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PII: S0143-7208(19)32907-9

DOI: https://doi.org/10.1016/j.dyepig.2020.108193

Reference: DYPI 108193

To appear in: Dyes and Pigments

Received Date: 11 December 2019

Revised Date: 7 January 2020

Accepted Date: 7 January 2020

Please cite this article as: Coelho FL, da Costa Duarte R, de Ávila Braga Clá, Toldo JM, Bruno Gonçalves PF, da Silveira Santos F, Rodembusch FS, Benzothiazole merocyanine dyes as middle pH optical sensors, *Dyes and Pigments* (2020), doi: https://doi.org/10.1016/j.dyepig.2020.108193.

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Abstract

Substituted merocyanine dyes were obtained by Knoevenagel condensation between formylated arylbenzothiazole and guaternary 2-alkylbenzothiazoles. The condensations were performed in the presence of sodium methoxide and the products were obtained with excellent yields after simple purification. The merocyanines presented absorption maxima in the range 576-605 nm (green to orange-red region). The calculated Strickler-Berg parameters indicated fully spin and symmetry allowed electronic transitions with ${}^{1}\pi$ - π * character. These compounds show significant fluorescence emission at the orange red region with a very small Stokes shift. Surprisingly, although the benzothiazole core possesses a structural moiety (o-hydroxyaryl moiety) which should facilitate proton transfer in the excited state, no evidence was found of this phototautomerism in the compounds studied. TD-DFT calculations show that ESIPT is not probable to happen due to the high barriers to transfer the proton. In addition, the aliphatic chain does not affect the absorption and emission maxima location. For both, ground and excited states, a negative solvatochromic effect was observed. Theoretical calculations also show that this effect is due to the larger dipole moment in the S_0 than in the S_1 state. In addition, these compounds presented large shifts on the absorption spectra

changing the pH (yellow to violet color), which allowed studying successfully as optical sensor for middle pH values.

Keywords: ESIPT, optical sensor, benzothiazole, merocyanine, colorimetric sensor

1. Introduction

Merocyanines can be classified as polymethine dyes that are characterized by neutral unsymmetrical structures composed of a nitrogen donor and an oxygen acceptor moieties linked by one or more ethylene group [1]. These compounds present photophysical and photochemical properties such as pronounced solvatochromism, high photostability and nonlinear optical and electrical responses [2]. These properties can be related to the charge transfer from donor to acceptor fragments through the polymethine chain and are modulated depending on the substitution of end and lateral groups [2]. In this sense, several applications can be envisaged for these structures, including optical data storage [3], solvatochromic sensor [4], biological markers [5-8], photodynamic therapy [9-11] and solar cell devices (Figure 1) [12,13].



Figure 1. Selected examples of merocyanine derivatives and their main applications presented in the literature and the merocyanines presented in this work.

It can be found in the literature that the synthetical approaches to merocyanine vary depending on the number of methylene groups in the polymethine chain [14]. The general synthetic protocol to merocarbocyanines (n = 1) involves Knoevenagel condensation between heterocyclic enamines or their corresponding salts with substituted aldehydes [15], while the preparation of simple merocyanines (n = 0) is mainly performed through condensation of nitrogen heterocycles containing leaving groups in the 2- or 4-positon with activated methylene group [16,17]. In case of meropolycarbocyanines (n > 1), similar methods are used according to structural feature and reactivity of the reactants [18,19].

In general, synthetic protocols for these dyes are robust and side reactions and byproducts formation are not reported. Moreover, the broad accessibility of starting materials, as well as the easy isolation of products, in contrast with polymethine chain analogues (meropolycarbocyanines) become their obtention more attractive [1,2].

For specific application as optical sensor for pH, the acidity constant plays a fundamental role, where the pKa value is a fundamental parameter to predict the ionization state concerning the pH [20-22]. However, organic dyes are poorly water-soluble compounds. In order to overcome this drawback, that difficult the pKa determination, mixtures of aqueous-organic solvents are used. In this methodology, it is reported in the literature polar protic cosolvents [23-25], as well as aprotic media, such as dimethylsulfoxide [26], *N*,*N*-dimethylformamide [27], or acetonitrile [28]. In this sense, ethanol/water solutions usually 1:1 (v/v) presents very good results for poorly water-soluble compounds [27,29,30], where the cosolvent ionization constant (pK_a) are measured and the ionization constant in water (wpK_a) is obtained by extrapolation [31-34].

