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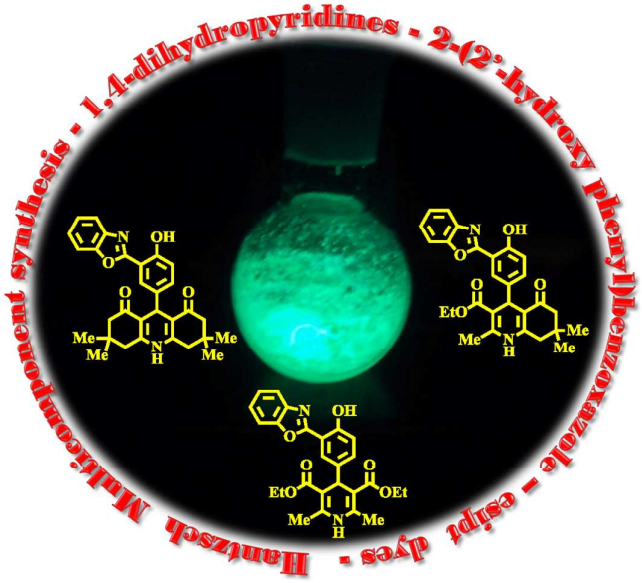
Table of Contents



Photoactive ESIPT dyads with high Stokes' shift were obtained by a multicomponent one-pot Hantzsch synthesis

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ARTICLE

Synthesis and fluorescence properties of benzoxazole-1,4-dihydropyridine dyads achieved by multicomponent reaction

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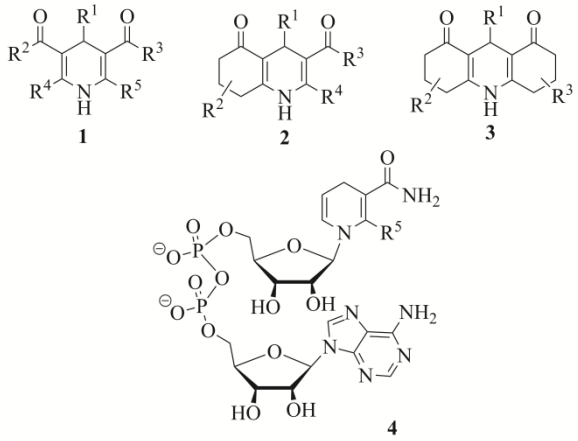
Photoactive 2-(2'-hydroxyphenyl)benzoxazole-1,4-dihydropyridine dyads (HBO-DHP) were obtained by a multicomponent one-pot Hantzsch synthesis using a fluorescent aldehyde, a 1,3-dicarbonylic compound and ammonium acetate. The key step in this synthetic methodology was the synthesis of the formyl benzoxazole derivative through a Duff-modified functionalization protocol. UV-Vis absorption and fluorescence emission spectroscopies were also applied to better understand the photophysics of these compounds. The three novel fluorescent compounds were obtained in moderate yields as stable solids with absorption in the UV region and emission in the blue greenish region. Preliminar results indicate that after excitation both HBO and DHP fluorophores behave independently in the HBO-DHP structure.

Introduction

The multicomponent synthesis is an important tool for the construction of compound libraries with high atom economy, allowing a large number of derivatives from combinations of different reagents, being also explored by the combinatorial chemistry field.[1] Instead of multistep linear strategy, these one-pot reactions are versatile and efficient for achievement of small molecules for biological screening and drug design.[2]

1,4-dihydropyridines (1,4-DHP) **1** are small molecules that present pronounced biological activity and are synthesized by the Hantzsch multicomponent reaction.[3] Their activity as calcium channel modulators has important consequences on the treatment of heart diseases such as hypertension and angina.[4] Other promising activities include antioxidant, bronchodilator, antiartherosclerotic and AD therapeutics.[5] The scope of the Hantzsch reaction is not only restricted to 1,4-dihydropyridine synthesis, but also polycyclic derivatives of the aromatic analogues can be obtained, such as polyhydroquinolines **2** and polyhydroacridinediones **3** (Scheme 1).[6] The NADH coenzyme system **4** is structurally related to 1,4-dihydropyridine compounds found in living cells and is responsible for electron transport and energy production in metabolic reactions. It possesses two oxidation states – i.e. NAD^+ and NADH – that differ in one proton and two electrons. NADH is also a powerful natural antioxidant. The different photophysical behaviour of the two species make them useful in protein identification in enzymatic colorimetric essays.[7] Additionally, it can also be applied as fluorescence sensor for explosives identification[8] and monitoring of biological reactions and microbial fermentations.[9]



Scheme 1 Chemical structure of the dihydropyridine core **1**, polyhydroquinoline **2**, polyhydriacridinedione **3** and NADH **4**.

