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Synthesis and evaluation of the europium^{III} and zinc^{II} complexes as luminescent bioprobes in high content cell-imaging analysis

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ABSTRACT

Novel phenanthroline derivatives and their europium(III) and zinc(II) complexes have been prepared in up to 92%. In contrast to the stable zinc complexes, the europium compounds exhibit a strong luminescence in THF solution. However, quenching of the emission is observed in DMSO indicating complete dissociation of the complexes back to free ligands in this solvent. ^{1}H NMR studies of the Eu(III)-complexes **5** and **6** also confirmed the existence of different states depending on the solvent used. Moreover, it was found that compound **5** is stable in EtOH–PBS solutions; here a strong signal in the emission spectra corresponding to the europium ion was detected. No spectral changes were observed for the zinc(II) complexes, they were shown to be stable in the media. These metal complexes can be used as fluorescence markers for the diagnosis of oesophageal squamous carcinoma (OE21) cells at low concentrations. Cell images were acquired using the compounds **5**, **7**–**9** as luminescent agents. The first images were taken already after 20 min incubation time at a very low concentration range (0.7–1.6 μM).

1. Introduction

In past decades, molecular imaging technology has become a central tool in biology, particularly in bioassays and cell imaging, cancer research, and clinical trials in medicine [1–5]. The development of novel luminescent probes for intracellular analysis, that are efficient and kinetically stable, is one of the vital goals. Rare earth metal complexes are now versatile targets in many areas of research, such as photochemistry, biomedicine and nanotechnology [6–13]. Special attention has been given to the luminescence properties of lanthanide ions emitting at a millisecond lifetime-scale and in the visible region for europium(III) and terbium(III) complexes [14–17]. These remarkable photophysical properties are key factors to make them molecules of choice for cellular imaging and diagnostics [18–21]. A variety of the different Ln(III) complexes have been synthesised, however a major problem is their instability in aqueous media. The lanthanide ion and its neutral ligands are linked via weak coordination bonds and generally exist in an equilibrium of "free ligand" ⇆ "metal complex", which can be shifted in a medium via solvent exchange [22–23]. The metal complexes are prone to dissociation in the presence of solvent molecules. Disadvantageously, in some cases this can lead to an irreversible dissociation into the "free ligand" form in water through completion of the first coordination sphere [22–25] of a lanthanide ion with solvent molecules. This normally prevents the use of Ln(III) complexes as luminescent markers for bioassays. Nevertheless, only limited information is available on the real state of such metal complexes in different media.

Zinc compounds are known to be highly fluorescent and present an alternative to lanthanide complexes. Thus, many photochemical studies targeted at modelling the natural situation use more stable zinc complexes. The propensity of zinc to act as an acceptor atom is used in many supramolecular approaches and/or for the modulation of chromophore properties via ligand binding. Due to their biological relevance, technical importance and good stability, they have found significant use as industrial pigments, electron transfer components, for photochemical transformations and in photobiotechnology [26].

Here, we have studied the stability and photophysical efficiency of europium and zinc complexes of phenanthroline derivatives. These molecules are strongly absorbing chromophores often used as antennas for indirect excitation of metal ions, where direct excitation of a metal is inefficient. Phenanthroline itself is known to inhibit metallopeptidases and its various metal complexes have been prepared and tested as bioprobes [27–37]. Here we present results based on the synthesis, behaviour indifferent solvents monitored by NMR spectroscopy, photophysical analysis and cellular imaging of novel metal complexes at low concentrations.

2. Experimental

2.1. Materials and methods

A 1 mM stock solution was prepared in THF, MeOH, DMSO and EtOH for each compound. Dilutions were carried out directly before starting the analyses. For cell stain imaging a stock solution prepared

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in EtOH was diluted with phosphate buffered saline (PBS) to different concentrations. Buffer solutions prepared at the desired concentrations were added directly to the cell culture. Total EtOH concentration was lower than 0.1%. After adding compounds, cells were incubated at 37 °C in 5% CO_2 for 20 min to 24 h. Cells (human esophageal squamous carcinoma OE21) were routinely cultured in RPMI 1640 (Hyclone, USA) with 10% inactivated foetal bovine serum (Hyclone, USA) and 1% Penicillin/(Hyclone, USA). These cultures were grown in sterile filtered top cell culture flasks (Nunc, Denmark). Cultures were split 1:8 when 70–80% confluency was reached. Cells were split using typsin 0.25% solution, with EDTA (Hyclone, USA) and kept at 37 °C and 5% CO_2 in a humidified atmosphere. Culture medium was changed every 3 to 4 days until confluency. The cell lines were plated at a concentration of 1.0×10^5 cells/mL into sterile 96-well plates (Nunc, Denmark) and left to attach overnight.

