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ORIGINAL PAPER

Synthesis of 1,8–Naphthyridines and Their Application in the Development of Anionic Fluorogenic Chemosensors

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Abstract Two 1,8–naphthyridines were synthesized and found to be fluorescent in solution. These compounds were studied in the presence of Cu^{+} and Cu^{2+} ions and it was verified that the metal causes the quenching of their fluorescence emission, due to the formation of complexes between the naphthyridine and the metal. A displacement assay was carried out in a DMSO–water mixture with the addition of various anions to the solutions of the complexes, and it was observed that these systems have a high capacity to selectively detect cyanide.

Keywords Displacement assays · Copper · Anion sensing · Cyanide · 1,8–naphthyridines · Fluorogenic chemosensors

Introduction

The recognition and detection of anions [1–7] is a field that has recently attracted increasing interest due to the fact that these species are of fundamental importance in many chemical and biological processes. Therefore, many methodologies based on optical chemosensors have been studied to perform selective naked–eye and quantitative detection of anionic species [1–17]. Of these methodologies, a strategy that has been applied for the detection of anionic analytes involves the concept of displacement assays [8–17]. In this strategy, a compound especially tailored to act as a receptor for a given analyte interacts with an indicator (a signalizing unit) forming a complex, which causes an optical change in the system. The addition of a particular analyte causes a competition scenario, which results in the displacement of the indicator from its initial complex, causing a perceptible change in the optical signal of the system. Various displacement assays for the detection of anions based on chromogenic and fluorogenic units have been reported in the literature [8–17].

Of the anionic species to be recognized and detected, CN^{-} has attracted considerable interest because it has many applications in metallurgy, fishing, mining, and the fabrication of polymers [18, 19]. This anion is lethal in very small concentrations due to its ability to bind strongly to the active site of cytochrome–oxidase, which leads to the inhibition of the mitochondrial electron transport chain, and consequently to a decrease in the oxidative metabolismo [18, 19]. CN^{-} is released through hydrolysis from some fruit seeds and roots [20–22]. The chemical warfare agent known as tabun, delivers CN^{-} through hydrolysis and this feature may be of importance in the development of chemosensors for the detection of this neurotoxic compound [23, 24]. Therefore, many chemosensors for the detection of CN^{-} have been studied in the recent years [20–22, 25–35].

1,8–Naphthyridine and its derivatives [36] are compounds that have attracted considerable interest in recent years because they exhibit various types of biological activity, which include their action as chemotherapeutic and anti–

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infective agents, growth regulators, fungicides, nematocides, and insecticides [37]. 1,8–Naphthyridine can also act as a bidentate ligand, using the lone electron pairs of the nitrogen atoms, leading to the formation of metal complexes [38]. Moreover, some of these compounds or their complexes display interesting photophysical properties [39–43], and therefore have the potential to function as fluorogenic chemosensors for the detection of metal ions [42, 44–47]. Thus, the importance of these compounds has led to the development of many synthetic methods aiming at their preparation and the study of their applications [37, 38, 48–51]. It is important to note that although 1,8–naphthyridines have been applied as fluorogenic chemosensors for metal ions [42, 44–47], no literature reference on the use of these compounds to detect simple anionic species is available.

In this study, the 1,8–naphthyridines 1 and 2 were synthesized and characterized. Their photophysical properties were investigated and these compounds were then used in studies to evaluate their potential as selective fluorogenic chemosensors for CN^-, in a dimethyl sulfoxide (DMSO)–water mixture, by means of a displacement assay.

Experimental

Materials and Methods

All chemicals used were high–purity commercial reagents. DMSO was purified according to a procedure described in the literature and then stored over molecular sieves (4 Å, Sigma–Aldrich) [52]. Karl–Fischer titrations were performed with this solvent and demonstrated the presence of water in a concentration of 5.1×10^{-3} mol dm^{-3} (0.04 % water). Acetonitrile (HPLC grade, Sigma–Aldrich) was dried with calcium hydride (Sigma–Aldrich), distilled and stored over 4 Å molecular sieves (Sigma–Aldrich), according to the literature [52]. Deionized water was used in all measurements. This solvent was boiled and bubbled with nitrogen and kept under a nitrogen atmosphere to avoid the presence of carbon dioxide. Tetrahydrofurane (THF) was distilled from sodium using benzophenone as an indicator [52]. Dichloromethane was distilled from calcium hydride [52]. All other solvents employed in the reactions were used as received. All anions (HSO_4^-, $H_2PO_4^-$, NO_3^-, CN^-, CH_3COO^-, F^-, Cl^-, Br^-, and I^-) were used as tetra–*n*–butylammonium salts with purity greater than 97–99 %. The anions were purchased from Fluka (F^-, >97 %; Cl^-, >98 %; NO_3^-, >97 %; and $H_2PO_4^-$, >97 %), Vetec (Br^-, >99 %; I^-, >99 %; and HSO_4^-, >99 %) and Sigma–Aldrich (CH_3COO^-, >97 %). They were dried over phosphorous pentoxide under vacuum before use. Karl–Fischer experiments were performed for the following tetra–*n*–butylammonium salts in order to determine the content of water in each salt: CN^- (0.116 % water), F^- (1.125 % water), $H_2PO_4^-$ (0.111 % water), and CH_3COO^- (0.067 % water). Buffered solutions were prepared with 2–amino–2–hydroxymethyl–propan–1,3–diol (tris, Sigma–Aldrich). 2,6–Diaminopyridine (Sigma–Aldrich) was recrystallized from trichloromethane before use. Meldrum's acid was prepared by a procedure described in the literature [53], through a reaction of malonic acid in acetic anhydride with acetone in the presence of sulfuric acid. Tetrakis(triphenylphosphine)palladium (0), $[Pd(PPh_3)_4]$, with purity of 99 %, was purchased from Sigma–Aldrich. $CuBF_4.4CH_3CN$ was prepared by reaction of $Cu(BF_4)_2$ with copper in powder in anhydrous acetonitrile, through a procedure reported in the literature [54].

The melting points were obtained on a Kofler hot stage and were uncorrected. UV–vis measurements were performed with a Varian Cary Bio 50 spectrophotometer and the emission spectra were obtained with a Shimadzu RF–5301PC spectrofluorimeter, both equipped with thermostatted cell compartments at ±0.1 °C, using 1 cm quartz square cuvettes closed with rubber septums to avoid the evaporation of the solvent. The maxima of the UV–vis spectra (λ_{max}) were calculated from the first derivative of the absorption spectrum. The pH values were determined with a Gehaka model PG 2000 pH meter. The NMR spectra were recorded on Brucker AC–300 and Varian AS–400 spectrometers. Chemical shifts were recorded in ppm with the solvent resonance as the internal standard. Data are reported as follows: chemical shift, multiplicity (*s* = singlet, *d* = doublet, *t* = triplet), coupling constants (Hz) and integration. IR spectra were obtained on a Shimadzu model Prestige–21 spectrometer, with KBr pellets. Microanalysis was performed by Central de Análises–UFSC, using a CHNOS elemental analyzer model EA 1110 CHNS–O from CE Instruments.

Synthesis and Characterization

N–(6–Amino–2–pyridinyl)acetamide (3) [55] A solution of 2,6–diaminopyridine (19 g; 174 mmol) and triethylamine (20.5 cm^3; 148 mmol) was prepared in THF (230 cm^3). A solution of acetic anhydride (14.1 cm^3; 148 mmol) in THF (60 cm^3) was then added slowly, drop by drop, under stirring. The reactional mixture was stirred for an additional 14 h. Water (260 cm^3) was then added to the mixture and the THF was rotary–evaporated. The mixture was left in a freezer overnight, and the white crystals formed were filtered and dried under vacuum without heating. Yield: 11.9 g (45 %); mp: 157–159 °C. IR (KBr) ν_{max}/cm^{-1}: 3458 (ν N–H), 3358 (ν N–H), 3224 (ν N–H), 1672 (ν C=O), 1647, 1631, 1618, 1562, 1541, 1369, 1303, 1261, 1242, 1166, 989, 792. ^{1}H–NMR (300 MHz, $CDCl_3$): δ 2.02 (*s*, 3H, $-CH_3$); 5.69 (*s*, 2H, $-NH_2$); 6.16 (*d*, *J*=9 Hz, 1H, CHAr); 7.20 (*d*, *J*=9 Hz, 1H, CHAr); 7.32 (*t*, *J*=6 Hz, 1H, CHAr); 9.83 (*s*, 1H, −NH). ^{13}C–NMR (75 MHz, $CDCl_3$): δ 23.92 ($-CH_3$); 100.79; 103.21; 138.80; 150.50;

158.42; 168.79 (−C=O). Anal. calcd. for $C_7H_9N_3O$: C, 55.62; H, 6.00; N, 27.80. Found: C, 55.47; H, 6.02; N, 27.80.