We have recently described a photophysical study of 4-aryl-substituted dihydropyridines allied with theoretical calculations, which showed that these systems are close to the well-known NADH. Their fluorescence emission can be ascribed to a normal relaxation process or an intramolecular charge transfer in dimethylamino-substituted compounds.[10] Albini *et al*. have also studied the mechanistic pathways on the excited state of

these dihydropyridines by ultraviolet absorption, fluorescence and phosphorescence spectroscopies, leading to different roles between substituted 1,4-dihydripyridines.[11-14] It is desirable that a fluorophore shows fluorescence in a well-defined region of the electromagnetic spectrum and a high value of Stokes' shift, i.e. a large separation between emission and absorption maxima wavelengths, since the interaction with biological systems can result in a hypsochromic shift of fluorescence favoring self-absorption and fluorescence quenching.[15]

Hydroxyphenylbenzazole heterocycles assume an important role as wide-application dyes hence they show a high Stokes' shift due to an excited state intramolecular proton transfer (ESIPT) mechanism (Fig 1), a phototautomerization strongly influenced by the solvent, where usually a dual fluorescence emission takes place in solution or in the solid state.[16] this mechanism can be related to a normal specie (or enol band) and a keto tautomer (or ESIPT band). This behavior confers to these compounds physical and chemical properties that are attractive from a synthetic and technological point of view.[17] Fluorescent properties of 1,4-dihydropyridines could be explored for determination and characterization of pharmacological mechanisms through interactions between molecules and biological systems, such as membrane calcium channels in cardiac cells with fluorimetric essays or other specific targets.[18]

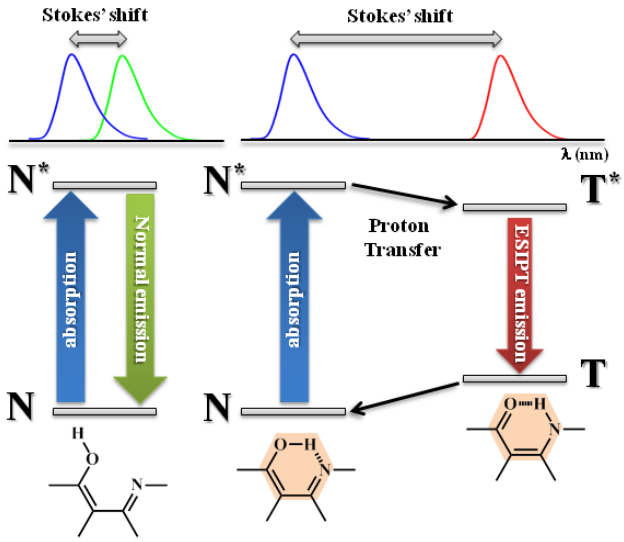


Fig 1. Photophysical pathways from ESIPT-exhibiting dyes: normal (or enol) emission (green) and ESIPT (or tautomer) emission (red). The asterisk indicates the excited state.

Concerning the high Stokes' shift originating from the 2-(2'-hydroxyphenyl)benzoxazole (HBO), we decided to establish a synthetic methodology for its functionalization to make it a suitable component for one-pot synthesis of new 1,4-dihydropyridine derivatives starting from an ESIPT derivative. This paper also deals with the photophysical characterization of the new compounds by means of UV-Visible absorption and fluorescence emission spectroscopies in solution.

Experimental

General procedures

All reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions were monitored using thin-layer chromatography (TLC) carried out on silica gel 60 F254 pre-coated aluminium plates. The visualization was achieved under UV light (254 nm) or staining with I_2. Chromatographic separations were achieved on silica gel columns (70-230 mesh). An ultrasound bath of 40 kHz (50 W) was used for mixture homogenization. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 300 MHz and 75.4 MHz, respectively. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS (0.00 ppm) for ^1H NMR and to the central line of $CDCl_3$ (77.0 ppm) for ^{13}C NMR. Coupling constants *J* are reported in hertz (Hz). The following abbreviations are used for the multiplicities: s, singlet; d, doublet; dd, double-doublet; dq, double-quartet; t, triplet; q, quartet; m, multiplet; br s, broad signal. FTIR spectra were obtained as KBR pellets with resolution of 4 cm^{-1} between 400 and 4000 cm^{-1}. Fragmentation patterns were obtained by 70 eV electronic impact with quadrupole analyzer by direct sample insertion of diluted solutions in chloroform (1 μL).

Spectroscopic grade solvents dichloromethane, acetonitrile, 1,4-dioxane and ethanol were used in fluorescence emission and UV-Vis absorption spectroscopy measurements. UV-Vis absorption spectra were determined using a Shimadzu UV-2450 spectrophotometer and steady state fluorescence spectra were measured using a Shimadzu spectrofluorometer model RF-5301PC. Spectrum correction was performed to enable measuring of a true spectrum by eliminating instrumental response such as wavelength characteristics of the monochromator or detector using Rhodamine B as an internal standard (quantum counter). The quantum yield of fluorescence (ϕ_{fl}) was made in spectroscopic grade solvents using the dilute optical methodology. Quinine sulphate (Riedel) in H_2SO_4 1M (ϕ_{fl} = 0.55) was used as quantum yield standard.[19] All experiments were performed at room temperature. High Resolution Mass Spectrometry with Electrospray Ionization (HRMS-ESI) data, in positive mode, were collected using a Micromass Q-Tof instrument. Samples were infused by a 100 μL syringe at a flow rate 30 $\mu L \cdot min^{-1}$ for all samples. The following typical operating conditions were used: 2980 V capillary voltage, 30 V sample cone voltage, 3.0 V extraction cone voltage, and desolvation gas temperature of 60°C. N_2 was used as the desolvation gas and HPLC grade acetonitrile was the solvent used with the samples.