2.2. General

^{1}H NMR spectra were recorded on a Bruker DPX 400 (400 MHz and 600 MHz for ^{1}H NMR). Chemical shifts are reported in ppm referred to TMS set at 0.00 ppm. All photophysical measurements were performed in THF, MeOH, EtOH, DMSO and PBS. All emission spectra were recorded on Fluorolog Horiba Jobin Yvon spectrometer. High resolution mass-spectra (HRMS) were measured on MaldiQ-Tof Premier Micromass and Micromass/Waters Corp. USA liquid chromatography time-of-flight spectrometer equipped with ES source. UV–visible (UV–vis) measurements were performed on Specord 250 Analytik Jena instrument. Melting points were acquired on Stuart SMP10 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (Merck) precoated aluminium sheets. Spectroscopy grade solvents were used in all cases. All commercial chemicals and solvents were supplied by Sigma-Aldrich and used without further purification. Abbreviations "s" — singlet, "d" — doublet, "dd" — doublet of doublets, "m" — multiplet, "br" — broad, ES MS — electrospray mass-spectrometry, TOF MS — time-of-flight mass-spectrometry, THF — tetrahydrofuran, MeOH — methanol, EtOH — ethanol, DMSO — dimethylsulfoxide, PBS — phosphate buffered saline were used.

2.3. Synthesis of 1,10-phenanthroline-5,6-dione [38] 1

To a dry mixture of 1,10-phenanthroline (2.0 g, 0.01 mol) and KBr (10.0 g, 0.08 mol), H_2SO_4 (40 mL) followed by HNO_3 (20 mL) was added dropwise at 0 °C. The resulting mixture was heated at 100 °C until the bromine vapours disappeared. The solution was poured carefully onto ice and slowly neutralised to pH>7 with Na_2CO_3 (saturated solution and powder). The product was extracted with dichloromethane and dried over Na_2SO_4. The solvents were evaporated to give a yellow solid that was dried under vacuum (88–95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.16 (dd, $J=4.6$ Hz, $J=1.7$ Hz, 2H); 8.54 (dd, $J=7.9$ Hz, $J=1.6$ Hz, 2H); 7.62 (dd, $J=7.9$ Hz, $J=4.7$ Hz, 2H).

2.4. Synthesis of imidazo[4,5-f]1,10-phenanthroline derivatives 2–4

1,10-Phenanthrolin-5,6-dione **1** (1.0 g, 4.76 mmol) and ammonium acetate (11.7 g, 0.15 mol) were dissolved in hot glacial acetic acid (50 mL) at ca. 70 °C and an appropriate aldehyde (4.76 mmol), dissolved in 20 mL of glacial acetic acid, was added. The resulting mixture was heated at 70–80 °C for 2 h (TLC-control). Then the solution was allowed to cool to room temperature and was neutralised with 1 N NaOH. The yellow compound was filtered, washed with water and dried under vacuum.

2.4.1. 2-(4-Bromophenyl)imidazo[4,5-f]-1,10-phenanthroline 2

Yellow solid (1.43 g, 80%); analytical data were in accordance with the literature [39].