N–[6–[[(2,2–Dimethyl–4,6–dioxo–1,3–dioxan–5–ylidene) methyl]amino]–2–pyridinyl] acetamide (4) Meldrum's acid (17.03 g; 118 mmol) was refluxed with trimethyl orthoformate (71.5 cm^3) for 2 h [56]. After this period compound 3 (8 g; 53 mmol) was added and the reflux was applied for a further 20 min. The reactional mixture was cooled a little, only to form the crystals, and the still hot mixture was filtered using a Büchner funnel, the crystals being washed with cold ethanol. The solid was dried under vacuum, yielding 13.25 g (82 %). mp: 228.9–230.0 °C. IR (KBr) ν_{max}/cm^{-1}: 3255 (ν N–H), 1734 (ν C=O), 1691 (ν C=O), 1668 (ν C=O), 1620, 1570, 1544, 1444, 1394, 1313, 1276, 1255, 1244, 1190, 933, 804, 786. ^{1}H–NMR (300 MHz, $CDCl_3$): δ 1.76 (*s*, 6H, $-CH_3$); 2.28 (*s*, 3H, $-CH_3$); 6.73 (*d*, *J*=9 Hz, 1H, CHAr); 7.74 (*t*, *J*=6 Hz, 1H, CHAr); 8.06 (*d*, *J*=9 Hz, 1H, CHAr); 8.10 (*s*, 1H, −NH); 9.27 (*d*, *J*=15 Hz, 1H, CHAr); 11.18 (*d*, *J*=15 Hz, 1H, −NH). ^{13}C–NMR (75 MHz, $CDCl_3$): δ 24.70 ($-CH_3$); 27.09 ($-CH_3$); 88.65; 105.29; 107.89; 110.95; 141.40; 147.32; 150.64; 151.16; 163.36 (−C=O); 165.35 (−C=O); 168.87 (−C=O). Anal. calcd. for $C_{14}H_{15}N_3O_5$: C, 56.56; H, 5.05; N, 14.14. Found: C, 56.08; H, 5.06; N, 13.97.

N–(5,8–Dihydro–5–oxo–1,8–naphthyridin–2–yl)acetamide (5) Diphenyl ether (200 cm^3) was refluxed at 250 °C to add slowly the Meldrum's acid adduct 4 (5 g; 16.4 mmol). The reflux was applied for a further 15 min. After cooling, ethyl ether (80 cm^3) was added. The suspension was filtered using a Büchner funnel and washed with ethyl ether. The solid obtained was washed with hot hexane, yielding 2.17 g (84 %). mp: >300 °C (lit. [57] 310–315 °C). IR (KBr) ν_{max}/cm^{-1}: 3332 (ν N–H), 2953 (ν C–H), 1676 (ν C=O), 1612 (ν C=O), 1508, 1307, 1192, 815, 599. ^{1}H–NMR (300 MHz, DMSO): δ 2.14 (*s*, 3H, $-CH_3$); 6.02 (*d*, *J*=7.6 Hz, 1H, CHAr); 7.80 (*d*, *J*=7.6 Hz, 1H, CHAr); 8.03 (*d*, *J*=8.8 Hz, 1H, CHAr); 8.37 (*d*, *J*=8.4 Hz, 1H, CHAr); 10.69 (*s*, 1H, −NH); 11.64 (*d*, *J*=4.8 Hz, 1H, −NH). ^{13}C–NMR (75 MHz, DMSO): δ 24.91 ($-CH_3$); 110.50; 111.09, 117.53; 137.68; 140.47; 150.20; 154.78; 170.76 (−C=O); 177.59 (−C=O).

N–(5–Chloro–1,8–naphthyridin–2–yl)acetamide (6) [58, 59] $POCl_3$ (18 cm^3) and compound 5 (1 g; 4.93 mmol) were heated at 90–95 °C for 1.5 h. The mixture was cooled and ice was added under vigorous stirring. NH_4OH was added to the mixture until pH 8, the solid was filtered with a Büchner funnel, and washed with ice cold water. The solid was dried under vacuum, yielding 0.69 g (63 %; lit. [58] 79.7 %; lit. [59] 32–37 %). mp: >250 °C. (lit. [58] 265–266 °C; lit. [59] 240 °C with previous decomposition). IR (KBr) ν_{max}/cm^{-1}: 3450 (ν N–H), 2920 (ν C–H), 1697 (ν C=O), 1601, 1537, 1489, 1435, 1393, 1306, 1240, 1134 (ν C–Cl), 851, 827. ^{1}H–NMR (300 MHz, $CDCl_3$): δ 2.31 (*s*, 3H, $-CH_3$); 7.48 (*d*, *J*=3 Hz, 1H, CHAr); 8.59 (*d*, *J*=9 Hz, 1H, CHAr); 8.65 (*d*, *J*=9 Hz, 1H, CHAr); 8.88 (*d*, *J*=6 Hz, 1H, CHAr); 9.47 (*s*, 1H, −NH). ^{13}C–NMR (75 MHz, $CDCl_3$): δ 24.99 ($-CH_3$); 116.24; 119.34; 120.84; 136.26; 142.85; 153.16; 154.61; 155.39; 169.86 (−C=O).

N–[5–(4–Methoxyphenyl)–1,8–naphthyridin–2–yl]acetamide (2) Compound 6 (0.4 g; 1.806 mmol), 4–methoxyphenylboronic acid (0.329 g; 2.167 mmol), and $Pd(PPh_3)_4$ (0.104 g; 0.09 mmol) were suspended in benzene (4.5 cm^3), ethanol (0.6 cm^3), and Na_2CO_3 (2 mol dm^{-3}; 1.6 cm^3; 3.25 mmol). This mixture was refluxed (80 °C) for 9 h and, after this time, more 4–methoxyphenylboronic acid (0.055 g; 0.361 mmol) was added and the reflux was maintained for a further 5 h. The suspension formed was washed with water (3×20 cm^3) and extracted with dichloromethane (3×30 cm^3). The organic phase was preserved and the aqueous phase was also washed with dichloromethane (2×60 cm^3). The organic phases were combined to be later washed with water (3×75 cm^3), dried with anhydrous $MgSO_4$, and rotary–evaporated. The dark–colored solid was purified through flash column chromatography using silica gel (60 G, 5–40 μm) as the adsorbent. The elution was initiated with acetonitrile/ethyl acetate (1:1) and the polarity was gradually increased during the elution using acetonitrile:ethyl acetate:etanol (4:4:1). The eluate was dried with $MgSO_4$ and rotary–evaporated. The product obtained was dried, yielding 286 mg (54 %). mp 256–257 °C. IR (KBr) ν_{max}/cm^{-1}: 3420 (ν N–H), 3285 (ν C–H, aromatic), 2930 (ν C–H, aliphatic), 1701 (ν C=O), 1607, 1584, 1516, 1497, 1422, 1395, 1304, 1283, 1254 (ν C–O–C, asymmetric), 1233, 1180, 1026, 835. ^{1}H–NMR (400 MHz, $CDCl_3$): δ 2.30 (*s*, 3H, $-CH_3$); 3.91 (*s*, 3H, $-O-CH_3$); 7.08 (*d*, *J*=8.0 Hz, 2H, CHAr); 7.33 (*d*, *J*=4.0 Hz, 1H, CHAr); 7.44 (*d*, *J*=8.0 Hz, 2H, CHAr); 8.36 (*d*, *J*=9.2 Hz, 1H, CHAr); 8.47 (*d*, *J*=9.2 Hz, 1H, CHAr); 9.00 (*d*, *J*=4.0 Hz, 1H, CHAr); 9.35 (*s*, 1H, −NH). ^{13}C–NMR (100 MHz, $CDCl_3$): δ 24.97 ($-CH_3$); 55.46 ($-O-CH_3$); 114.36; 115.34; 119.26; 120.79; 129.08; 130.84; 138.03; 149.42; 153.14; 153.78; 155.32; 160.30; 170.02 (−C=O). Anal. calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.48; H, 5.19; N, 14.24.