Syntheses

5-Formylsalicylic acid (6). To an equimolar mixture of salicylic acid **(5)** (14.5 mmol, 2.00 g) and hexamethylenetetramine (14.5 mmol, 2.03 g) in a 100 mL round-bottom vessel were added 22 mL of glacial acetic acid and the solution was heated to reflux for 8 hours. After this time, 16 mL of hot distilled water and 10 mL of concentrated hydrochloric acid was added with vigorous

stirring. The solution was then cooled to ambient temperature and the formed precipitate was filtered in a sintered funnel followed by washings of 50 mL of distilled water (40°C and ambient temperature). The pale yellow solid was dried leading to 0.24 g of 5-formylsalicylic acid in 16% yield and its characterization is in agreement with the literature.[20] M.p. 250ºC (decomp.). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 7.12 (d, J = 8.5 Hz, 1H); 7.99 (dd, ^{3}J = 8.5 and ^{4}J = 2.0 Hz, 1H); 8.34 (d, J = 2.0 Hz, 1H); 9.84 (s, 1H). ^{13}C NMR (DMSO-d_6, 75.4 MHz): δ = 114.3; 118.9; 128.9; 134.6; 135.8; 166.4; 171.8; 191.9.

2-(2'-Hydroxyphenyl)benzoxazole (8). An equimolar mixture of salicylic acid **(5)** (18.3 mmol, 2.53 g) and 2-aminofenol **(7)** (18.3 mmol, 2.00 g) in polyphosphoric acid (10 mL) was stirred at 180°C for 5 h. After cooling, the mixture was poured onto ice-cold water and the precipitate was filtered, washed with water and dried in a stove. The solid was purified by column chromatography with dichloromethane eluant leading to 2.09 g of the product (54%). M.p. = 125-126°C. ^{1}H NMR ($CDCl_3$, 300MHz): δ = 6.89 (t, J = 8.1 Hz, 1H); 7.01 (dd, ^4J = 8.2 and ^3J = 0.9 Hz, 1H); 7.24-7.35 (m, 3H); 7.46-7.49 (m, 1H); 7.58-7.62 (m, 1H); 7.90 (dd, ^4J = 7.9 and ^3J = 1.8 Hz, 1H); 11.37 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.4MHz): δ = 110.8; 117.6; 119.4; 119.7; 125.1; 125.5; 127.3; 133.7; 140.1; 149.2; 158.9; 163.0; 164.9.

2-(5'-Formyl-2'-hydroxyphenyl)benzoxazole (9). Method A (cyclization): polyphosphoric acid (10 mL) followed by 6.4 mmol of 5-formylsalicylic acid **(6)** (1.06 g) and 6.4 mmol of 2-aminofenol **(7)** (0.70 g) was added to a round-bottom flask. The mixture was heated at 170°C for 5 hours. The crude mixture was poured onto ice-cold water with vigorous stirring and the precipitate was filtered yielding a dark brown solid. The solid was extracted by Soxhlet with dichloromethane and then purified by column chromatography with dichloromethane as eluent. The extract was then purified by column chromatography with dichloromethane eluant, yielding after solvent evaporation 0.46 g of a white solid featuring an intense green fluorescence when UV exposed (30%). Method B (formylation): 10 mL polyphosphoric acid (10 mL) followed by 4.7 mmol of previously synthesized HBO **(8)** (0.99 g) and 5 mmol of hexamethylenetetramine (0.70 g) was added to a round-bottom flask. The temperature was raised to 100°C and the mixture stirred for 4 hours. After the heating, 30 mL of ice-cold water was added leading to a white precipitate, which was then filtered and washed with water before drying. The solid was purified by column chromatography with dichloromethane eluant leading to 0.38 g of product (34%). White needles crystals. M.p. = 165-170°C. ^{1}H NMR ($CDCl_3$, 300MHz): δ = 7.22 ppm (d, 2H, J = 8.6 Hz); 7.39-7.45 (m, 2H); 7.62-7.66 (m, 1H); 7.73-7.76 (m, 1H); 7.96 (dd, ^3J = 8.6 Hz and ^4J = 2.0 Hz); 8.55 (d, J = 2.0 Hz); 9.95 (s, 1H); 12.17 (br s, 1H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 111.2; 118.6; 119.7; 125.7; 126.3; 129.2; 130.5; 134.3; 139.7; 149.4; 161.9; 163.7; 165.0; 190.2. FTIR (KBr, ν = cm^{-1}): 3061, 2848, 2735, 1699, 1630, 1490. MS: m/z (%) = 240.1 (16), 239.1 (100) [M$^+$], 238.1 (90), 210.2 (10), 182.0 (29), 63.1 (32). HRMS (ESI-qTOF) m/z: [M+H]$^+$ Calcd for $C_{14}H_9NO_3$ 240.0660; Found 240.0620 (ppm = 4.2).

Diethyl 2,6-dimethyl-4-[2-(2'-hydroxyphenyl) benzoxazolyl]-1,4-dihydropyridine 3,5-dicarboxylate (15). To a round-bottom vessel, 0.106 g of In/SiO_2 (10 mol%), followed by 2.5 mmol of ethyl acetoacetate **(13)** (0.317 mL), 1.25 mmol of previously synthesized **9** (0.299 g) and 2.5 mmol of ammonium acetate **(12)** (0.193 g) were added. The mixture was dissolved in isopropanol (10 mL) and refluxed for 3 hours. The solution was cooled and the precipitate filtered and washed with cold isopropanol (4x5 mL). The catalyst was separated from the product by redissolution of the solid with dichloromethane and micropore (0.45 μm) filtration. The solution obtained was concentrated and dried leading to 0.26 g of the pure product (46%), a white solid with blue fluorescence when UV exposed. M.p. = 233-236°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.27 (t, 6H, J = 7.1 Hz); 2.39 (s, 6H); 4.07-4.22 (m, 4H); 5.05 (s, 1H); 5.75 (s, 1H); 7.00 (d, J = 8.7 Hz, 1H); 7.38-7.4 (m, 3H); 7.60-7.63 (m, 1H); 7.72-7.73 (m, 1H); 7.83 (d, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 14.5; 19.9; 39.3; 60.0; 104.2; 109.8; 110.8; 117.1; 119.3; 125.1; 125.3; 126.6; 133.9; 139.6; 144.1; 149.3; 157.4; 163.5; 164.9; 167.8. FTIR (KBr, ν = cm^{-1}): 3323 (NH), 3096, 2980, 1681, 1649, 1548, 1491, 1302, 1212. MS: m/z (%) = 462.2 (10) [M$^+$], 433.1 (10), 389.3 (19), 252.1 (100), 211.0 (5), 196.0 (25). HRMS (ESI-qTOF) m/z: [M+H]$^+$ Calcd for $C_{26}H_{26}N_2O_6$ 463.1869; Found 463.1865 (ppm = 0.9).