2.4.2. 2-{4-[1′-(2′-Trimethylsilyl)ethynyl]phenyl}imidazo[4,5-f]-1,10-phenanthroline 3

Yellow solid (1.24 g, 66%). Mp 258 °C; δ_H (400 MHz, DMSO-d_6) 9.06 (m, 2H, H_{phenan}), 8.92 (d, $J=6.9$ Hz, 2H, H_{phenan}), 8.29 (d, $J=8.4$ Hz, 2H, H_{Ph}), 7.86 (dd, $J=8.0$ Hz, $J=4.3$ Hz, 2H, H_{phenan}), 7.70 (d, 2H, $J=8.4$ Hz, H_{Ph}), 0.27 (s, 9H, TMS); δ_C (150.2 MHz, DMSO-d_6) 149.9, 148.3, 144.0, 132.6, 130.4, 129.9, 126.6, 123.3, 105.2, 96.5 (m); *m/z* (TOF MS) 393.152 (M + H. $C_{24}H_{21}N_4Si$ requires 393.153); UV–visible (UV–vis) (CH_3OH): λ_{max} (lgε) 283 (4.3), 335 (4.4).

2.4.3. 2-(4-Pyridinyl)imidazo[4,5-f]-1,10-phenanthroline [40] 4

Brown solid (1.07 g, 76%); δ_H (400 MHz, DMSO-d_6) 9.20 (m, 2H, H_{phenan}), 8.92 (m, 4H, H_{phenan} and H_{Py}), 8.27 (m, 2H, H_{Py}), 7.97 (m, 2H, H_{phenan}). UV–visible (UV–vis) (CH_3OH): λ_{max} (lgε) 273 (4.6), 322 (4.3), 387 (3.6).

2.5. Synthesis of the imidazo[4,5-f]-1,10-phenanthroline europium (III) complexes 5–7

To an appropriate imidazo[4,5-*f*]-1,10-phenanthroline derivatives **2**–**4** (0.63 mmol) was dissolved in hot ethanol (100 mL) and an ethanolic solution of $Eu(NO_3)_3{\bullet}5H_2O$ (90 mg, 0.21 mmol) was added. The resulting mixture was heated at reflux until the starting material disappeared (TLC-control, ca. 30 h). The yellow solid formed was filtered, washed with ethanol and dried under vacuum to give the desired product **5**–**7**.

2.5.1. 2-(4-Bromophenyl)imidazo[4,5-f]-1,10-phenanthroline europium (III) complex 5

Yellow solid (0.47 g, 58%). Mp>300 °C; δ_H (400 MHz, THF-d_8, TMS) 13.19 (3H, br s, NH), 8.86 (6H, br m, H_{phenan}), 8.08 (16H, br m, H_{phenan} and H_{Ph}), 6.50 (8H, br m, H_{phenan}); *m/z* (TOF MS) 1274.971 (M. $C_{57}H_{33}Br_3EuN_{12}$ requires 1274.976).

2.5.2. 2-{4-[1′-(2′-Trimethylsilyl)ethynyl]phenyl}imidazo[4,5-f]-1,10-phenanthroline europium(III) complex 6

Yellow solid (0.77 g, 92%). Mp>300 °C; δ_H (400 MHz, THF-d_8, TMS) 13.00 (br s, 3H, NH), 8.75 (br m, 6H, H_{phenan}), 8.18 (br m, 10H, H_{phenan} and H_{Ph}), 7.63 (m, 6H, H_{Ph}), 6.26 (br m, 8H, H_{phenan}), 0.20 (s, 27H, TMS); *m/z* (TOF MS) 1302.292 (M-3Me + H_2O. $C_{69}H_{53}EuN_{12}OSi_3$ requires 1302.299.

2.5.3. 2-(4-Pyridinyl)imidazo[4,5-f]-1,10-phenanthroline europium(III) complex 7

Yellow solid (0.36 g, 55%). Mp>300 °C; δ_H (400 MHz, D_2O + DMSO-d_6, TMS) 7.82 (br m, 30H, H_{phenan} and H_{Ph}), 0.52 (br, 3H, NH); *m/z* (ES) 968.17 (M-Py + H. $C_{50}H_{30}EuN_{14}$ requires 968.21), *m/z* (TOF MS) 906.18 (M-2Py + H_2O). $C_{44}H_{27}EuN_{13}O$ requires 906.17, 1044.23 (M). $C_{54}H_{33}EuN_{15}$ requires 1044.44, 1064.30 (M + H_2O + 2H). $C_{54}H_{37}EuN_{15}O$ requires 1064.25.