In this way, this investigation focus on the synthesis and photophysical characterization in solution of merocyanine dyes containing a proton-transfer moiety aiming an optical sensing application allowed by the chemical structure of these compounds. In addition, their photophysics in the ground state was evaluated against pH in order to obtain their ionization constants, as well as to explore their ability as optical sensors for pH. Theoretical calculations were also

applied to better understand their photophysics and also the unprecedented absence of proton transfer reaction in the excited state.

2. Experimental Section

2.1 Materials and methods

Purification by column chromatography was carried out on silica gel 60 (230–400 mesh). Analytical thin-layer chromatography (TLC) was conducted on aluminum plates with 0.2 mm of silica gel 60F-254. The solvents were purified using standard procedures [35] or used as received. Spectroscopic grade solvents (Merck or Aldrich) were used for the photophysical characterization (UV-Vis absorption and fluorescence emission). FTIR spectra were recorded on a Varian 640-IR using KBr disc or at ATR mode. ¹H (400 MHz) and ¹³C (100 MHz) NMR were obtained in $CDCI_3$ or $DMSO-d_6$ on a Bruker Avance III NMR spectrometer. The chemical shifts are expressed as δ (ppm) relative to tetramethylsilane (TMS). Splitting patterns are designed as follows; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet. All of the detected signals were in accordance with the proposed structures. UV-Vis absorption spectra were obtained using a Shimadzu UV-2450 spectrophotometer. Steadyfluorescence measured Shimadzu spectra using а state were spectrofluorometer model RF-5301PC. Fluorescence quantum yields (QY) were measured at 25°C using solutions at the optical dilute regime and using Quinine sulfate in H₂SO₄ 1 N as the quantum yield standards [36]. The ionization constant was obtained in accordance with the literature [28]. The pH values were measured on a Digimed DM-22 pH-meter with flow-through glass-silver chloride electrodes filled with a KCI saturated solution. The ionization constants were determined by UV-Vis absorption spectroscopy [23,33,34,29,30,37]. The UV-Vis titrations were performed with constant ionic strength of the solutions $(\mu=0.1)$ by the addition of KCI (0.15 M) under nitrogen atmosphere at 25°C [38]. The measurements were performed in EtOH/H₂O mixtures (1:1 v/v) with different pH values. The initial solution was acidified using HCI (1 Molar) up to pH 0.5 followed by additions of different amounts of KOH (1 Molar) up to pH 14. Stock solutions of the studied in methanol (~10⁻⁶M) were prepared. Different amounts of these solutions were added to the previous ethanol/water mixture at different pH. The calculations were carried out minimizing the sum of squared

residuals of the absorbance values as a function of the wavelength under different pH conditions by Boltzmann Function. The pH value vs. absorbance data was adjusted with good correlation coefficients, where the inflection point indicates where the pH=pK_a in the Henderson-Hasselbach equation [39].

2.2 Synthesis and characterization

2-(2'-hydroxy-3'-methylphenyl)benzothiazole (3)

In a round-bottom vessel 3-methylsalicylic acid (**2**) (5.5 mmol) and aminothiophenol (**1**) (5.0 mmol) were added. Then polyphosphoric acid (12 mL) was added and the viscous mixture was stirred at 120°C for 2 h. The temperature was increased to 170°C and stirring was continued for another 3 h. After this, the reaction mixture was cooled and crude poured into ice-cold water and neutralized with NaHCO₃. The precipitate was filtered and crude solid was dried prior to purification by column chromatography using AcOEt/hexane (5:95) as eluent. The desired product was obtained as a white solid with 62% yield (0.364 g). M.p. = 134-136 °C. ¹H NMR (400 MHz, CDCl₃, δ ppm) 12.77 (s, 1H), 7.95 (d, *J*=8.05 Hz, 1H), 7.87 (d, *J*=7.97 Hz, 1H), 7.53 (d, *J*=7.88 Hz, 1H), 7.48 (dt, *J*=7.2, 1.2 Hz, 1H), 7.38 (dt, *J*=7.2, 1.1 Hz, 1H), 7.26-7.22 (m, 1H), 6.85 (t, *J*=7.6 Hz, 1H), 2.35 (s, 3H). ¹³C NMR (100 MHz, CDCl₃), (400 MHz, CDCl₃, δ ppm) 169.8, 156.3, 151.8, 133.7, 132.7, 126.9, 126.6, 125.4, 122.0, 121.5, 119.0, 116.0, 16.0. FTIR (ATR, v = cm⁻¹) 1618, 1260. HRMS (ESI/Q-TOF) m/z: [M + H]⁺ calcd for C₁₄H₁₂NOS 242.0640; found 242.0635.