5–(4–Methoxyphenyl)–1,8–naphthyridin–2–amine (1) A suspension of 2 (0.5 mmol; 150 mg) in H_2SO_4 (10 %; 2 cm^3) was heated and after solubilization was refluxed for 5 min. The pH of the reactional mixture was increased until 9 and the pale–yellow precipitate obtained was filtered in a Büchner funnel and dried under vacuum at 60 °C to yield

110 mg (85 %). mp: 243.6–246.2 °C (lit. [60] 233–234 °C). IR (KBr) ν_{max}/cm^{-1}: 3456 (ν N–H), 3302 (ν N–H), 3157 (ν C–H, aromatic), 1635, 1606, 1562, 1502, 1431, 1404, 1244, 1177, 1028, 822, 555. ^{1}H–NMR (300 MHz, DMSO): δ 3.83 (*s*, 3H, −CH_3); 6.80 (*d*, *J*=9.6 Hz, 1H, CHAr); 6.83 (*s*, 2H, −NH_2); 7.05 (*d*, *J*=4.5 Hz, 1H, CHAr); 7.09 (*d*, *J*=8.4 Hz, 2H, CHAr); 7.41 (*d*, *J*=8.4 Hz, 2H, CHAr); 7.85 (*d*, *J*=9.0 Hz, 1H, CHAr); 8.66 (*d*, *J*=4.5 Hz, 1H, CHAr). ^{13}C–NMR (75 MHz, $CDCl_3$): δ 55.26 (−CH_3); 113.35; 114.20; 114.48; 117.45; 129.42; 130.64; 135.10; 147.85; 151.34; 157.26; 159.49; 160.39. Anal. calcd. for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.47; H, 5.25; N, 16.65.

Photophysical Properties of 1 and 2

Buffered aqueous solutions were prepared using tris in a concentration of 1×10^{-3} mol dm^{-3}, the pH being adjusted to 8.5 and 6.0 with a solution of HCl 0.1 mol dm^{-3}. Subsequently, solutions of 1 and 2 were prepared in the respective buffers and in DMSO, in a concentration of 1×10^{-5} mol dm^{-3}. From these solutions, UV–vis spectra were obtained at 25 °C and the absorbance values were collected at the maximum wavelength for 1, at λ_{max}=330 nm for water (pH 8.5) and λ_{max}=350 nm for DMSO, while for 2 the values were obtained for λ_{max}=321 nm (pH 6.0) and λ_{max}=327 nm in DMSO.

Samples were excited at the λ_{max} values for 1 and 2 at 25 °C, with excitation and emission slit width settings of 5.0 and 1.5 nm, respectively. The quantum yields (Φ_f) were determined for the dye using quinine sulfate ($\Phi_{f,ref}$=0.577 em H_2SO_4 0.1 mol dm^{-3}) as an internal standard, with the use of the expression $\Phi_f=\Phi_{f,ref}\ (A_{ref}/A)(\eta/\eta_{ref})(a/a_{ref})$, A being the absorbance of the sample, A_{ref} the absorbance of the internal standard, η and η_{ref} the refractive index of the solvent and the internal standard and a and a_{ref} the area under the fluorescence peak of the sample and the internal standard, respectively [61, 62].

pK_a Values for 1 and 2 in Aqueous Solution

Solutions of 1 and 2 were prepared in a concentration of 4×10^{-3} mol dm^{-3} in $CHCl_3$, and stored in glass flasks closed with rubber stoppers to avoid the evaporation of the solvent. An aliquot of the solution, sufficient to give a concentration of the compound of 1×10^{-5} mol dm^{-3} was collected with a microsyringe and placed in a flask. After the evaporation of the solvent, water at pH 2.22 (adjusted with HCl 0.1 mol dm^{-3}) was added and the fluorescence emission spectrum of the naphthyridine was recorded. The excitation of 1 and 2 was applied at 330 nm and 321 nm, respectively, at 25 °C. The pH of the solution was measured and the fluorescence spectrum was obtained after each addition of small amounts of KOH 0.1 mol dm^{-3} until pH 10–12.

Compounds 1 and 2 as Anionic Fluorogenic Chemosensors

Two solutions of 1 and 2 ($c=1\times10^{-6}$ mol dm^{-3}) in DMSO–water (7:3 *v/v*; tris/HCl, $c=1\times10^{-3}$ mol dm^{-3}; pH 8.5) were prepared and then used in two experiments, in the selectivity assay with the anions and for the titration of the complexes involving Cu^{2+} and the naphthyridines with CN^-. The selectivity assay was performed obtaining the fluorescence emission spectra at 25 °C for the solutions of the free naphthyridines, and the fluorescence emission intensity (I_F) was collected for their maxima at the λ_{em} values (λ_{em}=408 nm for 1 and λ_{em}=440 nm for 2). A solution of the complexes 1:Cu^{2+} and 2:Cu^{2+} was then prepared, with Cu^{2+} in a concentration of 4.0×10^{-3} mol dm^{-3}. The solution of each complex was used to prepare 2 cm^3 of each solution of the anion (NO_3^-, CN^-, F^-, Cl^-, Br^-, I^-, HSO_4^-, and $H_2PO_4^-$) in a concentration of 8×10^{-3} mol dm^{-3}. The fluorescence emission spectra were recorded for each solution and the I_F values were collected as previously described.

For the titrations with CN^-, solutions (6 cm^3) of 1:Cu^{2+} and 2:Cu^{2+} were performed with the solutions of 1 and 2, with the same concentrations as those used in the selectivity assays. From the solution of the complex, 1 cm^3 was used to prepare a stock solution of CN^- ($c=6\times10^{-2}$ mol dm^{-3}) and 1.5 cm^3 was placed in a quartz cuvette closed with a rubber septum. After being thermostatized at 25 °C, a spectrofluorimetric reading was taken. The titration was performed by adding small aliquots of the solution of the anion with a microsyringe. After each addition a spectrum was obtained, the I_F values being collected as shown in the preceding paragraph. This procedure was followed until the return to the I_F value obtained for the free naphthyridine in solution.

A similar procedure was employed for the assays using Cu^+, but the concentration of the metal ion was 3×10^{-3} mol dm^{-3} and the concentration of the anions was 3×10^{-3} mol dm^{-3}.

The CN^- residues were treated by adding 5 mL of 10 % NaOH (2.5 mol dm^{-3}) and 70 cm^3 of household bleach for each 50 mL of solution of CN^- in the concentration of 2 % (*w/v*).

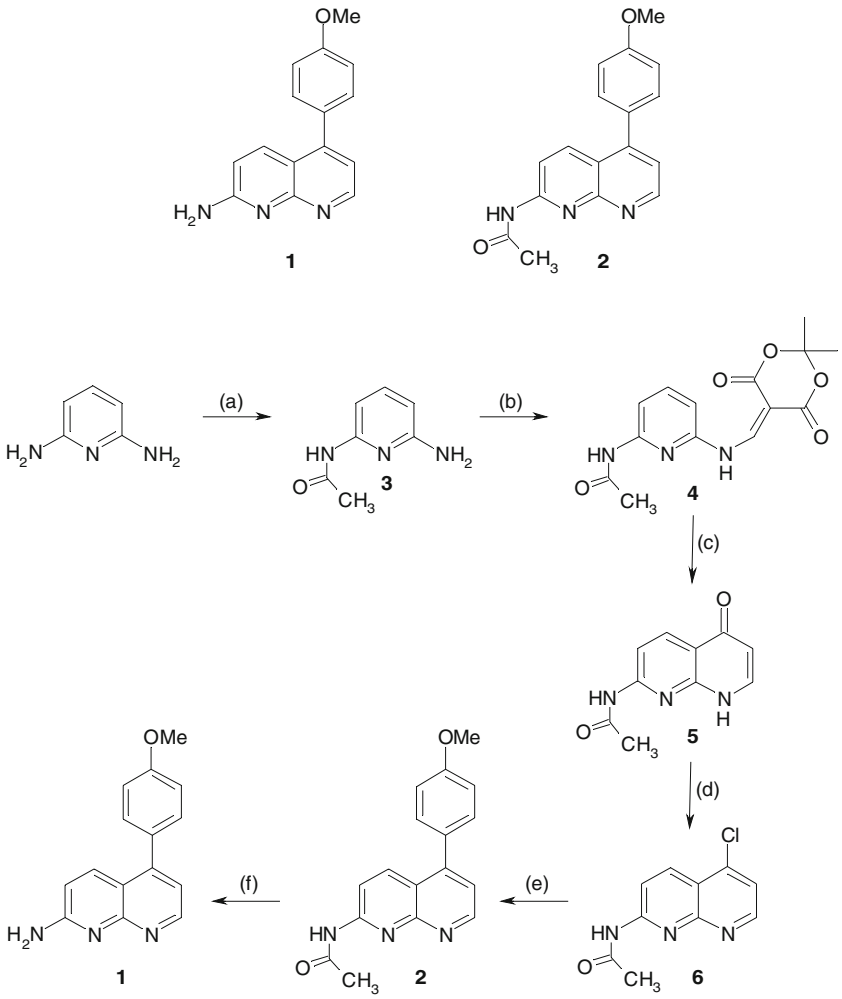
Calculations

The binding constants were calculated through the fitting of the least–squares regression curves using the ORIGIN 6.1 program.

