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

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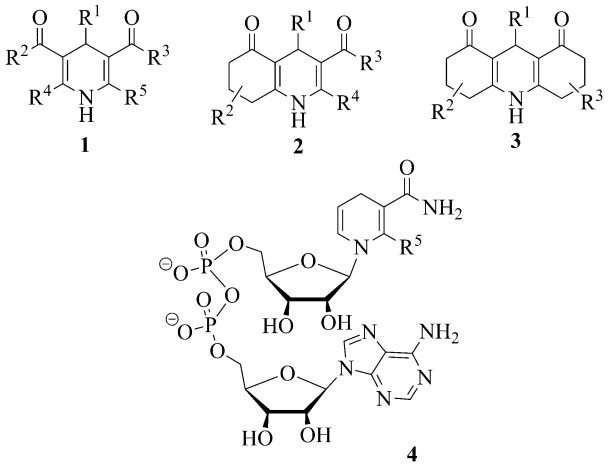
Photoactive 2-(2′-hydroxyphenyl)benzoxazole-1,4-dihydropyridine (HBO–DHP) dyads were obtained by a multicomponent one-pot Hantzsch synthesis using a fluorescent aldehyde, a 1,3-dicarbonylic compound and ammonium acetate. The key step in this synthetic methodology was the synthesis of the formyl benzoxazole 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-green region. Preliminary 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, also being explored by the combinatorial chemistry field.[1] In contrast to the multistep linear strategy, these one-pot reactions are versatile and efficient at achieving small molecules for biological screening and drug design.[2]

1,4-Dihydropyridines (1,4-DHP) **1** are small molecules that exhibit 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 their role as antioxidants, bronchodilators, antiartherosclerotic and AD therapeutic agents.[5] The scope of the Hantzsch reaction is not restricted to 1,4-dihydropyridine synthesis only, but polycyclic derivatives of the aromatic analogues can also be obtained, such as polyhydroquinolines **2** and polyhydroacridinedione **3** (Scheme 1).[6]



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

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 behaviours of the two species make them useful for protein identification in enzymatic colorimetric essays.[7] Additionally, it can also be applied as a fluorescence sensor for identification of explosives[8] and monitoring of biological reactions and microbial fermentations.[9]

We have recently described a photophysical study of 4-aryl-substituted dihydropyridines allied with theoretical calculations,

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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 for the excited state of these dihydropyridines by ultraviolet absorption, fluorescence and phosphorescence spectroscopies, leading to different roles of substituted 1,4-dihydropyridines.[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, in which usually a dual fluorescence emission takes place in solution or in the solid state.[16] This mechanism can be related to a normal species (or enol band) and a keto tautomer (or ESIPT band). This behavior confers to these compounds physical and chemical properties that are attractive from both synthetic and technological points of view.[17] Fluorescence 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]

Considering the high Stokes' shift originating from 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.

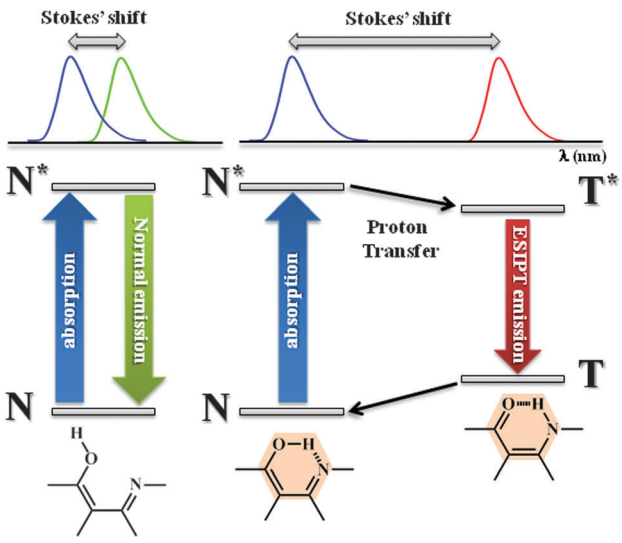


Fig. 1 Photophysical pathways for ESIPT-exhibiting dyes: normal (or enol) emission (green) and ESIPT (or tautomer) emission (red). The asterisk indicates the excited state.