Ethyl 4-[2-(2'-hydroxyphenyl)benzoxazolyl]-1,4,5,6,7,8,-hexahydro-2,7,7-trimethylquinolin-5(1H,4H,6H)-one 3-carboxylate (16). To a round-bottom vessel, 0.106 g of In/SiO_2 (10 mol%), followed by 1.25 mmol of 5,5-dimethylcycloexane-1,3-dione **(14)** (0.175 g), 1.25 mmol of ethyl acetoacetate **(13)** (0.158 mL), 1.25 mmol of previously synthesized **9** (0.299 g) and 2.5 mmol of ammonium acetate **(12)** (0.193 g) were added. The mixture was dissolved in isopropanol (10 mL) and refluxed for 3 hours. The solution was cooled and the precipitate filtered and washed with cold isopropanol (4x5 mL). The catalyst was separated from the product by redissolution of the solid with dichloromethane and micropore (0.45 μm) filtration. The solution obtained was concentrated and dried leading to 0.39 g of the pure product (66%), a white solid with green fluorescence when UV exposed. M.p. >250°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.96 (s, 3H, CH_3); 1.09 (s, 3H, CH_3); 1.23 (t, J = 7.1 Hz, 3H, CH_3); 2.14-2.40 (m, 4H, 2xCH_2); 2.4 (s, 3H, CH_3); 4.08 (dq, J = 1.1 Hz and J = 7.1 Hz, 2H, CH_2); 5.08 (s, 1H); 5.97 (s, 1H); 6.97 (d, J = 8.7 Hz, 1H); 7.34-7.39 (m, 3H); 7.58-7.61 (m, 1H); 7.69-7.72 (m, 1H); 7.99 (d, J = 2.4 Hz, 1H); 11.34 (br s,1H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 14.2; 19.5; 27.1; 29.4; 32.7; 35.9; 41.1; 50.6; 59.9; 106.0; 109.6; 110.6; 112.0; 117.0; 119.1; 124.8; 125.1; 126.5; 133.5; 138.6; 140.1; 143.4; 147.9; 149.1; 157.1; 163.2; 167.3; 196.0. FTIR (KBr, ν = cm^{-1}): 3289, 3222, 2963, 1706, 1608, 1487, 1379, 1262. MS: m/z (%) = 472 (39) [M$^+$], 399 (20), 262 (100), 234 (28). HRMS (ESI-qTOF) m/z: [M+H]$^+$ Calcd for $C_{26}H_{28}N_2O_5$ 473.2076; Found 473.2058 (ppm = 3.8).

3,3,6,6-Tetramethyl-9-[2-(2'-hydroxyphenyl) benzoxazolyl]-3,4,6,7-tetrahydro acridine-1,8-(2H,5H,9H,10H)-dione (17). To a round-bottom vessel, 0.106 g of In/SiO_2 (10 mol%), followed by 2.5 mmol of 5,5-dimethylcyclohexane-1,3-dione **(14)** (0.350 g), 1.25 mmol of previously synthesized **9** (0.299 g) and 2.5 mmol of ammonium acetate **(12)** (0.193 g) were added. The mixture was dissolved in isopropanol (10 mL) and refluxed for 3 hours. The solution was cooled and the precipitate filtered and washed with cold isopropanol (4x5 mL). The catalyst was separated from the product by redissolution of the solid with dichloromethane and micropore (0.45 μm) filtration. The solution obtained was concentrated and dried leading to 0.30 g of the pure product (50%), a white solid with blue fluorescence when UV exposed. M.p. = 212-215°C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.15 (s, 6H, 2xCH_3); 1.35 (s, 6H, 2xCH_3); 2.33-2.55 (m, 8H, 4xCH_2); 5.53 (s, 1H); 7, 03 (d, *J* = 8.7 Hz, 1H); 7. 16 (d, *J* = 8.7 Hz); 7.36-7.38 (m, 2H); 7.48-7.51 (m, 1H); 7.71-7.74 (m, 1H); 7.78-7.81 (m, 1H); 11.23 (br s, 1H, NH); 12.01 (s, 1H, OH). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ = 27.4; 30.2; 31.6; 32.3; 48.7; 47.3; 110.6; 115.7; 117.5; 119.5; 125.2; 125.5; 126.8; 129.2; 132.4; 140.4; 149.2; 157.0; 163.1; 165.0; 169.7; 180.9. FTIR (KBr, ν = cm^{-1}): 2958, 2925, 1593, 1372. MS: m/z (%) = 361.1 (100) [M^+], 318.0 (8), 277.0 (16), 263.0 (9), 249.0 (30), 140.0 (13), 83.0 (49). HRMS (ESI-qTOF) m/z: $[M+H]^+$ Calcd for $C_{30}H_{30}N_2O_4$ 483.2284; Found 483.2293 (ppm = 1.9).