Results and Discussion

Synthesis and Characterization

Compound 1 was synthesized in six steps, through a synthetic route described in Scheme 1. Firstly, 2,6–diaminopyridine was acetylated, according to a procedure described in the literature [55], with acetic anhydride in THF to generate compound 3. This compound was refluxed with 5–



Scheme 1 Synthetic route for the preparation of the 1,8–naphthyridines 1 and 2. Reagents and conditions: (a) acetic anhydride, THF, triethylamine, 12 h, 45 %; (b) Meldrum's acid + $HC(OCH_3)_3$, reflux, 82 %; (c) diphenyl ether, 250 °C, 15 min, 84 %; (d) $POCl_3$, 90–95 °C, 1.5 h, 63 %; (e) 4–methoxyphenylboronic acid, $Pd(PPh_3)_4$, benzene, ethanol, Na_2CO_3, reflux, 54 %; (f) H_2SO_4 (aq) 10 %, reflux, 5 min, 85 %

methoxymethylene Meldrum's acid (generated *in situ* by refluxing Meldrum's acid with trimethyl orthoformate) [56] to afford compound 4, which was cyclized by means of thermolysis in diphenyl ether at 250 °C, to generate *N*–(5,8–dihydro–5–oxo–1,8–naphthyridin–2–yl)acetamide (5). The reaction of 5 with $POCl_3$ led to the formation of *N*–(5–chloro–1,8–naphthyridin–2–yl)acetamide (6), applying a classical procedure [58, 59]. In the next step, 1,8–naphthyridine 2 was obtained through Suzuki coupling [63], which involved the reflux of 6, 4–methoxyphenylboronic acid, and $Pd(PPh_3)_4$ in benzene, ethanol, and Na_2CO_3. Finally, compound 2 was hydrolyzed by reflux with H_2SO_4 (10 %) for 5 min to form the 1,8–naphthyridine 1. The identity and purity of the compounds 1 and 2 were verified through their characterization using IR, NMR, and elemental analyses.

Photophysical Properties of 1 and 2

Table 1 shows experimental data obtained from the analysis of UV–vis and fluorescence emission spectra for compounds 1

Table 1 Photophysical properties of compounds 1 and 2

Compound	Solvent	λ_{max} (nm)	ε_{max} (dm^3mol^{-1} cm^{-1})	λ_{em} (nm)[a]	Φ[b]
1	DMSO	350	1.74×10^4	408	0.37
1	DMSO–water[c]	340	8.84×10^3	408	0.31
1	water, pH 8.5[d]	330	8.82×10^3	401	0.19
2	DMSO	327	2.24×10^4	426	0.03
2	DMSO–water[e]	325	1.17×10^4	447	0.07
2	water, pH 6.0[d]	321	7.88×10^3	470	0.06

[a] The solutions were excited using absorption λ_{max} values. [b] Relative to the standard quinine sulfate in 0.1 mol dm^{-3} of H_2SO_4 (λ_{exc}=350 nm; Φ=0.577). [c] 7:3 (*v/v*), buffer tris–HCl, pH 8.5. [d] Buffer tris–HCl. [e] 7:3 (*v/v*), buffer tris–HCl, pH 6.0

and 2 in DMSO, water and in a DMSO–water (7:3, *v/v*) mixture. The solution of naphthyridine 1 absorbs in the UV range with a maximum in the wavelength (λ_{max}) at 330 nm in water (HCl/tris; pH 8.5) and at 350 nm in DMSO, with a molar absorptivity of 1.74×10^4 dm^3 mol^{-1} cm^{-1}. For compound 2, the maxima in the wavelengths were at 321 nm in water (HCl/tris; pH 6.0) and 327 nm in DMSO, with a molar absorptivity in DMSO of 2.24×10^4 dm^3 mol^{-1} cm^{-1}.

Solutions of 1 and 2 were excited using their respective absorption λ_{max} values, and it was observed that these compounds are fluorescent. The quantum yields (Φ) were obtained in DMSO and water (HCl/tris at pH 8.5 for 1 and pH 6.0 for 2) and are similar to other results found in the literature related to 1,8–naphthyridines [42, 44, 64]. Compound 1 exhibits a higher Φ value than compound 2, in DMSO and in buffered water, which is in agreement with the well documented fact that 1,8–naphthyridines with electron–donating groups in their molecular structure have higher Φ values in comparison with those possessing weaker electron–donating groups [61]. The λ_{max} values for the absorption of 1 change if the polarity of the medium is altered, a hypsochromic shift of 20 nm being observed on comparing the spectrum obtained in DMSO with that in water. This is due to the fact that water is able to act as a hydrogen–bond donating solvent, interacting strongly with the lone electron pair of the nitrogen atom in the 2–position of 1. This hinders the electronic transition involving the amino group, leading to the negative solvatochromism observed. This observation is corroborated by the fact that 2 is less sensitive to the medium polarity, because this compound has an acetyl bound to the amino group in its molecular structure, which diminishes the electronic availability of the nitrogen atom. This explanation also aids an understanding of the reduction in the Φ values of compound 1 verified in the presence of water. Interestingly, according to the emission data, the band of compound 1 is slightly changed with the change in the polarity of the medium ($\Delta\lambda$=7 nm on comparing data obtained in DMSO and in water), while compound 2 shows an important bathochromic shift of 44 nm on comparing the emission spectrum obtained in DMSO with that in water. This observation suggests that compound 2 can be used as a solvatofluorochromic probe [65–68] in the investigation of the polarity of pure solvents and binary mixtures.

pKa Values of 1 and 2 in Aqueous Solution

Figure 1(a) shows the fluorescence emission spectra for compound 1 at several pH values. The fluorescence emission band at 401 nm predominates for pH values between 5.8 and 12.0 and this band disappears at pH values below 4.0, simultaneously with the appearance of another band with low intensity in the region of 500 nm. Figure 1(b) shows the titration curve, as a plot of the fluorescence emission intensity (I_F) at 401 nm as a function of pH.

Figure 2(a) shows the influence of pH on the fluorescence emission spectrum of 2, where it can be observed that for pH values between 4.8 and 10.0 a maximum fluorescence occurs at 470 nm and for low pH values, more precisely in the pH range of 2.5–3.3, a fluorescence quenching occurs without the bathochromic shift verified in the case of compound 1. Figure 2(b) shows the corresponding titration curve obtained from the emission spectra.

The titration curves were used to obtain the pK_a values by means of a theoretical fitting of the experimental data using a sigmoidal equation. The pK_a value for the conjugated acid of 1 was 6.18±0.04 (S.D.=2.73×10^{-4}; r^2=0.999) and for 2 this value was 3.83±0.12 (S.D.=9.60×10^{-4}; r^2=0.997). A pK_a value of 3.36 was obtained by Perrin for the conjugated acid of non–substituted 1,8–naphthyridine in water [69].