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 by 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 ^{1}H 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 ^{1}H 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 using KBR pellets with a resolution of 4 cm^{-1} between 400 and 4000 cm^{-1}. Fragmentation patterns were obtained using a quadrupole analyzer equipped with a 70 eV electron impact ionization source by direct insertion of samples diluted 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 recorded using a Shimadzu UV-2450 spectrophotometer, and steady state fluorescence spectra were measured using a Shimadzu spectrofluorometer model RF-5301PC. Spectral correction was performed to enable measurements of true spectra by eliminating instrumental response such as wavelength characteristics of the monochromator or the detector using Rhodamine B as an internal standard (quantum counter). The fluorescence quantum yield (ϕ_{fl}) measurement was made in spectroscopic grade solvents using the dilute optical methodology. Quinine sulphate (Riedel) in 1 M H_2SO_4 (ϕ_{fl} = 0.55) was used as a quantum yield standard.[19] All experiments were performed at room temperature. High resolution mass spectrometry electrospray ionization (HRMS-ESI) data, in a positive mode, were collected using a Micromass Q-Tof instrument. Samples were infused by a 100 μL syringe at a flow rate 30 μL min^{-1} for all samples. The following typical operating conditions were used: a capillary voltage of 2980 V, a sample cone voltage of 30 V, an extraction cone voltage of 3.0 V, and a 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). 22 mL of glacial acetic acid was added 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 and the solution was heated to

reflux for 8 hours, after which 16 mL of hot distilled water and 10 mL of concentrated hydrochloric acid were added with vigorous stirring. The solution was then cooled to ambient temperature and the formed precipitate was filtered using a sintered funnel followed by washings with 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. 250 °C (decomp.). ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 7.12 (d, J = 8.5 Hz, 1H); 7.99 (dd, ^3J = 8.5 and ^4J = 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-aminophenol (**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 using dichloromethane as an eluant leading to 2.09 g of the product (54%). M.p. = 125–126 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): 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 ($CDCl_3$, 75.4 MHz): 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-aminophenol (**7**) (0.70 g) were 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 using a Soxhlet extractor with dichloromethane and then purified by column chromatography using dichloromethane as the eluent, yielding, after solvent evaporation, 0.46 g of a white solid (30%) exhibiting intense green fluorescence when exposed to UV radiation. Method B (formylation): 10 mL of 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) were 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 using dichloromethane as an eluant leading to 0.38 g of the product (34%). White needles crystals. M.p. = 165–170 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): 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 (Δppm = 4.2).

Diethyl 2,6-dimethyl-4-[2-(2′-hydroxyphenyl) benzoxazolyl]-1,4-dihydropyridine 3,5-dicarboxylate (15). 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 to a round-bottom vessel. 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 (4 × 5 mL). The catalyst was separated from the product by redissolution of the solid in 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 exposed to UV radiation. 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 (Δppm = 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). 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 to a round-bottom vessel. 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 (4 × 5 mL). The catalyst was separated from the product by redissolution of the solid in 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 exposed to UV radiation. 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, $2CH_2$); 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 (Δppm = 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). 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 to a round-bottom vessel. 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

(4 × 5 mL). The catalyst was separated from the product by redissolution of the solid in 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, $2CH_3$); 1.35 (s, 6H, $2CH_3$); 2.33–2.55 (m, 8H, $4CH_2$); 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 (Δppm = 1.9).

Results and discussion

Synthesis

We first attempted to apply aminobenzazoles on benzoxazolylquinoline synthesis without success using multicomponent and even classical cyclization protocols involving anilines, such as Skaup glycerol acidic reactions and Combes, Doebner-Miller and Conrad-Limpach approaches (see ESI†).

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 3,5-formyl-hydroxyphenylbenzoxazole has been recently described as an intermediate for a fluorescent pyrophosphate sensor.[21] In Hantzsch multicomponent condensation giving 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 for the synthesis of fluorescent dyad HBO–DHP, the structure of which is depicted in Fig. 2.