Results and discussion

Synthesis

We first attempted to apply aminobenzazoles on benzoxazolyl-quinoline synthesis without success with multicomponent and even classical cyclization protocols involving anilines, such as Skaup glycerol acidic reactions, Combes, Doebner-Miller and Conrad-Limpach approaches (see Electronic Supplementary Information).

Therefore it was decided to bypass this drawback by preparing a formyl derivative, which has, to the best of our knowledge, not been described in the literature, although the 3,5-formyl-hydroxyphenylbenzoxazole has been recently described as an intermediate for a fluorescent pyrophosphate sensor.[21] In Hantzsch multicomponent condensation to 1,4-dihydropyridines, it has been observed that aromatic and heteroaromatic aldehydes bearing both electron withdrawing as well as electron releasing substituted groups do not significantly affect the reaction yields.[22] On the other hand, the synthesis of 2-(2'-hydroxyphenyl)benzoxazole (HBO) is well established in the literature.[23] In this way, a synthetic method was proposed to afford functionalization of HBO by introducing a formyl group, which was predicted to react with two equivalents of a 1,3-dicarbonyl compound and ammonia, aiming the fluorescent dyad HBO-DHP, which structure is depicted in Figure 2.

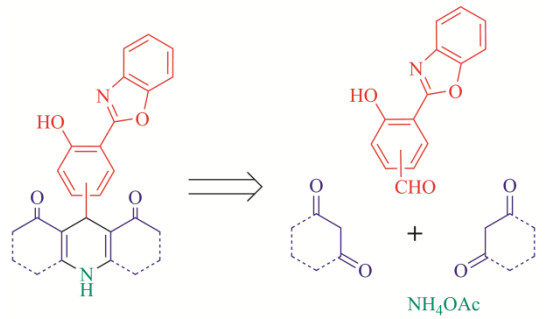
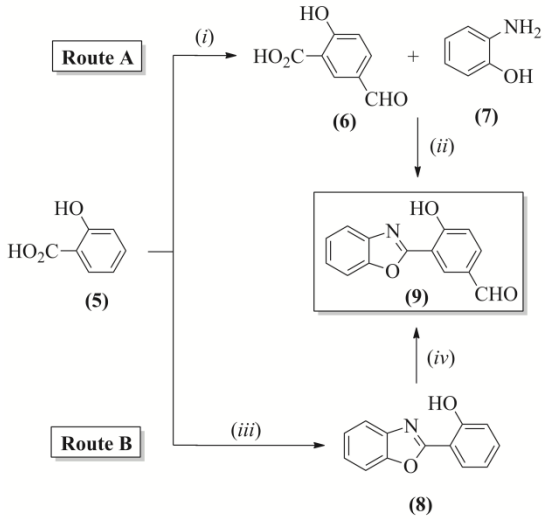


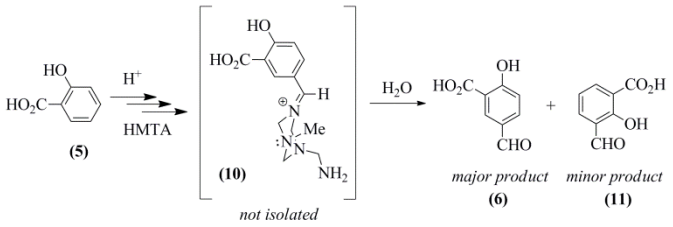
Fig 2. Proposed HBO-DHP structure.

In this way, as depicted in Scheme 2, two different methodologies (Route A and B) were used to synthesize the formyl functionalized fluorophore **9**. Duff's formylation for salicylic acid **(5)** using hexamethylenetetramine (HMTA) involves stable and low-cost reagents, although low yields are usually obtained.[20] The procedure was employed for salicylic acid **(5)** and later condensation of 5-formylsalicylic acid **(6)** with 2-aminophenol **(7)**. At the same time, formylation of HBO **8** obtained by condensation of salicylic acid **(5)** and 2-aminophenol **(7)** was tested.

It was expected that the rate of the Duff's formylation reaction was highly dependent on the successful hydrolysis of an iminium cation **10** formed by protonated HMTA and the activated phenol derivative in acidic media (Scheme 3). We performed this reaction refluxing in acetic acid for 8 hours the salicylic acid **(5)** and an excess of HMTA. After several attempts, a mixture of the 3-formyl and 5-formyl derivatives (**11** and **6**, respectively) could be observed, whose separation was quite difficult. In this way, a simple procedure was chosen: filtering the solid precipitate after hydrolysis and washing with hot water portions. This method has proved to be useful since 5-formylsalicylic acid **(6)** is less soluble in water than 3-formylsalicylic acid **(11)**; however the product 6 was obtained in low yield (16%).



Scheme 2 Reaction pathways for obtention of formyl functionalized HBO **(9)**. Conditions: (i) Hexamethylenetetramine (HMTA), acetic acid (AcOH), reflux, 8h; (ii) and (iii) 7, polyphosphoric acid (PPA), 170ºC, 5h, (iv) HMTA, PPA, 100ºC, 4h.



Scheme 3 Iminium hydrolysis in Duff's formylation of salicylic acid **(5)**.

^{1}H-NMR analysis of the pale yellow precipitate produced a spectrum in agreement with pure 5-formylsalicylic acid **(6)**. Pure 3-formylsalicylic acid **(11)** could also be obtained by recrystallization (m.p. = 178-179ºC). It is worth noting that no formylated product was observed when the reaction was carried in pure water up to 16 hours.