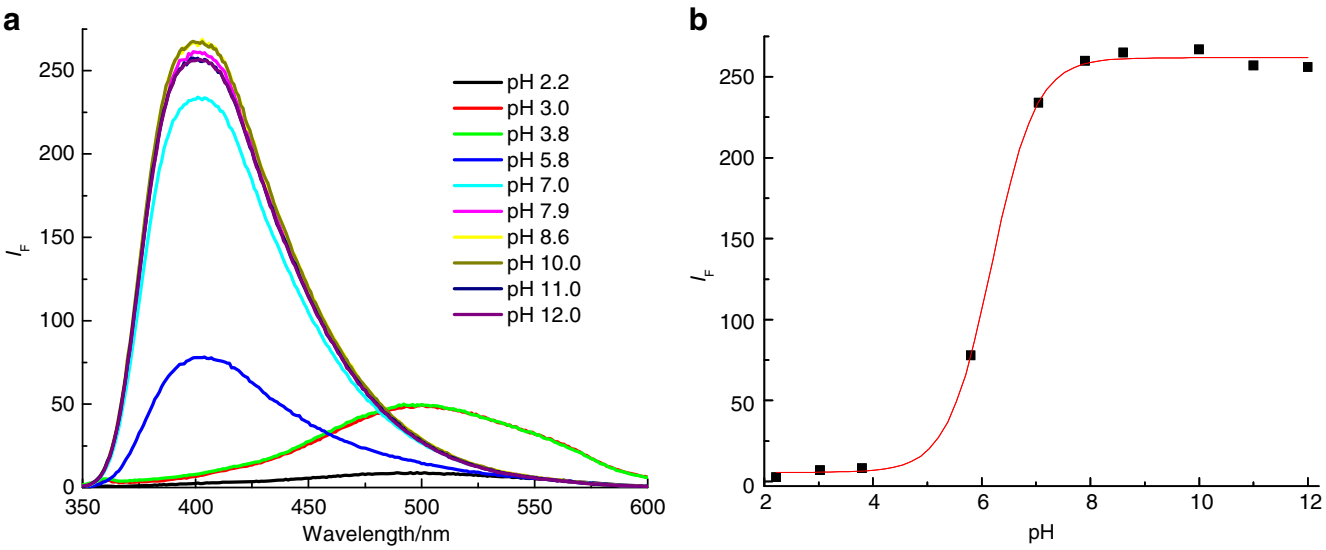


Fig. 1 (**a**) Fluorescence emission spectra of 1 at different pH values and (**b**) I_F values for 1 at 401 nm as a function of pH

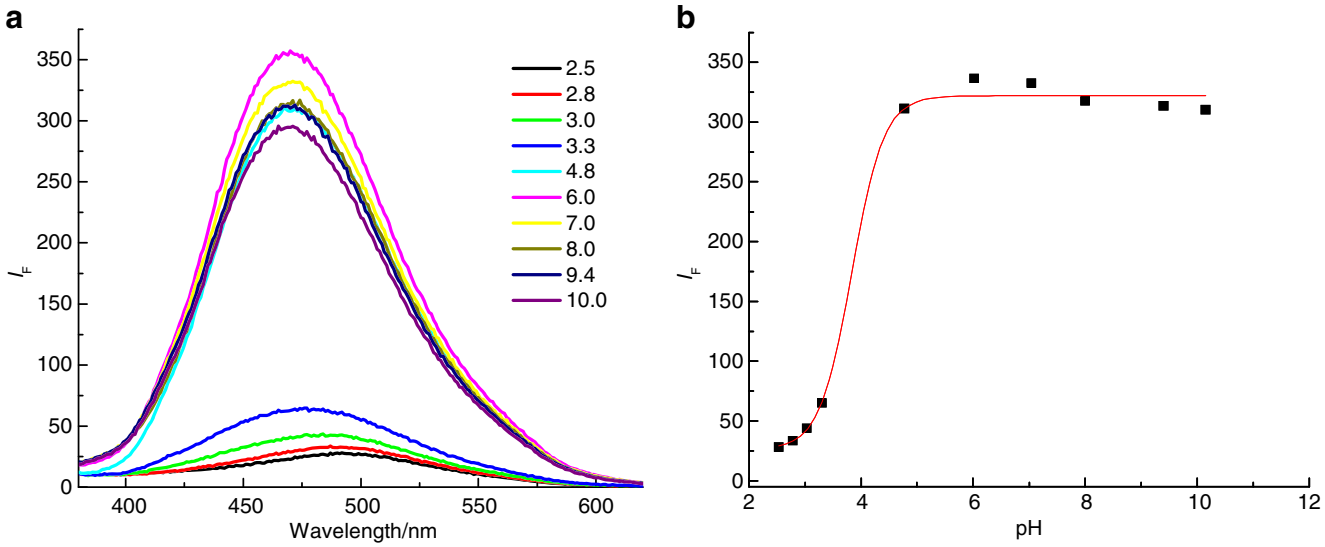


Fig. 2 (**a**) Fluorescence emission spectra of 2 at different pH values and (**b**) I_F values for 2 at 470 nm as a function of pH

Compound 1 has an amino electron–donor group in its molecular structure, which is responsible for increasing the electronic density at the nitrogen atoms of the naphthyrine ring. This may be the reason for the higher p*K*a value for 1 in comparison with 2 and with the non–substituted naphthyridine. The intermediate value obtained for 2 in comparison with the other two naphthyridines can be explained considering the acetamide substituent as a moderate electron–releasing group.

Influence of Cu^{2+} on the Fluorescence of 1 and 2

Figure 3(a) shows a group of fluorescence emission spectra relating to the titration of 1 with Cu^{2+}. It can be observed that with the addition of Cu^{2+} the fluorescence emission of the naphthyridine is quenched, until the complete disappearance of the band with a maximum at 401 nm. The I_F values at 401 nm were collected from the spectra, as shown in Fig. 3(b), and indicate that saturation occurs at a Cu^{2+} concentration of 4.0×10^{-3} mol dm^{-3}. Similar results were obtained for the titration of compound 2 with Cu^{2+}, as shown in Fig. 4.

The titration curves showed behavior typical of a 1:1 naphthyridine:Cu^{2+} stoichiometry. The binding constants were calculated by fitting the experimental data with a nonlinear regression to eq 1, which considers the participation of one molecule of the naphthyridine to one Cu^{2+} ion [70, 71].

$$I_F = \left[I_{Fo} + I_{F11}K_{11}c(Cu^{2+})\right]/\left[1 + K_{11}c(Cu^{2+})\right] \quad (1)$$

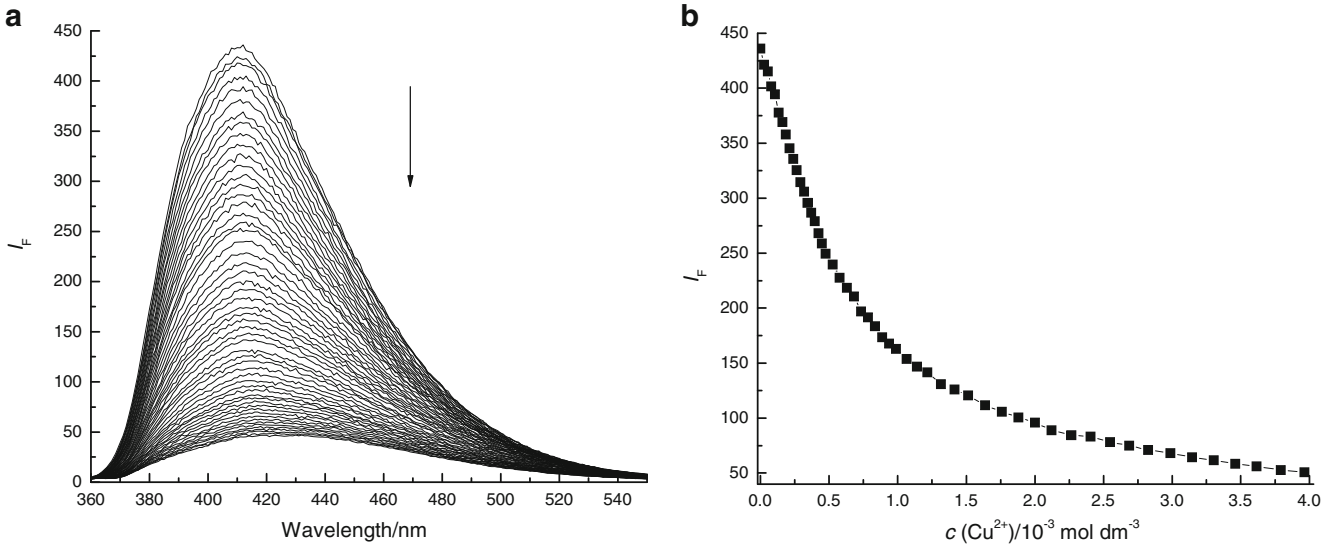


Fig. 3 (**a**) Influence of the addition of increasing amounts of Cu^{2+} on the fluorescence emission spectrum of 1 ($c=1\times10^{-6}$ mol dm^{-3}) in DMSO–water 7:3 (*v*/*v*) at 25 °C. The final concentration of Cu^{2+} was 4×10^{-3} mol dm^{-3}. (**b**) Variation in the I_F of 1 at 408 nm with the addition of increasing amounts of Cu^{2+}