2-(5'-formyl-2'-hydroxy-3'-methylphenyl)benzothiazole (4)

In Schlenk vessel, compound 3 (0.15 а mmol) and hexamethylenetetramine (10 eq.) were added followed by the addition of trifluoroacetic acid (2.5 mL). The mixture was heated at reflux for 5 h. The progress of the chemical reaction was monitored by TLC. Water (2.5 mL) was added and the mixture was refluxed for 60 min. The reaction mixture was kept overnight at 5°C. The obtained precipitate was filtered in silica, washed with water, extracted in ethyl acetate affording desired product as a pale yellow solid with 84% yield (0.038 g). M.p. = 180-186°C. ¹H NMR (400 MHz, CDCl₃, δ ppm) 13.42 (s, 1H), 9.78 (s, 1H), 7.90-7.81 (m, 3H), 7.63 (s, 1H), 7.48-7.34 (m, 2H),

2.32 (s, 3H). ¹³C NMR (100 MHz, CDCl₃, δ ppm) 189.9, 168.4, 161.3, 151.0, 133.7, 132.4, 128.6, 128.0, 127.9, 126.7, 125.8, 122.0, 121.4, 115.9, 15.9. FTIR (ATR, $\nu = \text{cm}^{-1}$) 1687, 1610, 1273. HRMS (ESI/Q-TOF) m/z: [M + H]+ calcd for C₁₅H₁₂NO₂S 270.0589; found 270.0587.

2.3 General protocol for quaternary indoles 6-7

To degassed *N*,*N*-dimethylformamide (15 mL) was added 2methylbenzo[*d*]thiazole (0.20 g, 1.34 mmol) and respective alkyl iodide (8.0 mmol) under N₂ atmosphere. The reaction mixture was stirred at 55 °C for 24h. Crude was diluted with ethyl acetate (50 mL) and the precipitate was filtered and washed with hot ethyl acetate (100 mL).

2,3-dimethylbenzo[*d*]thiazol-3-ium iodide (6)

Yield: 90 %. ¹H NMR (400 MHz, DMSO- d_6 , δ ppm) 8.45 (d, J=8.4 Hz, 1H), 8.27 (d, J=8.4 Hz, 1H), 7.85 (t, J=8.5 Hz, 1H), 7.76 (t, J=8.5 Hz, 1H), 4.20 (s, 3H), 3.19 (s, 3H). ¹³C NMR (100 MHz, CDCl₃, δ ppm) 177.5, 142.0, 129.7, 129.1, 128.5, 125.0, 117.2, 37.1, 18.1.

2-methyl-3-octylbenzo[*d*]thiazol-3-ium iodide (7)

Yield: 44 %. ¹H NMR (400 MHz, CDCl₃, δ ppm) 8.49 (d, *J*=8.2 Hz, 1H), 8.10 (d, *J*=8.5 Hz, 1H), 7.82 (t, *J*=8.5 Hz, 1H), 7.72 (t, *J*=8.5 Hz, 1H), 4.85 (d, *J*=9.9 Hz, 2H), 3.44 (s, 3H), 2.05-1.88 (m, 2H), 1.50 (m, 2H), 1.37-1.19 (m, 8H), 0.91-0.79 (m, 3H). ¹³C NMR (100 MHz, CDCl₃, δ ppm) 174.6, 140.8, 130.0, 129.0, 128.6, 125.0, 116.5, 51.3, 31.6, 29.0, 28.9, 28.7, 26.7, 22.4, 19.8, 14.0.

2.4 General procedure for merocyanine dyes 8-9

A solution of **4** (0.05 g, 0.18 mmol) in ethanol (10 mL) was added dropwise to a previously prepared solution of corresponding quaternary indole **6-8** (0.22 mmol) and sodium methoxide (0.011 g, 0.20 mmol) in ethanol (5 mL). The reaction mixture was stirred under reflux for 24h. Desired product precipitate as a fine powder and was centrifuged and washed with cold ethanol. No additional purification was performed.

(*E*)-2-(3-(benzo[*d*]thiazol-2-yl)-4-hydroxy-5-methylstyryl)-3methylbenzo[*d*]thiazol-3-ium iodide (**8**)

Yield: 97%. ¹H NMR (400 MHz, DMSO- d_6 /TFA, δ ppm) 8.38 (d, *J*=7.4 Hz, 2H), 8.34 (s, 1H), 8.26 (d, *J*=15.8 Hz, 1H), 8.19 (m, 2H), 8.09 (d, *J*=7.9 Hz, 1H), 7.91 (d, *J*=15.8 Hz, 1H), 7.83 (m, 1H), 7.73 (t, *J*=7.2 Hz, 1H), 7.56 (m, 1H), 7.48 (m, 2H), 4.34 (s, 3H), 2.35 (s, 3H). ¹³C NMR (100 MHz, DMSO- d_6 /TFA, δ ppm) 172.4, 168.2, 159.5, 151.2, 148.5, 142.4, 133.6, 133.1, 130.5, 129.7, 128.6, 128.13, 128.09, 127.5, 126.4, 126.1, 124.5, 122.7, 122.3, 117.1, 117.0, 112.1, 36.6, 16.2. HRMS (ESI) calcd. for C₂₄H₁₉N₂OS₂⁺ (M⁺) 415.0939, found 415.0936.