2.6. Synthesis of imidazo[4,5-f]-1,10-phenanthroline zinc complexes 8–10

To an appropriate imidazo[4,5-*f*]-1,10-phenanthroline derivatives **2** or **4** (0.54 mmol) dissolved in 10–20 mL of THF–MeOH (1:1, v/v) was added a solution of $ZnCl_2$ (0.27 mmol) in MeOH. A yellow precipitate was formed immediately after addition of the metal salt. The mixture was stirred at room temperature for 24 h and filtered. The solid residue was washed with ethanol and dried in vacuum.

2.6.1. 2-(4-Pyridinyl)imidazo[4,5-f]-1,10-phenanthroline zinc(II) complex 8

Pale yellow solid (36 mg, 20%). Mp>300 °C; δ_H (600 MHz, DMSO-d_6) 10.09 (s, 2H, NH), 9.16 (br, 8H, H_{phenan}), 8.96 (d, $J=4.6$ Hz, 4H,

H_{phenan}), 8.27 (br, 8H, H_{Py}); δ_C (150.2 MHz, DMSO-d_6) 162.1, 151.4, 144.1, 134.8, 133.3, 121.0; *m/z* (TOF MS ES+) 397.008 (M-L-Cl + H). $C_{18}H_{12}N_5ClZn$ requires 397.007; UV–visible (UV–vis) (CH_3OH): λ_{max} (lgε) 277 (4.0), 319 (3.9).

2.6.2. 2-(4-Bromophenyl)imidazo[4,5-f]-1,10-phenanthroline zinc(II) complex 9

Pale yellow solid (152 mg, 69%). Mp > 300 °C. δ_H (600 MHz, DMSO-d_6) 14.22 (br, 2H, NH), 9.17 (br, 4H, H_{phenan}), 8.82 (m, 4H, H_{phenan}), 8.23 (d, 4H, H_{Ph}), 8.08 (dd, 4H, H_{Ph}), 7.84 (m, 4H, H_{phenan}); δ_C (150.2 MHz, DMSO-d_6) 150.8, 146.6 (m), 137.7, 132.1, 128.8, 125.6, 123.4; *m/z* (TOF MS) 812.968 (M + H). $C_{38}H_{23}N_8ZnBr_2$ requires 812.970; UV–visible (UV–vis) (CH_3OH): λ_{max} (lgε) 283 (4.8), 390 (4.8), 318 (4.7).

2.7. (2-(4-Bromophenyl)-N-hexyl)imidazo[4,5-f]-1,10-phenanthroline zinc complex 10

To a solution of 2-(4-bromophenyl)imidazo[4,5-*f*]-1,10-phenanthroline **2** (230 mg, 0.61 mmol) in anhydrous DMF (10 mL), K_2CO_3 (170 mg, 1.21 mmol) was added under argon. The mixture was heated at 60 °C for 2 h and 1-bromohexane (0.13 mL) was added. The reaction was refluxed at 80 °C for 48 h and quenched with 10 mL of water. The product was extracted with 3 × 20 mL of $CHCl_3$, washed with water and dried over Na_2SO_4. The solvents were evaporated to give the crude product. To the residue (80 mg, 0.17 mmol) dissolved in 10 mL of THF–MeOH (1:1, v/v) was added a solution of $ZnCl_2$ (12 mg, 0.087 mmol) in MeOH. The reaction gave the complex **10** as a pale yellow solid (5 mg, 6%). Mp 260 °C. δ_H (400 MHz, DMSO-d_6): 9.79 (d, *J* = 8.3 Hz, 2H, H_{phenan}), 9.54 (br, 2H, H_{phenan}), 9.23 (m, 4H, H_{phenan}), 8.47 (m, 2H, H_{phenan}), 8.32 (d, *J* = 8.3 Hz, 4H, H_{Ph}), 8.14 (m, 2H, H_{phenan}), 7.89 (d, *J* = 8.3 Hz, 4H, H_{Ph}), 5.92 (t, *J* = 7.0 Hz, 4H, CH_2), 2.09 (m, 4H, CH_2), 1.55 (m, 4H, CH_2), 1.37 (m, 8H, CH_2), 0.91 (t, *J* = 7.0 Hz, 6H, CH_3). *m/z* (TOF MS) 981.154 (M + H. $C_{50}H_{47}N_8ZnBr_2$ requires 981.158); UV–visible (UV–vis) (CH_3OH): λ_{max} (lgε) 294 (4.2), 301 (4.2).