In this way, as depicted in Scheme 2, two different methodologies (routes 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 for 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 Duff's formylation reaction was highly dependent on the successful hydrolysis of iminium cation **10** formed by protonated HMTA and the activated phenol derivative in acidic media (Scheme 3). We performed this reaction by refluxing salicylic acid (**5**) and an excess of HMTA in acetic acid for 8 hours. After several attempts, a

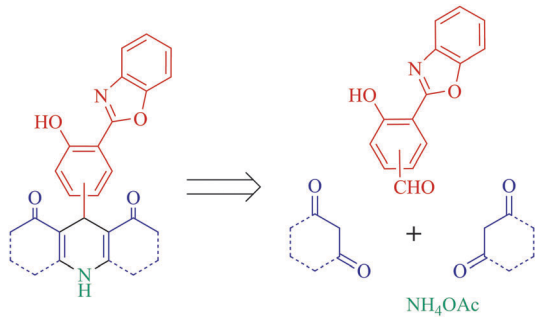
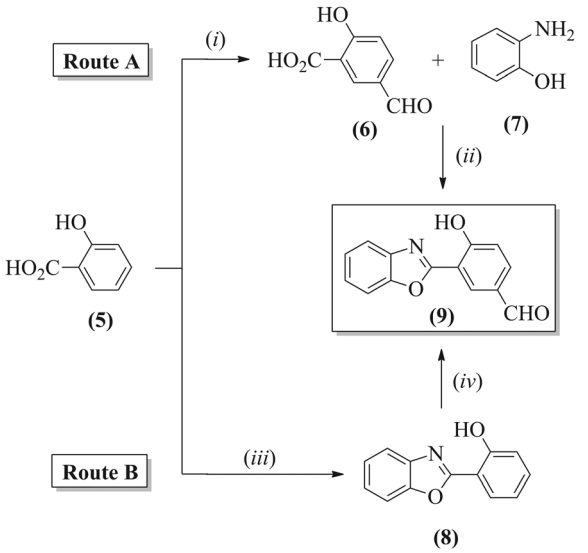


Fig. 2 Proposed HBO–DHP structure.

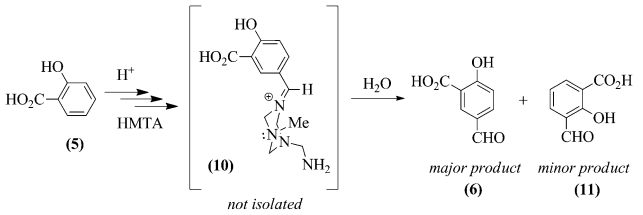


Scheme 2 Reaction pathways for obtaining formyl functionalized HBO (**9**). Conditions: (i) Hexamethylenetetramine (HMTA), acetic acid (AcOH), reflux, 8 h; (ii) and (iii) 7, polyphosphoric acid (PPA), 170 °C, 5 h, (iv) HMTA, PPA, 100 °C, 4 h.

mixture of the 3-formyl and 5-formyl derivatives (**11** and **6**, respectively) could be obtained, whose separation was quite difficult. Therefore, 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, product 6 was obtained in low yield (16%).

^{1}H-NMR analysis of the pale yellow precipitate produced a spectrum in agreement with that of pure 5-formylsalicylic acid (**6**). Pure 3-formylsalicylic acid (**11**) could also be obtained by recrystallization (m.p. = 178–179 °C). 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 170 °C 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



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

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).

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 to be suitable for both steps of cyclization and formylation. The structure of **9** was confirmed by ^{1}H and ^{13}C NMR, FTIR and MS analyses (see ESI†). The signal pattern in the region between 7 and 9 ppm in the ^{1}H NMR spectrum (Fig. 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 250 °C.

The Hantzsch three-component synthesis of 4-(2-(2′-hydroxyphenyl)benzoxazolyl)-1,4-dihydropyridine **15** was performed by 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 acetylacetone was used, a complex mixture was obtained.

Considering the antimicrobial activity of decahydroacridinediones and hexahydroquinolines with a fused dimedone