(*E*)-2-(3-(benzo[*d*]thiazol-2-yl)-4-hydroxy-5-methylstyryl)-3-octylbenzo[*d*]thiazol-3-ium iodide (**9**)

Yield: 78%. ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm) 8.45 (d, *J*=8.1 Hz, 1H), 8.38 (s, 1H), 8.33 (d, *J*=15.7 Hz, 1H), 8.28 (d, *J*=8.5 Hz, 1H), 8.28 (m, 2H), 8.12 (d, *J*=5.4 Hz, 1H), 7.96-7.86 (m, 2H), 7.78 (t, *J*=11.7 Hz, 1H), 7.6 (td, *J*=5.0, 3.7 Hz, 1H), 7.51 (m, 1H), 4.96 (t, *J*=7.2 Hz, 2H), 2.38 (s, 3H), 1.86 (m, 2H), 1.41 (m, 2H), 1.32 (m, 2H), 1.20 (s, 6H), 0.78 (t, *J* = 6.71 Hz, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm) 172.2, 168.1, 151.2, 149.0, 141.6, 133.5, 133.2, 131.0, 129.9, 128.7, 128.5, 128.2, 127.6, 126.5, 126.1, 124.8, 122.9, 122.8, 122.5, 122.3, 117.2, 111.5, 49.1, 31.6, 29.1, 29.0, 28.9, 26.1, 22.5, 16.4, 14.34. HRMS (ESI) calcd. for $C_{31}H_{33}N_2OS_2^+$ (M⁺) 513.2034, found 513.2031.

2.5 Theoretical calculations

In this investigation, merocyanine **8** was used as a model. In this sense, the structures of the ground (S_0) and first excited (S_1) state were optimized using Density Functional Theory (DFT) and Time-dependent DFT (TD-DFT), respectively. For the optimizations, the functional B3LYP [40] including D3 Grimme's dispersion corrections was used together with 6-31G(d,p) basis set [41]. The equilibrium geometries were verified by vibrational analysis at the same level of theory. At the equilibrium geometries, single-point calculations were done at B3LYP-D3/6-311+G(d,p) level of theory. The vertical absorption and emission energies were benchmarked against the experimental values

using several different DFT functionals. The better agreement between those values was found at B3LYP-D3/6-311+G(d,p) level. The solvent effects were included for the optimizations and vertical transitions by using the integral equation formalism of the polarizable continuum model (IEF-PCM) and the dielectric constants corresponding to 1,4-dioxane, dichloromethane, methanol, and acetonitrile [42,43,44]. The ground and excited states molecular electrostatic potential surfaces (MEP), charges, and dipole moment were obtained by population analysis using the Charges from Electrostatic Potential using a Grid-based method ChelpG [45]. The frontier molecular orbitals and molecular structures were plotted using Chemcraft Software [46]. To evaluate the intramolecular proton transfer, relaxed scans were done in the S₁ state at B3LYP/6-31G(d,p) level employing 1,4-dioxane and acetonitrile for the solvent effect. For those, the geometries were optimized by increasing the O-H bond distance by 0.05 Å each step. All DFT and TD-DFT calculations were performed using Gaussian 16 Revision A.03 [47].

3. Results and Discussion

3.1 Synthesis

The merocyanine dyes **8-9** investigated in this work were synthesized by condensation reaction between formylated benzothiazole **4** and quaternary benzothiazole ammonium salts **6-7** as shown in Scheme 1. Preparation of **4** was performed through the previously described protocol in two steps [48]. First, the classical method of condensation between *o*-aminothiophenol (**1**) and 3-methylsalicylic acid (**2**), in equimolar amounts, in polyphosphoric acid (PPA) leads to formation of benzothiazole **3** [49]. A second step of Duff formylation reaction with hexamethylenetetramine (HMTA) in trifluoroacetic acid (TFA) followed by the addition of water afforded formylated benzothiazole **4** with 84 % yield. Noteworthy, formylation only occurs in 5' position since ortho positions of the phenolic ring are substituted with a methyl group and a benzothiazole moiety [50].