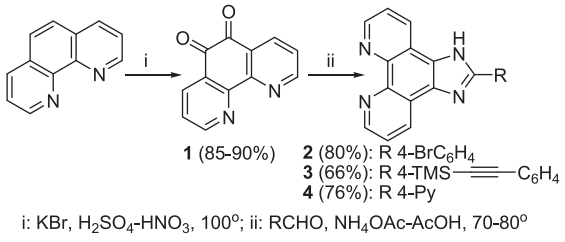
3. Results and discussion

3.1. Synthesis

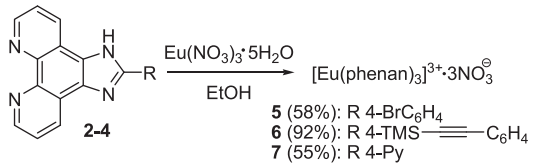
The neutral bidentate ligands **2–4** were synthesized using a simple two-step approach starting from commercially available 1,10-phenanthroline. 1,10-Phenanthroline-5,6-dione **1** readily reacted with various aldehydes to give compounds **2–4** in up to 80% yield (Scheme 1).

The metal complexes **5–10** were prepared using the derivatives **2–4** and the corresponding metal salts in good yields. Formation of the europium(III) complexes was achieved in ethanol in the presence of europium(III) trinitrate with the ligands **2–4** in a stoichiometric ratio of 1 to 3 yielding the compounds **5–7** in 58%, 92%, and 55% yields, respectively (Scheme 2). The structure of the new europium compounds have been confirmed by MS-spectroscopy and ^{1}H NMR (in THF) analysis.

Synthesis of the zinc(II) complexes was carried out using $ZnCl_2$ in THF–MeOH in a similar manner to produce the compounds **8–10** in up to 69% yield (Scheme 3). Noteworthy, the different stoichiometric salt-ligand ratios result only in the formation of the 2 to 1 complexes **8** and **9**; neither 1:1 nor 3:1 complexes were observed, as reported in some cases.



Scheme 1. Synthesis of 1,10-phenanthroline-5,6-dione **1** and derivatives **2–4**.



Scheme 2. Synthesis of europium(III) complexes **5–7**.

3.2. Photophysical behaviour of compounds 5–10

Influence of the structural differences in complexes **5–10** on their photophysical properties was investigated by comparing the spectroscopic profiles of the free ligands, zinc and europium compounds. The complexes **5–10** exhibited absorption bands between 280 and 400 nm that strongly depended on the ligation state and type of metal inserted. Slight shifts, intensity and shape differences were observed in the absorption spectra for the free ligand **2** and its europium **5** and zinc **9** complexes (Fig. 1).

Emission spectra for this series of compounds were recorded at various irradiation energies. All three types of compounds exhibited an emission band at 405 nm which is characteristic for the ligand's emission followed by an additional band corresponding to metal–ligand transitions (Fig. 2).

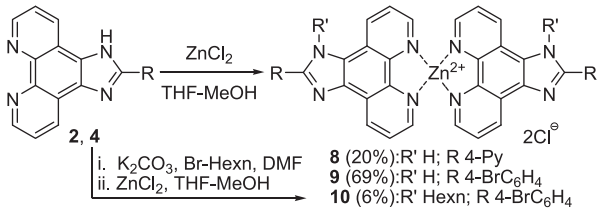
The europium complex **5** showed the characteristic bands of lanthanide ion emission between 670 nm and 710 nm. In the case of the zinc complex **9**, a splitting of the band at 410 nm was observed with the new maximum at 465 nm as the result of complex formation (Table 1).

In addition to the emission profile requirements, fluorescence imaging of the biomaterials requires reasonable Stokes shifts. Indeed, according to the results reported in Table 1, all compounds show large Stokes shifts.