Table 1 Experimental conditions for the HBO's formylation

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

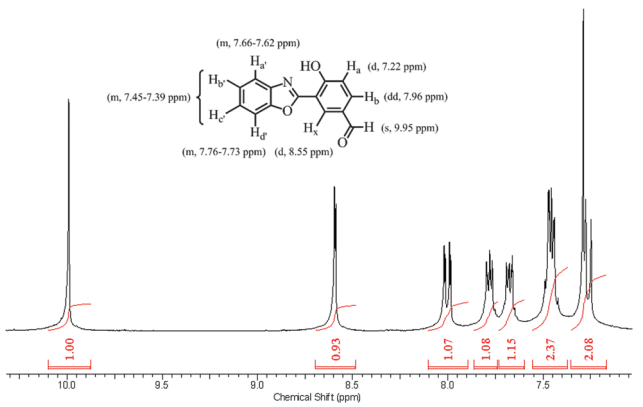
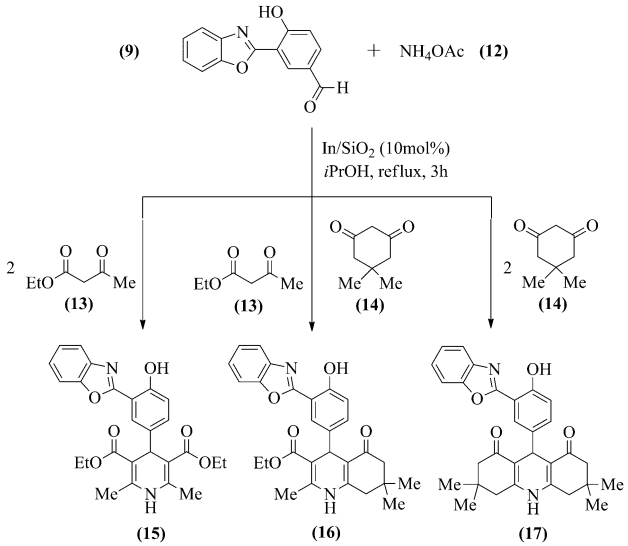


Fig. 3 ^{1}H NMR spectrum of **9**.



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

moiety,[25] for further investigations, two N-heterocycles, **16** and **17**, bearing the HBO moiety and the DHP core were synthesized using 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 were white solids and fluorescent in the blue-green region when exposed to UV 365 nm radiation (solid or in solution).

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. Fig. 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 are summarized in Table 2.

The UV-Vis absorption spectra of **9** in all solvents exhibit an intense band located at 266 nm and a red-shifted 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 ($\sim$9150 cm^{-1}) can be ascribed to the ESIPT mechanism.[17] Due 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 a 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 ← (T*)S_1 and consequently the absence of a blue-shifted band from the locally excited enol species (N)S_0 ← (N*)S_1 is quite similar to that of the HBO

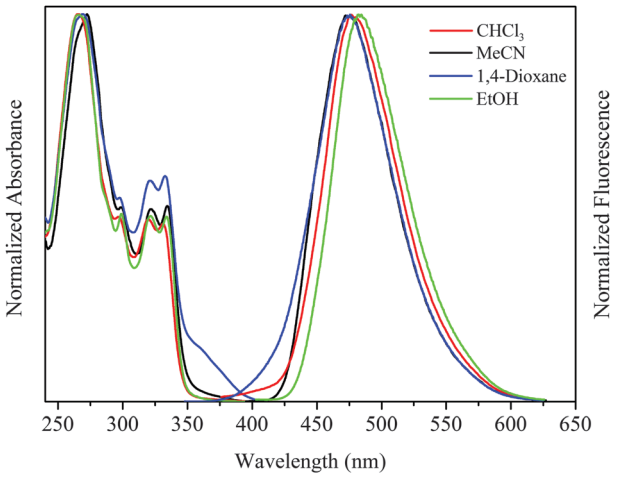


Fig. 4 Normalized UV-Vis absorption and fluorescence emission spectra of **9** in different solvents.

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 mol^{-1} cm^{-1}

Solvent	λ_{abs} (ε)	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 maxima ($\Delta\lambda_{ST} = \lambda_{em} - \lambda_{abs}$).

spectra.[24] These results strongly suggest that the formyl group in 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 ESI†), where the structured bands ascribed to the HBO (322 and 334 nm) and benzoxazolyl moieties (~270 nm) can be observed.

Fig. 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.

The compounds exhibit 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 not to affect the photophysics of these compounds in the ground state. The fluorescence emission was also obtained using the absorption maxima as the excitation wavelengths. The compounds in the solid state exhibit emission in the blue-green region (Fig. 6).