The quaternary benzothiazole ammonium salts were obtained by a substitution reaction between 2-methylbenzothiazole (5) and suitable alkylation agents. For compounds 6 and 7 either methyl iodide (90% yield) or octyl iodide (44% yield)

respectively were used. Lastly, condensation of **4** with **6** and **7** yielded merocyanine dyes **8** and **9** in 97 and 78%, respectively, after isolation by precipitation. The reactions were performed in ethanol (efflux temperature) and required one equivalent of base. Besides sodium methoxide, other bases as sodium hydroxide and sodium carbonate were also tested and no product was detected by TLC after 24 hours. During the reaction pathway, a color changing of crude reaction from yellow to reddish is observed. It is highlighted the presence of two doublets in ¹H NMR spectra, for both merocyanines, attributed to olefinic hydrogens with coupling constant (*J*) about 15.7 Hz that is in agreement with the proposed *trans* conformer.



Scheme 1. Synthetic route for the preparation of compounds 8 and 9.

3.2 Photophysical study

The electronic properties of the compounds **8** and **9** were evaluated by UV-Vis absorption and fluorescence emission spectroscopies in organic solvents with different dielectric constants. The obtained photophysical data are summarized in Table 2. In this investigation, the extinction coefficient ε was correlated to the oscillator strength f_e by the Strickler-Berg relations [51]. The rate constant k_e^0 was also obtained using Equation 1, where the integral is related to the area under the absorption curve corresponding to a single electron oscillator. In addition, the pure radiative lifetime τ^0 was also calculated, defined as $1/k_e^0$ [52].

$$k_e^0 \approx 2.88 x 10^{-9} \bar{v}_0^2 \int \varepsilon d\bar{v}$$
, (1)

Figure 1 presents the UV-Vis spectra from compounds **8** and **9** in different organic solvents. It can be observed that changes on the substituent (methyl or octyl) do not affect the absorption maxima wavelength due to the similar electronic contributions of these lateral groups. The absorption maximum is located between 585-605 nm with a medium negative solvatochromic effect ($\Delta\lambda_{abs}$ =30 nm). From the extinction coefficient and calculated radiative rate constant values, the observed electronic transitions are fully spin and symmetry allowed related to ${}^{1}\pi$ - π^{*} transitions. Moreover, the oscillator strength (*f_e*) values for **8** and **9** show almost unitary values, which corroborates with electronic dipole-allowed transitions, as expected [53]. Practically constant emission lifetime values (~10⁻⁹ s) indicate that after the absorption, the compounds populate the same excited state.



Figure 2. UV-Vis absorption spectra of **8** and **9** in different solvents [$\sim 10^{-6}$ M].

The normalized fluorescence emission spectra of compounds 8 and 9 are presented in Figure 2. The fluorescence emission data were obtained using the absorption maxima as excitation wavelengths. The relevant data from this investigation are also presented in Table 2. The studied compounds present emission maxima located between 610-628 nm, once again, with a small negative solvatochromic effect. Despite the presence of the 2-(2'hydroxyphenyl)benzothiazole core, able to proton-transfer in the excited state (ESIPT) (Scheme 2), the studied merocyanines present a main and sharp fluorescence emission with small Stokes shift (<1000 cm⁻¹). This result is contrary to expectations for ESIPT-containing compounds where a dual fluorescence emission or even a main emission at longer wavelengths is observed [54,55]. Surprisingly, this result indicates that the fluorescence emission arises from a locally excited state, related to an enol conformer (Scheme 2), discarding the possibility of proton transfer as effective deactivating channel. This latter would lead to an emission with very large Stokes shift, usually called as keto or ESIPT emission (Scheme 2).

Moreover, studies with very similar structures indicate that proton transfer occurs in organic media and in PBS buffer [56-58], unlike what was observed in this study. Thus, in this case, it is believed that the substitution pattern in the phenolic ring tailor significantly the photophysical properties in these "virtuallyable-to-proton-transfer" merocyanines, indicating once again that the ESIPT

11

reaction is a particular and always interesting theme of investigation. It is worth mentioning that excitation at higher energy (~400 nm) produced fluorescence emission with the same maxima location, as expected for a compound that follows the Kasha's rule. In this way, these additional blueshifted absorption bands can be related to electronic transitions to excited states higher than S_1 (see ESI).



Figure 3. Normalized fluorescence emission spectra of **8** and **9** in different solvents [$\sim 10^{-6}$ M] (Exc./Em. slits 5.0nm/5.0 nm).



Scheme 2. Förster cycle for the ESIPT process using compound **8** as a model. The asterisk indicates the excited state and E and K indicate enol conformer and the keto tautomer, respectively.