3.3. Luminescence: solvents dependency and dissociation of the europium(III) complexes

A change in a photophysical profile of many optical materials such as a "switch-on" and "-off" can be achieved simply by varying a solvate medium through temporary quenching of the emission of one or more components of the system. However, in some cases these changes can be irreversible and can be used to detect a particular molecule or ion. We have analysed the behaviour of the phenanthrene-based europium complexes **5–7** under different conditions. The effects of the solvents can even be seen with the "naked eye". Simple illumination of the different solutions of complex **5** with a standard laboratory UV-lamp revealed the differences (Fig. 3).

A detailed analysis of compounds **5–7** was performed in various solvents including THF, D_2O, EtOH and DMSO. The presence of the metal complexes was clearly verified for THF and D_2O solutions.



Scheme 3. Synthesis of zinc(II) complexes **8–10**.

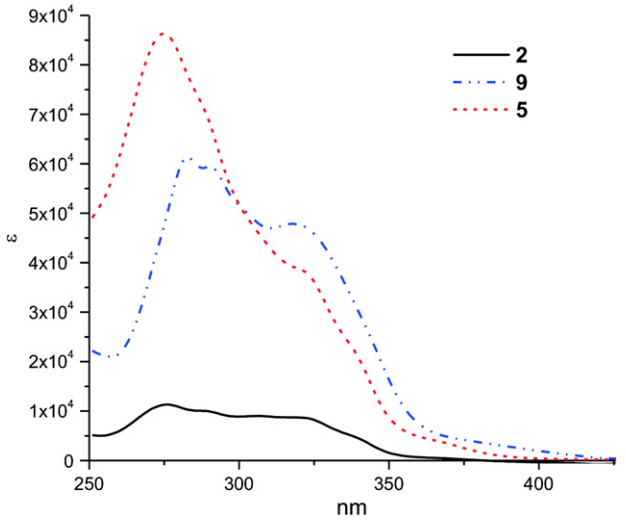


Fig. 1. Absorption spectra of compounds **2**, **5** and **9** (10^{-5} M) in THF.

Here the typical emission signals corresponding to $^5D_0 \rightarrow {}^7F_{0,1,2,3,4}$ transitions of the europium(III) ion in **5** and **6** were observed (Fig.4).

No lanthanide emission was found for **5** and **6** in DMSO. Here, only the fluorescence corresponding to the ligand unit was detected. These results indicate that the emission of the Eu(III) ion is either quenched by solvent molecules entering the first solvation sphere or indicate the complete dissociation of the complex back to a free ligand and lanthanide salt with solvent molecules as new ligands (Scheme 4).

Detailed studies on solvent exchange mechanisms have been carried out for the series of d- and f-metal complexes [22, 23]. The solvent exchange rates differ greatly between harder and softer atom donors of a solvent and different solvent exchange mechanisms (associative vs. dissociative activation) have been proposed [22, 23, 41]. Certainly, other factors such as different steric and electronic characteristics of the system components, the presence of an extensive π-moiety on the *NN* part of the ligand, an ease of the π-system to interact with nonbonding electrons of the metal, and the flexibility and stretching of the chelate bite mode have to be taken in account.

3.4. NMR studies

As the luminescence studies indicated a possible decomposition of the europium(III) complexes **5** and **6** in DMSO we employed NMR analysis to determine the existence of different states for these materials. For this, the effects of the different deuterated solvents were analysed by ^{1}H NMR spectroscopy. Fig. 5 gives an example of the different behaviours of compound **5** in THF-d_8, Methanol-d_4 and DMSO-d_6.

Clearly, europium complex **5** exists in its "metal complex"-form in THF solution. Here, the phenanthroline hydrogen atoms in closest proximity to the Eu(III)-centre are shifted up-field. This is in good agreement with the fact that europium(III) complexes have a low paramagnetic relaxation enhancement (*T*1 and *T*2 shortening effect) and small paramagnetic shifts [22, 23, 41, 42]. The spectrum of compound **5** in DMSO is identical to that of "free ligand" **2**. Clearly, this is the only form that exists in DMSO for compound **5**. Similar results were reported for europium(III) complexes with phosphine oxides in DMSO-d_6 [43]. However, complex **7** showed a notable stability in comparison to **5** and **6** in a deuterated DMSO-D_2O solvent mixture.