Although ESIPT emission could be observed in the studied HBO–DHPs, as already observed for compound **9**, the fluorescence spectra of compounds **15–17** seem to be more complex being dependent on both the solvent polarity and the DHP moiety. Generally, an emission spectrum presents a dual fluorescence emission located at around 425 nm and 488 nm,

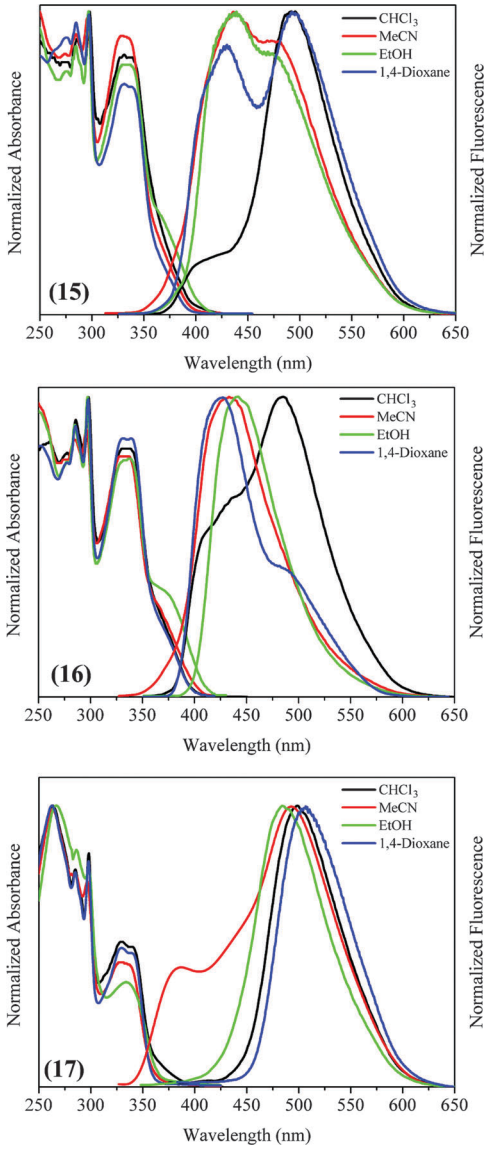


Fig. 5 Normalized UV-Vis absorption and fluorescence emission spectra of **15–17** in different solvents.

the so-called short wavelength (SW) and long wavelength (LW), respectively. In these compounds, the fluorescence emission can be influenced by 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' shifts was 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 in the case of 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 behave, after excitation, as independent fluorophores in the HBO–DHP structure. Concerning the intricate photophysics of the DHPs,[11–14]

Table 3 Relevant UV-Vis absorption and fluorescence emission data of the HBO–DHPs **15–17**, where the concentration is $\sim 10^{-6}$ 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

		λ_{em}		$\Delta\lambda_{ST}$ (cm^{-1})		
Solvent	λ_{abs} (ε)	SW	LW	SW	LW	ϕ_{fl}
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

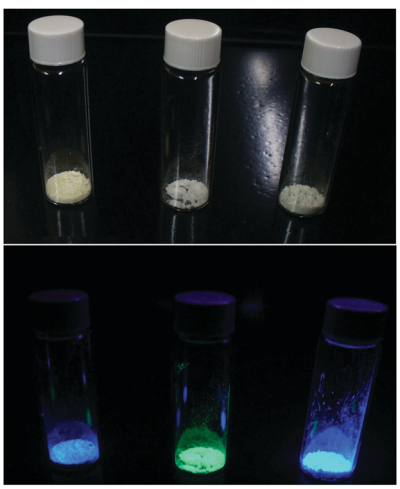


Fig. 6 Picture of compounds **15** (left), **16** (right) and **17** (middle) in the solid state under irradiation of normal light (above) and UV 365 nm (below).

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 used for investigations through the Förster resonance energy transfer (FRET) formalism involving a donor (probably a DHP core) and an acceptor (probably a 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 at using different ESIPT fluorescent amino derivatives as nitrogen sources 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 maximum in the UV region and fluorescence emission in the blue-green region depending on the compound. Preliminary results indicate that after excitation both HBO and DHP behave as independent fluorophores in the HBO–DHP structure. Since a solid synthetic methodology to incorporate ESIPT compounds into the DHP structures could be established, additional experiments are in progress to better understand the photophysics of these dyes.

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