	Dee			
		\mathbf{pr}		

Table 2. General photophysical data of merocyanines **8** and **9**, where ε is the molar extinction coefficient (x10⁴ M⁻¹·cm⁻¹), λ_{abs} is the absorption maxima (nm), f_e is the calculated oscillator strength, k_e^0 is the calculated radiative rate constant (10⁸ s⁻¹), τ^0 is the calculated pure radiative lifetime (ns), λ_{em} is the emission maxima (nm), $\Delta\lambda_{ST}$ is the Stokes shift (nm/cm⁻¹) and QY is the fluorescence quantum yield (%).

Merocyanine	Solvent	3	λ_{abs}	f _e	k ⁰ e	τ ⁰	λ_{em}	$\Delta\lambda_{ST}$	QY
	1,4-Dioxane	17.7	604	0.88	2.42	4.13	628	24/633	0.6
Q	Dichloromethane	3.96	599	0.85	2.38	4.20	622	23/617	1.9
0	Ethanol	17.3	576	0.90	2.72	3.68	611	35/994	1.5
	Acetonitrile	8.99	585	0.76	2.22	4.50	610	25/701	1.2
	1,4-Dioxane	14.8	605	0.83	2.26	4.43	626	21/554	0.8
0	Dichloromethane	2.80	600	0.58	1.61	6.23	621	21/564	1.4
5	Ethanol	11.2	577	0.54	1.62	6.19	611	34/964	1.6
	Acetonitrile	1.17	585	0.52	1.52	4.13	610	25/701	1.7

In order to investigate the presence of an intramolecular charge transfer (ICT) mechanism in these compounds, the Lippert-Mataga correlation was applied [59]. In this methodology, a solvent polarity plot function is obtained [59,60], allowing the calculation of the difference in the dipole moment between the excited and ground states ($\Delta \mu_{eg}^2$) (Equation 2). The linear plot of the absorbance, fluorescence emission or Stokes shift versus the solvent polarity function indicates the intramolecular charge transfer character [61].

$$\Delta \bar{v}_{st} = \frac{2\Delta \mu_{eg}^2}{hca^3} \Delta f + \Delta \bar{v}_0 \quad (2)$$

In Equation 2, *h* is the Planck's constant, *c* is the speed of light and *a* is the Onsager cavity radius. Aditionally, the orientation polarization function (Δf) can be obtained from Equation 3 [62]:

$$\Delta f = \frac{(\varepsilon - 1)}{(2\varepsilon + 1)} - \frac{(n^2 - 1)}{(2n^2 + 1)}, \quad (3)$$

where the dielectric constant (ε) and the refractive index (n) for a mixture of solvents can be calculated by Equations (4) and (5), where f_A and f_B are the volumetric fractions of the two solvents.

$$\varepsilon_{mix} = f_A \cdot \varepsilon_A + f_B \cdot \varepsilon_B (4)$$
$$n_{mix}^2 = f_A n_A^2 + f_B n_B^2 (5)$$

From the Lippert-Mataga correlation applied in the synthesized compounds **8** and **9**, there is no evidence for charge transfer in the studied merocyanines in both ground and excited states (data not shown, see ESI), which corroborates with the emission from the locally excited states. In addition, it can be observed that these compounds are more destabilized in more polar environments.

3.3 Ionization constant

The ionization constant (K_a) of these compounds was obtained by the Henderson-Hasselbach relation (Equation 2) [32,63] from the acid-base equilibrium in ethanol/water solutions (1:1 v/v).

$$pH = pK_a + log \frac{(A-A_a)}{(A_b-A)}$$
, (2)

where A_a and A_b are the absorbances of the protonated and the neutral species in basic media (KOH), respectively. In this equation, A is the absorbance from the protonated and neutral species [64-66]. The determination of the respective ionization constants by UV-Vis absorption spectroscopy takes the direct determination of the ratio of the neutral to ionized species into account. In this way, an absorption wavelength must be preferred where the difference between the absorbances of the two species is maximized, as already observed in the literature [20,67]. Thus, Figure 5a presents the UV-Vis absorption of merocyanines 8-9 in ethanol/water solution (1:1 v/v) in two different pH values. The neutral and protonated species show the maximum absorption wavelength located at 418 and 420 nm, respectively. Based on these results, it is believed that 560 (8) nm and 552 (9) nm are the most suitable wavelengths to calculate the pK_a for these compounds. Figure 5b shows the dependence of the absorbance on the pH observed at 560 nm (8) and 552 nm (9), probably due to the presence of the neutral and deprotonated species at different pH values. The obtained results could be fitted using a sigmoidal curve, where the pH values, 6.03 (8) and 6.18 (9), were obtained according to Equation (2) as the inflection point (Table 3). These results indicate that the merocyanines 8-9 can be explored for optical sensing in these pH values.