Interestingly, the mass spectrometric analysis of compounds **5** and **6** carried out with a DMSO solution did not give any molecular ion corresponding to these complexes. Only the signal of the free ligand was detected in all cases. These results were observed for the europium complexes **5** and **6** and can be explained by a potential dissociation of the complexes through ligand exchange with solvent molecules. No such changes were observed for the zinc complexes **8**–**10** in various solvents, including MeOH and THF.

Table 1
Photophysical parameters of **5** and **6** in THF and **8**–**10** in MeOH.

Entry	Absorption maxima, nm	Emission maxima, nm[a]
5	279, 325	405; 591; 617; 646; 681
6	282; 339	405; 591; 617; 650; 681
8	277; 319	400
9	283; 290; 318	560
10	294; 301	495

[a] Emission spectra recorded with 280 nm.

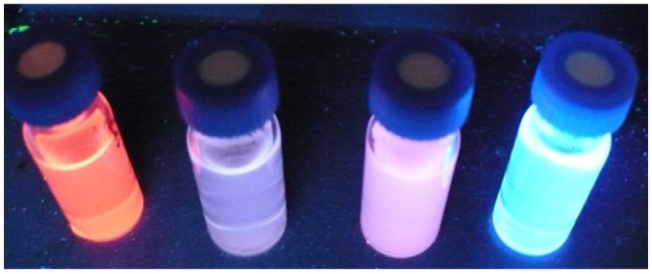


Fig. 3. Solvent dependency of solutions of **5** in THF, EtOH, D_2O and DMSO (from left to right) irradiated with UV-light (365 nm).

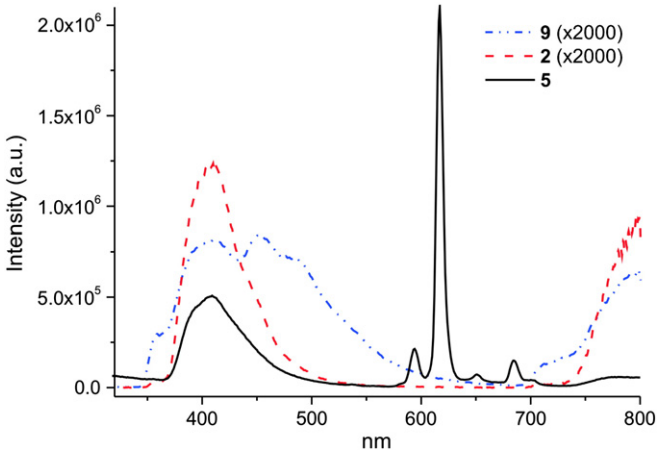


Fig. 2. Comparison of the emission spectra of compound **2** and its europium **5** and zinc **9** complexes (10^{-5} M) at excitation 280 nm in THF.

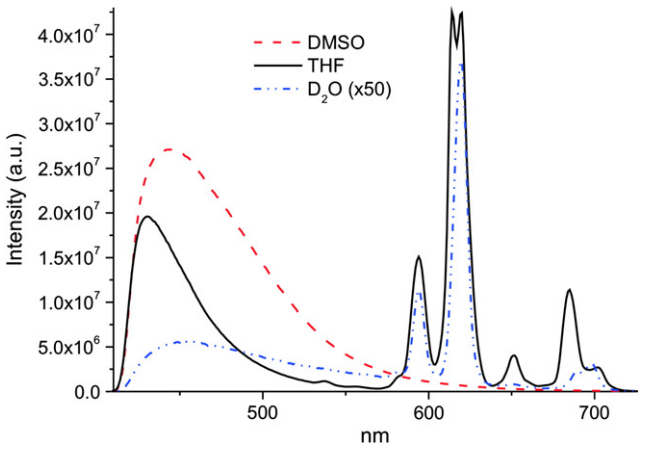


Fig. 4. Emission spectra of complex **5** in different solvents at 335 nm excitation and with a 420 nm filter.

$$[EuL_3]\cdot 3NO_3 + nDMSO \rightarrow 3L + [Eu(DMSO)_n]\cdot 3NO_3$$

Scheme 4. Dissociation of europium(III) complex in DMSO.