The condensation procedure to yield the novel fluorescent formylated compound **9** was adapted from the literature,[23] i.e. reacting the previously synthesized 5-formylsalicylic acid **(6)** and 2-aminophenol **(7)** in polyphosphoric acid (PPA) at 170ºC for 5 hours. The product was purified by column chromatography yielding **(9)** (Yield 30%), a white solid with green fluorescence, quite similar to the unfunctionalized HBO fluorophore **8**. It was expected that an inverse procedure starting with the HBO synthesis and its further formylation could improve the yields in both reaction steps (Scheme 2, Route B).

The precursor **8** was prepared as reported in the literature[24] (Yield 57%) and the formylation methodology was investigated in three acidic solvents. The results are summarized in Table 1. PPA was found suitable for both steps of cyclization and formylation. The structure of **9** was confirmed by ^{1}H and ^{13}C NMR, FTIR and MS spectra (see Electronic Supplementary Information). The signal pattern in the region between 7 and 9 ppm in the ^{1}H NMR spectrum (Figure 3) was crucial for the identification of the formyl group in the 5' position of the phenolic ring. The novel fluorophore precursor shows good thermal stability and no decomposition was observed up to 250ºC.

The Hantzsch three-component synthesis of 4-(2-(2'-hydroxyphenyl)benzoxazolyl)-1,4-dihydropyridine **15** was performed mixing one equivalent of **9** with ammonium acetate **(12)** and two equivalents of ethyl acetoacetate **(13)**, employing a In/SiO_2 heterogeneous Lewis-acid catalyst in refluxing isopropanol (Scheme 4).[22] The product was isolated after 3 hours as a blue fluorescent solid in moderate yield (46%) by simply filtering the heterogeneous isopropanol mixture. It is worth mentioning that when using acetylacetone, a complex mixture was obtained.

Table 1. Experimental conditions for the HBO's formylation.

Entry	Solvent	Time (h)	Temp. (ºC)	Yield (%)
1	Acetic acid (AcOH)	6	Reflux	8
2	Trifluoroacetic acid (TFAA)	6	Reflux	15
3	Polyphosphoric acid (PPA)	4	100	34

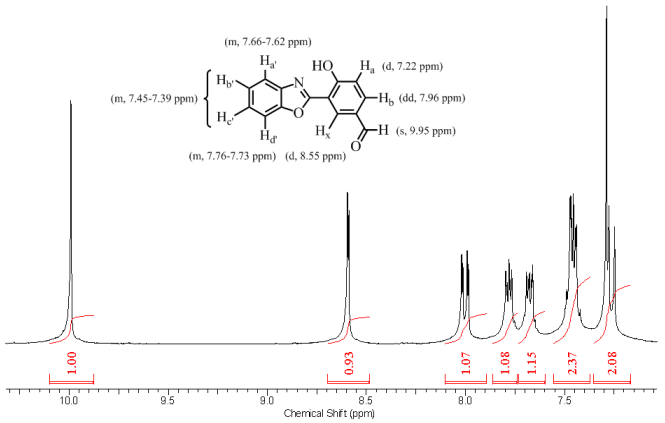
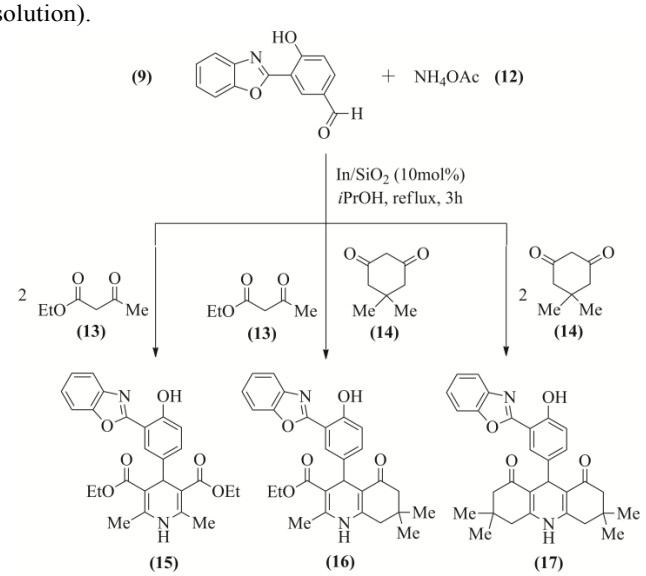


Fig 3. ^{1}H NMR spectrum of **9**.

Considering the antimicrobial activity of decahydroacridinediones and hexahydroquinolines with a fused dimedone moiety,[25] it was synthesized, for further investigations, two *N*-heterocycles bearing the HBO moiety and the DHP core **16-17** through the same methodology. The compounds were obtained with significant yields (66% and 50% for **16** and **17**, respectively) without any additional chromatographic purification. All synthesized novel heterocycles are white solids and fluorescent in the blue-green region when exposed to UV 365 nm irradiation (solid or in solution).



Scheme 4 Multicomponent synthesis of HBO-DHPs **15-17**.

Photophysics

The photophysical study of the synthesized compounds was performed in solution (10^{-5} M) using four different organic

solvents with a wide range of dielectric constants. This investigation was focused on the influence of the structural changes, as well as the oxidation state of the dihydropyridine core in the ESIPT emission. Figure 4 presents the UV-Vis absorption and fluorescence emission spectra of **9**. The fluorescence emission spectra were obtained using the absorption maxima as the excitation wavelengths. The relevant photophysical data is summarized in Table 2.