Figure 5. (a) UV-Vis absorption spectra of compounds **8** and **9** in ethanol/water solution (1:1 v/v) in extreme pH values at 25° C. (b) The plot of the absorbance intensity recorded at indicated wavelength *versus* pH. The dotted line was fitted according to the Boltzmann Function - sigmoidal curve. The colorimetric dependence of compounds **8** and **9** with the pH in 1:1 v/v ethanol/water solution at high (~14) and low pH (~0.5) values by naked eye detection was also presented for comparison..

Table 3. Photophysical data of **8** and **9**, where *Conc*. is the concentration (10^{-6} M) , λ_{abs} is the absorption maxima (nm), pK_a is the ionization constant ethanol/water solution (1:1 v/v) and wpK_a is the ionization constant extrapolated to water media.

Comp.	Conc.	λ _{abs} Neutral form	λ_{abs} Deprotonated form	рК _а	_w pK _a
8	3.61	418	556	6.03	5.82
9	5.15	420	555	6.18	5.97

The ionization constant in water ($_wpK_a$) was determined from the pK_a at the same conditions (ethanol/water solution (1:1 v/v)) by applying Equation (3):

$$\mathsf{pK}_{\mathsf{a}} = {}_{\mathsf{w}}\mathsf{pK}_{\mathsf{a}} - \delta, (3)$$

where δ is equal to 0.21 with 0.1 ionic strength by the addition of 0.15 mol·L⁻¹ KCI [31,32,68-71]. Although non-substituted phenolic compounds present pK_a values around 9, as can be observed in Table 3, pK_a ~6 were obtained, which can be related to the presence of their substituents. Finally, their absorption in the visible region, as well as the significative difference between the neutral and deprotonated species observed on their absorption spectra, make these compounds to present interesting and exploitable colorimetric response with the pH. The protonated specie is yellow with absorption shifted to shorter wavelengths while the deprotonated one is violet.

3.4 Theoretical calculations

Since changes in the lateral group R on the compounds **8** and **9** do not affect the location of the experimental absorption maxima (see Table 2), as already discussed, (TD)-DFT calculations were done only for the merocyanine **8**. In this way, two different optimized conformers were found in the ground and first excited states, which correspond to the sulphur atoms on the benzothiazole ring in *trans* or *cis* position each other. Due to the presence of the hydroxyl group attached to the central ring, enol and keto tautomeric structures were also analyzed in the S₁ state, as expected to ESIPT reactive compounds. The optimized geometries in acetonitrile are shown in Figure 6. The cartesian coordinates in acetonitrile, as well as the optimized structures in dichloromethane, 1,4-dioxane, and ethanol and respective energies can be found as supplementary data.



Figure 6. Optimized geometries (front and lateral view) in acetonitrile for the ground and the first excited state of merocyanine **8**.

In the S₀ state, in all the solvent media investigated, the most stable conformer presents the sulphur atoms oriented towards the same side (Conformer 2, *cis*). This conformer is about 2.2 to 2.6 kcal·mol⁻¹ more stable than the *trans* conformer (Conformer 1). This could be attributed to the more extended conjugation in Conformer 2. In the last one, the hemicyanine moiety is coplanar and conjugated with the phenyl moiety and HBT core through an ethylene bridge, while in Conformer 1 the cyanine moiety is about 30 degrees out-of-plane. As a consequence of the increased conjugation, the ethylene moiety in Conformer 2 has smaller bond length alternation (BLA) [72] than conformer 1 due to the most effective delocalization of π -electrons. The same effect is observed in the S₁ state (for the enol and keto tautomers), where the planar Conformer 2 is even more stable (by 3.3 to 3.5 kcal·mol⁻¹) when compared to Conformer 1. Going from the S₀ to the S₁ state (enol conformer)

the main geometric changes observed are the smaller BLA in the ethylene bridge, as well as changes in the bond lengths in the central part of the molecule comprised between the two thiazole rings (bond lengths are more similar than in the S_0 state). Interestingly, enol tautomer was found to be preferred in 1,4-dioxane by ~2.3 kcal·mol⁻¹, while in the remaining solvents the keto tautomer was favored by 2.3 to 2.7 kcal·mol⁻¹.