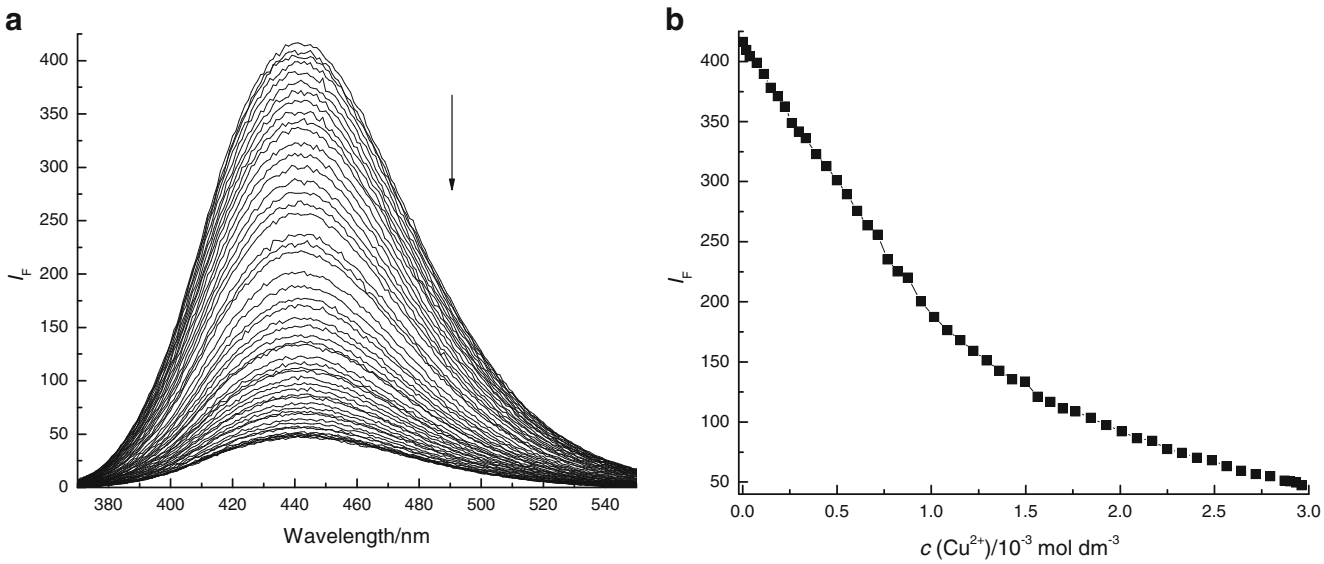


Fig. 4 (**a**) Influence of the addition of increasing amounts of Cu^{2+} on the fluorescence emission spectrum of 2 ($c=1\times10^{-6}$ mol dm^{-3}) in DMSO–water 7:3 (*v/v*) at 25 °C. The final concentration of Cu^{2+} was 3×10^{-3} mol dm^{-3}. (**b**) Variation in the I_F of 2 at 440 nm with the addition of increasing amounts of Cu^{2+}

where I_F is the fluorescence emission intensity after each addition of Cu^{2+}, c (Cu^{2+}) is the concentration of the metal after each addition of Cu^{2+}, I_{F11} is the minimum value of I_F obtained by the addition of Cu^{2+}, and K_{11} is the binding constant. The K_{11} value was obtained through this mathematical treatment and for compound 1 it was found to be $(1.54\pm0.43)\times10^3$ dm^3 mol^{-1} ($r^2=0.998$ and S.D.$=1.7\times10^{-4}$), while for 2 it was found to be $(6.25\pm0.35)\times10^2$ dm^3 mol^{-1}, ($r^2=0.996$ and S.D.$=4.1\times10^{-4}$). The K_{11} value for 1:Cu^{2+} is larger than that for 2:Cu^{2+} due to the electron–releasing effect of the amino group, which increases the electron density at the naphthyridine ring, making the electron pairs on the nitrogen atoms of the 1– and 8– positions of the naphthyridin ring 1 more available to interact with the metal ion. Compound 2 has in its molecular structure an acetamide group in the 2– position, and the carbonyl is responsible for reducing the electron density of the amino group through the resonance effect, leading consequently to the reduction in the complexation ability of the compound. The results obtained, in combination with data

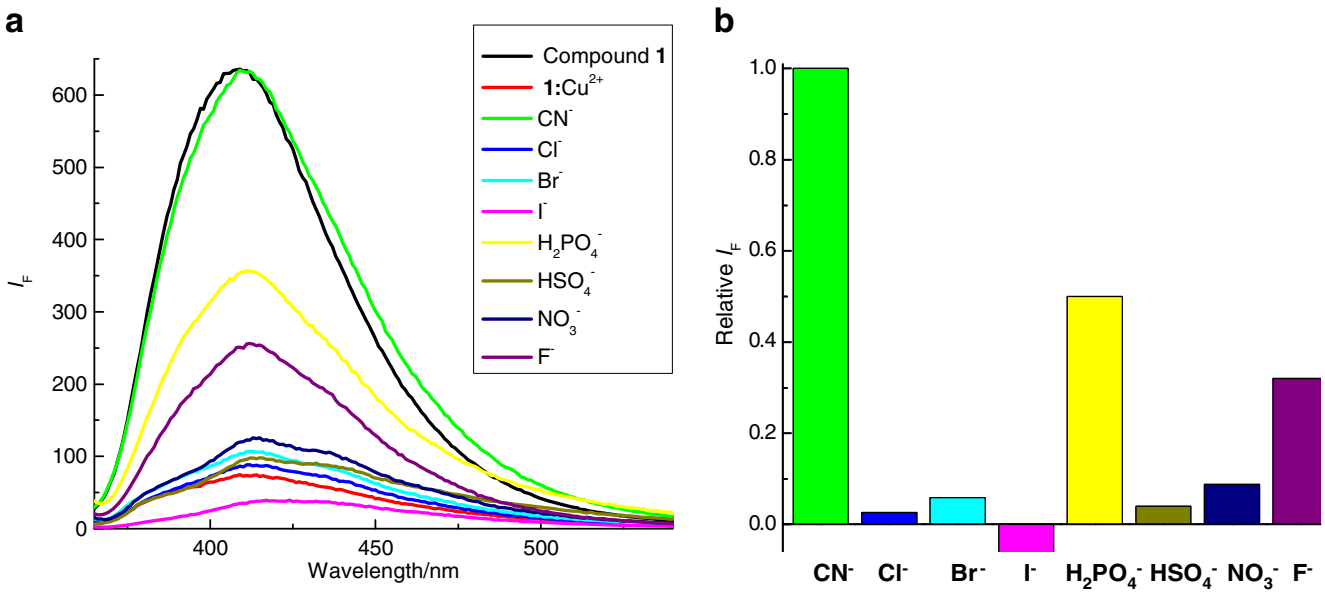


Fig. 5 (**a**) Fluorescence emission spectra at 25 °C of solutions of 1: Cu^{2+} in DMSO–water (7:3; *v/v*) in the presence of various anions. (**b**) I_F for the anions added to solutions of 1 at 408 nm relative to the CN^- added. A λ_{exc} value of 340 nm was used and the concentrations of the species were: c (1)$=1\times10^{-6}$ mol dm^{-3}, c (Cu^{2+})$=4\times10^{-3}$ mol dm^{-3}, and c (anion)$=8\times10^{-3}$ mol dm^{-3}