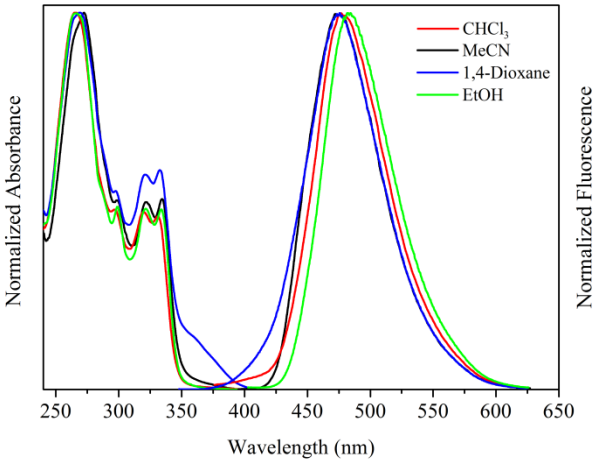


Fig 4. Normalized UV-Vis absorption and fluorescence emission spectra of **9** in different solvents.

The UV-Vis absorption spectra of **9** presents in all solvents an intense band located at 266 nm and a red-shifted structured one at 320-335 nm with molar absorptivity coefficient ε values in agreement with π–π* transitions. No significant solvatochromism in the ground state was observed for this dye.

The fluorescence emission spectra show a major emission band located at 474-483 nm. As already presented in the literature, the measured Stokes' shift (~9150 cm^{-1}) can be ascribed to the ESIPT mechanism.[17] To this dye the ground-state enol-*cis* conformer (N) absorbs UV radiation leading to the singlet excited state enol-*cis* (N^*), which tautomerizes to the excited-state keto (T^*). This conversion is responsible for energy loss with large Stokes' shift, and the excited keto decays to the ground state (T) emiting fluorescence. The single red-shifted emission band ascribed to $(T)S_0 \leftarrow (T^*)S_1$ and consequently the absence of a blue-shifted band from the locally excited enol species $(N)S_0 \leftarrow (N^*)S_1$ is quite similar to the HBO spectra.[24] This result strongly suggests that the formyl group on the 5'-position of the phenolic ring does not favour an equilibrium between the conformers in solution in the ground state, as observed for amino derivatives in the same position.[16] Excitation spectra show similar profiles to the absorption curves (not shown, see Electronic Supplementary Information), where the structured bands ascribed to the HBO (322 and 334 nm) and benzoxazolyl moiety (~270 nm) can be observed.

Table 2. Relevant UV-Vis absorption and fluorescence emission data of **9**, where λ_{abs} and λ_{em} are the absorption and emission maxima, respectively in nanometers, and ε is the molar extinction coefficient in 10^3 $L \cdot mol^{-1} \cdot cm^{-1}$.

Solvent	$\lambda_{abs}(\varepsilon)$	λ_{em}	$\Delta\lambda_{ST}{}^a$ (cm^{-1})
$CHCl_3$	334(9.68), 272(19.1)	474	8843
MeCN	330(13.3), 266(28.9)	478	9382
EtOH	332(10.9), 269(18.9)	475	9068
1,4-Dioxane	333(13.6), 265(28.5)	483	9326

[a]Stokes' shift was obtained from the difference of the emission and absorption maximum ($\Delta\lambda_{ST} = \lambda_{em}-\lambda_{abs}$)

Figure 5 presents the absorption and emission spectra of HBO-DHPs **15-17** in solution. The relevant data from the photophysical study of these compounds are summarized in Table 3.

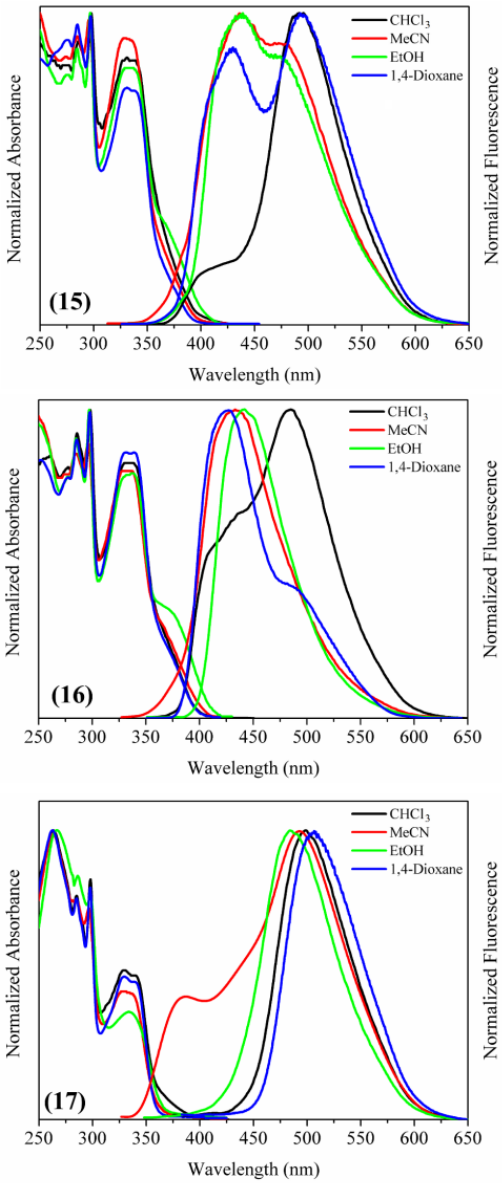


Fig 5. Normalized UV-Vis absorption and fluorescence emission spectra of **15-17** in different solvents.