The frontier molecular orbitals (FMOs) plotted in acetonitrile are shown in Figure 7. The data in 1,4-dioxane is presented as supplementary material. It can be observed that in the S₀ state, the HOMO is delocalized over the whole molecule, while the LUMO is mainly delocalized over the merocyanine and the vinyl bridge. The vertical electronic transitions have $\pi\pi^*$ character and large oscillator strengths indicating that the S₁ state is the bright state. Interestingly, in the S₁ state, the HOMO associated with the calculation in 1,4-dioxane is slightly different than in the other solvents, being more localized over the HBT unit while in the other solvents, HOMO is delocalized over the whole molecule. This result shows that, in 1,4-dioxane, the S_1 state presents a smaller locally excited (LE) character and it should have some ICT character. Besides polymethine dyes are known to have small Stokes shift (<25 nm) when they do not present excited-state ICT between the donor and acceptor units [73], results indicating ICT were presented by Li et al. for donor-two-acceptor hemicyanine-HBT based fluorophore [74]. Molecule 8 can be classified as one-donor-two-acceptors systems because of the presence of the hydroxyl group. Yet, due to the fact that the electron-donating hydroxyl group can be involved in both ICT and ESIPT processes, deprotonation can modulate both processes.



Figure 7. Frontier Molecular Orbitals (HOMO/LUMO) calculated for both ground and excited (Enol) states in acetonitrile for Conformer 2 of merocyanine **8**.

Despite the presence of 2-(2'-hydroxyphenyl)benzothiazole group, and the large literature about experimental evidence on proton transfer in this fluorophore in different structures [75-80] or media [Error! Bookmark not defined, 81-86], ESIPT was not observed in this compound. This can be explained by the larger barriers for the proton transfer (Figure 8). In fact, the calculations in 1,4-dioxane show again a different pattern, where the enol is more stable than keto tautomer, in contrast to the calculations in the other solvents. However, in all the cases, the presence of a barrier larger than 2.0 kcal·mol⁻¹ suggests that the enol tautomer should be the most probable one. In addition, looking at the charge distribution on the nitrogen and oxygen atoms potentially involved in the ESIPT (see Table S3), it can be seen that the negative charge on the oxygen atom slightly decreases in the S₁ state while the charge in the nitrogen is less affected. The small change in the charge distribution in N_1 and O_1 also indicates that excited state intramolecular proton transfer in this molecule is not probable to happens.



Figure 8. Potential energy surface in 1,4-dioxane and acetonitrile for the intramolecular proton transfer in the S_1 state.

The molecular electrostatic potential (MEP) surfaces of compound **8** in acetonitrile show that there is no substantial separation in the overall charge distribution in such media (Figure 9). Similar behavior was observed in 1,4-dioxane (data not shown, see Figure S29). This result agrees with the very small solvatochromic effect experimentally observed. When comparing the MEP surfaces in the S₀ and S₁ states, it is clear that the charge separation is higher in the S₀ than in the S₁ state. This is reflected on the larger dipole moment in the ground state ($\mu_{dioxane}$ = 13.0; $\mu_{acetonitrile}$ = 15.9) than in the excited state ($\mu_{dioxane}$ = 7.6; $\mu_{acetonitrile}$ = 7.4) and in the consequent small negative solvatochromic effect observed (see Table S2). In this case, solvents with higher polarity stabilize the S₀ better than the S₁ state, which increases the energy gap and, therefore, increases the maximum wavelength.



Figure 9. Molecular electrostatic potential (MEP) surfaces of **8** (conformer 2) in the ground (left) and excited (right) states calculated in acetonitrile.

4. Conclusions

The novel merocyanine dyes containing two benzothiazole conjugated units were easy synthesized by robust condensation protocols. The photoactive compounds were obtained in excellent yields and present and simple purification. The compounds present electronic transitions in the green-orange region ascribed to fully spin and symmetry allowed and fluorescence emission at the orange red region with very small Stokes shift. No evidence for ESIPT as well as for intramolecular charge transfer in the excited state was observed. Theoretical calculations show that the S₁ state has a local excited character and ESIPT is not probable to happen in these compounds due to the high energy barriers for transferring the proton. By changing the pH value, the studied compounds present significant changes in the absorption spectra (yellow to violet color), which allow their application as optical sensors for middle pH values

Acknowledgments

The authors would like to acknowledge FAPERGS (17/2551-0000968-1), CNPq (409855/2018-9) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 for the financial support.

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Merocyanines obtained by Knoevenagel condensation between arylbenzothiazole and 2-methylbenzothiazoles.

Intense fluorescence emission at the orange red region with very small Stokes shift

No evidence of proton transfer in the excited state

Absorption spectra tailored by pH (yellow to violet color)

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Declaration of interests

■ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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