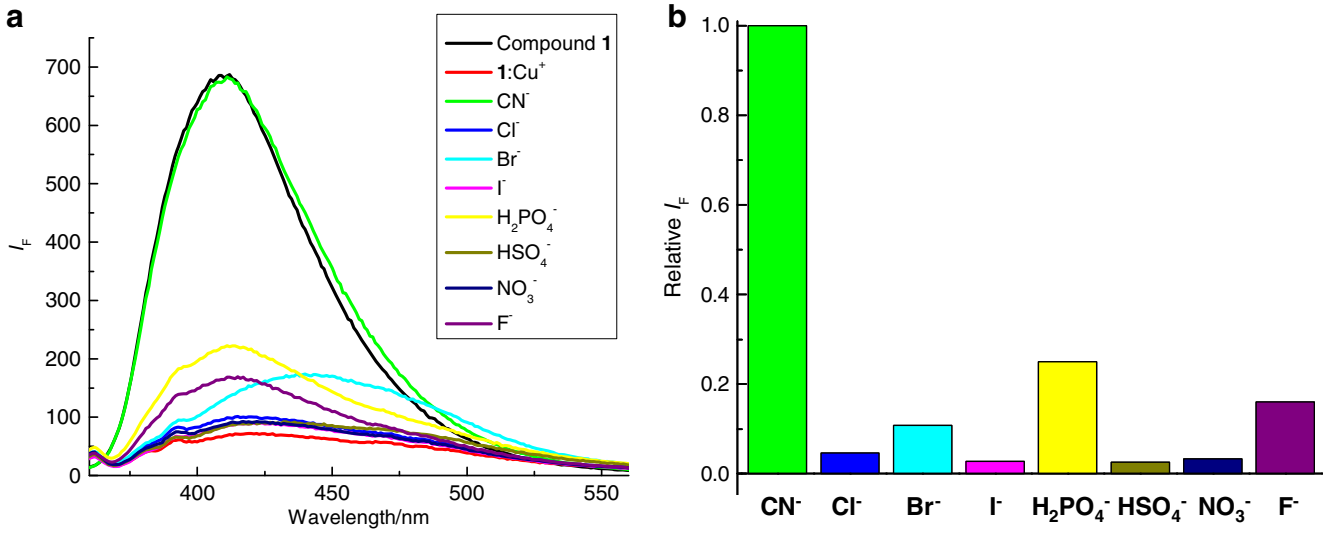


Fig. 6 (**a**) Fluorescence emission spectra at 25 °C of solutions of 1: Cu^{+} in DMSO–water (7:3; *v/v*) in the presence of various anions. (**b**) I_F for the anions added to solutions of 1 at 408 nm relative to the CN^{-} added. A λ_{exc} value of 340 nm was used and the concentrations of the species were: *c* (1)=1×10^{-6} mol dm^{-3}, *c* (Cu^{+})=3×10^{-3} mol dm^{-3}, and *c* (anion)=3×10^{-3} mol dm^{-3}

in the literature for the complexation of 1,8–naphthyridines with metal ions [42, 44, 72–74], suggest that the coordination of Cu^{2+} with compound 1 occurs in the naphthyridine center, at the nitrogens in the 1– and 8– positions. The coordination causes the quenching in the fluorescence of the system through a photoinduced electronic transfer (PET), which can occur between Cu^{2+} and the fluorophore. This situation is reported in the literature as involving the complexation of fluorescent compounds with metal ions [75–77].

Compounds 1 and 2 were also titrated with Cu^{+} and the pattern observed in these assays was very similar to that verified when Cu^{2+} was used. The experimental data were fitted with eq. (1), leading to $K_{11}=(2.50\pm0.11)\times10^{3}$ dm^{3} mol^{-1} ($r^{2}=0.996$ and S.D.=3.4×10^{-4}) for 1 while the K_{11} value for 2 was ($7.25\pm0.23)\times10^{2}$ dm^{3} mol^{-1} ($r^{2}=0.999$ and S.D.=4.1×10^{-5}).

Competition Assay Using the Complex Between the Naphthyridines and Cu^{2+} as an Anionic Fluorogenic Chemosensor

According to the literature, Cu^{2+} reacts with CN^{-} to form CuCN and cyanogen $(CN)_2$ [78]. Subsequently, complexes with a high stability constant of the type $[Cu(CN)_2]^{-}$, [Cu

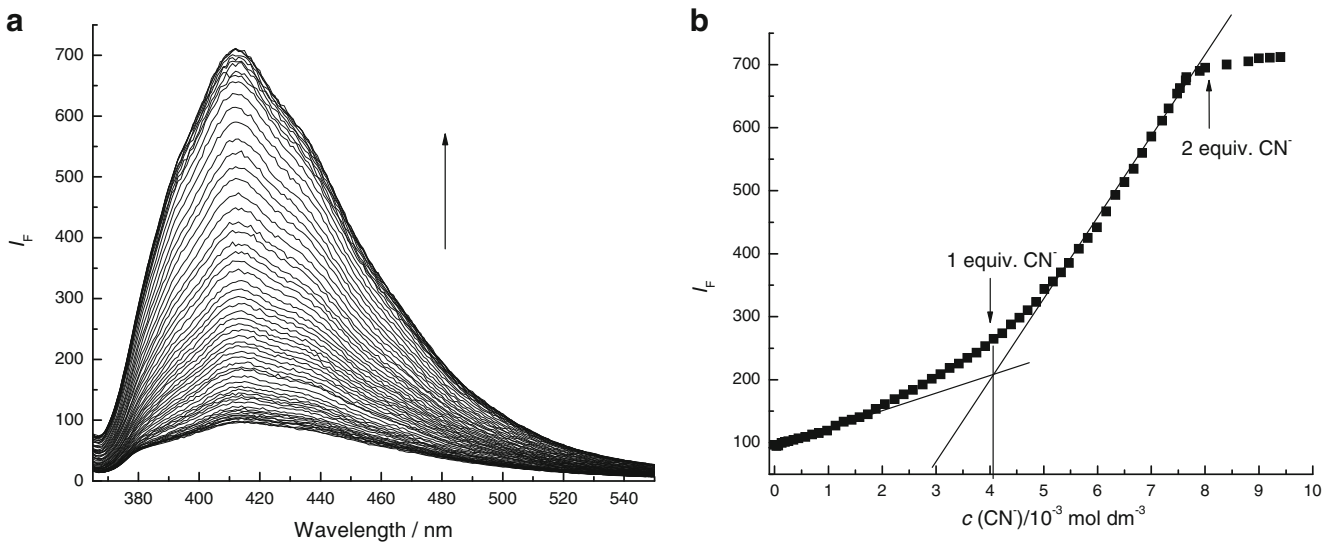


Fig. 7 (**a**) Influence of the addition of increasing amounts of CN^{-} on the fluorescence emission spectrum of 1:Cu^{2+} in DMSO–water 7:3 (*v/v*) at 25 °C. The final concentration of CN^{-} was 8×10^{-3} mol dm^{-3}. (**b**) Variation in the I_F of 1:Cu^{2+} at 408 nm with the addition of increasing amounts of CN^{-}. A λ_{exc} value of 340 nm was used and the concentrations of 1 and Cu^{2+} were 1×10^{-6} mol dm^{-3} and 4×10^{-3} mol dm^{-3}, respectively