Table 3. Relevant UV-Vis absorption and fluorescence emission data of the HBO-DHPs **15-17**, where the concentration is ~10^{-6} mol·L^{-1}, λ_{abs} and λ_{em} are the absorption and emission maxima, respectively in nanometers, $\Delta\lambda_{ST}$ is the Stokes' shift, ε is the molar extinction coefficient in 10^3 L·mol^{-1}·cm^{-1} and ϕ_{fl} is the fluorescence quantum yield.

Solvent	$\lambda_{abs}(\varepsilon)$	λ_{em}		$\Delta\lambda_{ST}$ (cm^{-1})		ϕ_{fl}
		SW	LW	SW	LW	
15						
$CHCl_3$	339(24.1)	424	483	5914	8795	0.19
MeCN	336(10.2)	433	-	-	6667	0.07
EtOH	336(15.5)	441	-	-	7086	0.11
1,4-Dioxane	339(24.5)	426	485	6024	8880	0.11
16						
$CHCl_3$	339(24.1)	424	483	5914	8795	0.31
MeCN	336(10.2)	433	-	-	6667	0.08
EtOH	336(15.5)	441	-	-	7086	0.08
1,4-Dioxane	339(24.5)	426	485	6024	8880	0.11
17						
$CHCl_3$	340(8.17)	-	499	-	7140	0.35
MeCN	337(13.7)	382	492	3456	9348	0.23
EtOH	334(21.3)	-	484	-	9279	0.33
1,4-Dioxane	339(23.9)	-	506	-	9736	0.17

The compounds present absorption maxima in the UV region (334-340 nm) with electronic transitions ascribed to π–π*. Changes in the solvent polarity, where only a slight solvatochromism was observed, as well as modifications in the DHP core, seem to not affect the photophysics of these compounds in the ground state. The fluorescence emission were also obtained using the absorption maxima as the excitation wavelengths. The compounds in the solid state present emission in the blue-green region (Figure 6).

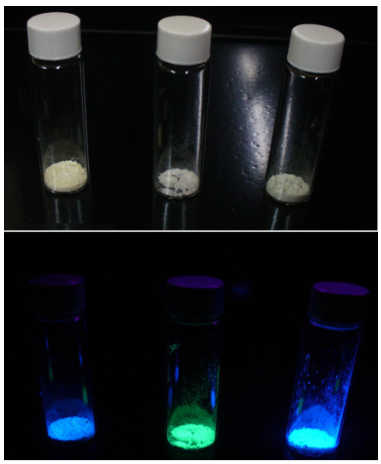


Fig 6. Picture of the compound **15** (left), **16** (right) and **17** (middle) in the solid state under irradiation of normal light (above) and UV 365nm (below).

Although it could be observed ESIPT emission in the studied HBO-DHPs, as already observed to compound **9**, the fluorescence spectra of compounds **15-17** seems to be more complex being both dependent on the solvent polarity and the DHP moiety. Generally, the emission spectrum present a dual fluorescence emission located around 425 nm and 488 nm, so-called short wavelength (SW) and long wavelength (LW), respectively. In these compounds, the fluorescence emission can be influenced by from internal electron transfer and/or the energy transfer character of the corresponding DHP core (blue-shifted band)[11-14] or by deactivation of the keto tautomer, which arises from the ESIPT mechanism (red-shifted band). It is worth mentioning that the normal emission from enol conformers was discarded since the measured Stokes' shift were much higher (~5200-7000 cm^{-1}) than those attributed to this emission in similar compounds (~3500 cm^{-1}).[16-17] An exception can be observed to compound **17** in acetonitrile, where the blue-shifted band could be related to the normal emission. The nature of the emission spectra of these compounds indicates that both HBO and DHP behaves, after excitation, as independent fluorophores in the HBO-DHP structure. Concerning the intricate photophysics of the DHPs,[11-14] which involves internal electron transfer and/or energy transfer, as well as the observed dual fluorescence emission from the HBO-DHPs, additional studies are in progress to better understand the photophysics of these dyes. Furthermore, the single C-C bond between the two fluorophores can also be useful to investigations through the Förster Resonance Energy Transfer (FRET) formalism involving a donor (probably DHP core) and an acceptor (probably, HBO moiety).

Conclusions

In summary, the synthesis of three new fluorescent dyads was accomplished through the multicomponent approach involving a fluorescent aldehyde, a 1,3-dicarbonylic compound and ammonium acetate. The first attempt using different ESIPT fluorescent amino derivatives as nitrogen source did not provide the desired cyclization compounds. A new photoactive aldehyde derivative of 2-(2'-hydroxyphenyl)benzoxazole was successfully obtained through a Duff-modified functionalization protocol.

The new HBO-DHPs were obtained in moderate yields as thermal and photostable solids. The photophysical study in solution shows an absorption maxima in the UV region and fluorescence emission in the blue greenish region depending on the compound. Preliminar results indicate that after excitation both HBO and DHP behave as independent fluorophores in the HBO-DHP structure. Since it could be established a solid synthetic methodology to incorporate ESIPT compounds into the DHP structures, additional experiments are in progress to better understand the photophysics of these dyes.

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Electronic Supplementary Information (ESI) available: spectral and HRMS data of the new compounds, as well as additional data for the fluorophore **9**, supplementary photophysical experiments and attempts to the quinolines synthesis and salicylic acid formylation are included. See DOI: 10.1039/b000000x/

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