysical investigations of squaraine and cyanine dyes and their interaction with bovine serum albumin. *J. Phys. Conf. Ser.* **2016**, *704*, 012012.
14. He, Q.; Fan, X.; Sun, S.; Li, H.; Pei, Y.; Xu, Y. Highly selective turn-on detection of (strept)avidin based on self-assembled near-infrared fluorescent probes. *RSC Adv.* **2015**, *5*, 38571–38576.
15. Lima, E.; Ferreira, O.; Gomes, V.S.D.; Santos, A.O.; Boto, R.E.; Fernandes, J.R.; Almeida, P.; Silvestre, S.M.; Reis, L.V. Synthesis and in vitro evaluation of the antitumoral phototherapeutic potential of squaraine cyanine dyes derived from indolenine. *Dyes Pigments* **2019**, *167*, 98–108.
16. Lima, E.; Ferreira, O.; Silva, J.F.; Santos, A.O.; Boto, R.E.; Fernandes, J.R.; Almeida, P.; Silvestre, S.M.; Reis, L.V. Photodynamic activity of indolenine-based aminosquaraine cyanine dyes: Synthesis and in vitro photobiological evaluation. *Dyes Pigments* **2020**, *174*, 108024.
17. Friães, S.; Silva, A.M.; Boto, R.E.; Ferreira, D.; Fernandes, J.R.; Souto, E.B.; Almeida, P.; Ferreira, L.F.V.; Reis, L.V. Synthesis, spectroscopic characterization and biological evaluation of unsymmetrical aminosquarylium cyanine dyes. *Bioorg. Med. Chem.* **2017**, *25*, 3803–3814.
18. Ogunsipe, A.; Maree, D.; Nyokong, T. Solvent effects on the photochemical and fluorescence properties of zinc phthalocyanine derivatives. *J. Mol. Struct.* **2003**, *650*, 131–140.