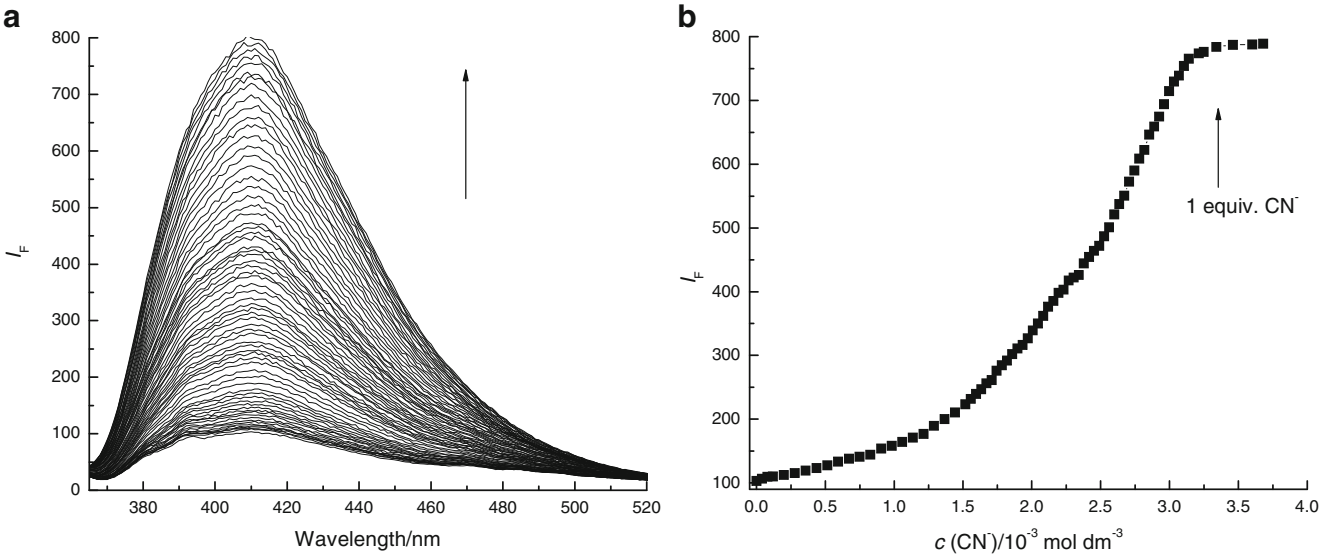


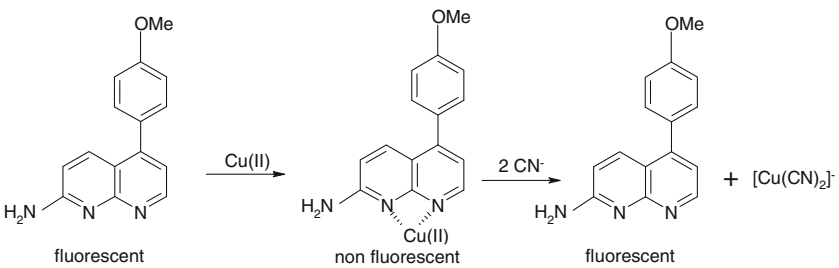
Fig. 8 (**a**) Influence of the addition of increasing amounts of CN^- on the fluorescence emission spectrum of 1:Cu^+ in DMSO–water 7:3 (*v/v*) at 25 °C. The final concentration of CN^- was 3×10^{-3} mol dm^{-3}. (**b**) Variation in the I_F of 1: Cu^+ at 408 nm with the addition of increasing amounts of CN^-. A λ_{exc} value of 340 nm was used and the concentrations of 1 and Cu^+ were 1×10^{-6} mol dm^{-3} and 3×10^{-3} mol dm^{-3}, respectively

$(CN)_3]^{2-}$, and $[Cu(CN)_4]^{3-}$are formed in excess of CN^-, and the species commonly found are $[Cu(CN)_2]^-$ ($K=1\times10^{24}$) and $[Cu(CN)_4]^{3-}$ ($K=1\times10^{30}$) [79–83]. This finding is the basis for the recent development of some anionic chemosensors based on displacement assays [24, 84–87]. Therefore, the potential of the complexes comprised of 1,8–naphthyridines and copper ion as anionic chemosensors was studied in a DMSO–water mixture. The strategy used here is based on the idea that an anion having a great affinity for the metal ion can displace the latter from the naphthyridine site, leading to the restoration of the fluorescence emission in solution.

Figure 5(a) shows the influence of various anions, at the same concentration, on the fluorescence emission spectrum of 1:Cu^{2+}. It can be observed that of the anions used, only CN^- was able to fully restore the original fluorescence of 1, confirming the initial expectation. Figure 5(b) shows the relative emission intensities for the same system in the presence of the anions, which verifies that CN^- recovers 100 % of the original fluorescence of 1, followed by $H_2PO_4^-$ (50 %) and F^- (32 %), while the other anions have practically no influence on the spectrum of the complex. Very similar results were obtained for compound 2. In addition, the fluorescence recovery process is very fast: only one addition of CN^- to the complex of 1 (or 2) with Cu^{2+} in a concentration twice the *c* (Cu^{2+}) immediately causes the return to the original fluorescence of the naphthyridine in solution.

Figure 6(a) illustrates the behavior of 1:Cu^+ in the presence of several anions. It can be observed at this time that the system becomes more selective toward CN^- in comparison with the other anions: the fluorescence emission is completely restored when CN^- is used while the influence of $H_2PO_4^-$ and F^- in terms of a return of the fluorescence is small (25 % and 16 % for $H_2PO_4^-$ and F^-, respectively). One reason for the high selectivity obtained for CN^- is related to the fact that the free energies of hydration for $H_2PO_4^-$ (−465 kJ mol^{-1}) and F^- (−465 kJ mol^{-1}) are high in comparison to that observed for CN^- (−295 kJ mol^{-1}) [88, 89], and some papers have reported the use of this strategy to make chemosensors selective to CN^- in solution [90–95]. Thus, $H_2PO_4^-$ and F^- in aqueous medium are preferentially solvated by water, hindering their interaction with Cu^{2+}. Another explanation that can be offered makes use of the hard and soft acids and bases (HSAB) theory of Pearson [96, 97]. Cu^+ is a soft acid which forms a complex with CN^- of high

Scheme 2 Representation for the displacement assay studied with the addition of increasing amounts of CN^- to the complex 1:Cu^{2+}



stability. $H_2PO_4^-$ and F^- are hard bases, and Cu^{2+} is a harder acid than Cu^+, which can aid an explanation of the differences between the results obtained in the two systems studied. Hence, the experimental data suggest two different mechanisms of interaction for the anionic species with the fluorogenic system. $H_2PO_4^-$ and F^- would displace the metallic ion from the naphthyridinic site, while CN^- would react with Cu^{2+} changing its oxidation state before the displacement of the metal.

Figure 7(a) shows a set of fluorescence emission spectra related to the titration of 1:Cu^{2+} with CN^-, where it can be verified that with the addition of the anion there occurs an increase in the I_F corresponding to the appearance of the free naphthyridine at 408 nm. The experimental data were used to obtain a plot of the I_F values at 408 nm as a function of c (CN^-), shown in Fig. 7(b). The titration curve obtained has a sigmoidal profile, which suggests that the addition of the anion has initially a small influence on the increase in the fluorescence and on the addition of larger amounts of the anion the fluorescence is restored, corresponding to the same value obtained for 1 prior to the addition of the metal. A careful analysis of the titration curve reveals that two equivalents of the anion need to be added to fully restore the fluorescence of the system. When two straight lines are placed tangentially to the two halves of the curve, it is verified that their intersection coincides with one equivalent of anion added. The data suggest that the first anion equivalent is used to change the oxidation state of the metallic ion, from Cu^{2+} to Cu^+, and in sequence the second anion equivalent is responsible for displacing the metal from the naphthyridine binding site, which leads to the full return of the fluorescence of the system. A very similar result was obtained for the system with compound 2, which can be explained by the fact that both the reduction of Cu^{2+} and its displacement by the anion are very fast–occurring events.

Figure 8(a) shows the fluorescence emission spectrum for the titration of 1:Cu^+ with CN^-, which reveal an increase in the fluorescence of the system on the addition the anion, corresponding to the appearance of the free naphthyridine. The corresponding titrating curve, shown in the Fig. 8(b), presents a sigmoidal shape, similar to that obtained for the titration of the Cu^{2+} complex. However, the data show that only one CN^- equivalent is needed to displace Cu^+ from the naphthyridine site, causing a complete return to the original fluorescence. Thus, these data reinforce the above suggestion regarding the role of CN^- in the change in the oxidation state of Cu^{2+}.

The results reported herein are summarized in Scheme 2, which illustrates the competition assay involving the addition of CN^- to the complex. Compound 1 is fluorescent in solution but the fluorescence is quenched with the addition of Cu^{2+}, due to the formation of a complex with 1:1 stoichiometry. On addition of CN^-, 1 is released from the metal and becomes free to exhibit its fluorescence.

Conclusions

1,8–Naphythyridines 1 and 2 were synthesized and they were found to be fluorescent in solution. In the presence of Cu^{2+} (and Cu^+) their fluorescence was quenched, due to a PET mechanism between the metallic ion and the fluorophore. The assays employing the complex of the naphthyridines with the copper ions in the presence of various anions showed both selectivity and the complete and immediate return of the original fluorescence with the addition of CN^-. In addition, the titration experiment demonstrated that two anion equivalents are needed for the complete restoration of the fluorescence of the naphthyridines. The first equivalent of the anion is needed to change the oxidation state of the metal to Cu^+, and the second equivalent is responsible for displacing the metallic ion from the binding site in the naphthyridine, making the latter free to fluoresce. This was confirmed with the titration of 1 using Cu^+, which revealed that only one equivalent of the anion is needed to completely restore the fluorescence of 1.

The strategy applied for the detection of CN^- in aqueous medium is easy to perform, but the range of detection of CN^- is in the order of 10^{-4} mol dm^{-3}, which is above the minimum concentration of CN^- allowed by the World Health Organization in potable water, that is 1.7×10^{-6} mol dm^{-3} [98]. Nevertheless, the potential of the assay studied is demonstrated herein, as well as the possibility to exploit a clever design to synthesize other modified 1,8–naphthyridines in order to change certain features, such as to increase the quantum yield values and the binding constants associated with the complexation of these compounds with metallic ions, which will improve the sensitivity of this methodology.

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