3.5. Aqueous solutions

The analysis of biological systems requires work under aqueous conditions. Some luminescent materials can be reversibly quenched by water via the formation of hydrogen bonds or directly by water molecules. Thus, it is important to establish the real state of the complex and its photophysical characteristics in aqueous solution before the substance enters a cell. It is necessary to establish whether any observed fluorescence quenching is the result of reduced emission of the europium centre or is due to irreversible decomposition of the metal complex.

Here, the emission spectrum of compound **5** in EtOH–PBS buffer at physiological pH (7.4) is similar to that in THF. It clearly shows the strong signals of the europium-ion bonded to the phenanthroline ligand (Fig. 6).

The materials **5** and **7** were stable in the buffer solution for quite some time (24–48 h); however, compound **6** does not exhibit any lanthanide-corresponding emission under these conditions.

3.6. Cell imaging

Oesophageal cancer is a common and aggressive form of cancer and has shown a dramatic increase in Europe and North America in the past few years [44–47]. Survival rates in Europe are only about 10% [48] and the incidence of this cancer, especially in the UK and Eire, is rising [49]. Thus, we chose the oesophageal SCC cell line OE21 (human oesophageal squamous carcinoma) for cell labelling studies [50].

The determination of the lowest staining concentration was performed in the 0.74 to 10 μM range. We have demonstrated that complexes **5** and **7** are stable (up to 48 h) in aqueous solutions (EtOH–PBS) and it still exhibits a strong luminescence (Fig. 6). The 1 mM stock ethanolic solutions of the compounds **5** and **7** were diluted with PBS buffer and an excess of crystals was filtered off to give concentrations of 0.74 μM for **5** and 8.2 μM for **7**. The fluorescence images

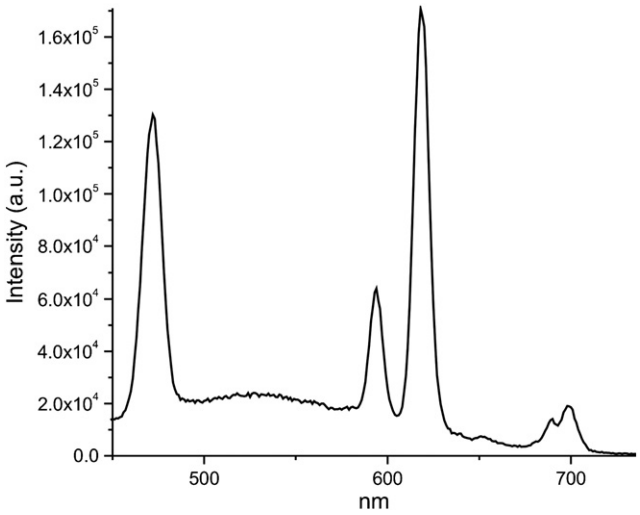


Fig. 6. Emission spectrum of complex **5** in EtOH–PBS at pH 7.4.

were taken after 20 min and 1 h incubation times at 405 nm irradiation energy and detection at 535 nm (Fig. 7).

The zinc complexes **8**–**10** were less fluorescent after 1 h incubation at these concentrations, the fluorescent images were obtained for the zinc compounds **8** (1.6 μM) and **9** (0.84 μM) after 2 and 6 h incubation, respectively (Fig. 8). Interestingly, the fluorescence of **9** diminished with time, and no intracellular fluorescence was observed for complex **10**.

Overall, the europium compounds represent a good intensity after 1 h incubation and are photostable over 6 h. In comparison to the europium materials, the zinc complexes required longer incubation times and show lower photostability. Furthermore, fluorescence decay occurred over time as a result of photobleaching.

4. Conclusion

We have demonstrated that the new zinc(II) and europium(III) complexes **5**–**10** can be synthesized in good yield. The emission spectra of compounds **5**–**7** show a strong signal corresponding to the Eu(III)-ion

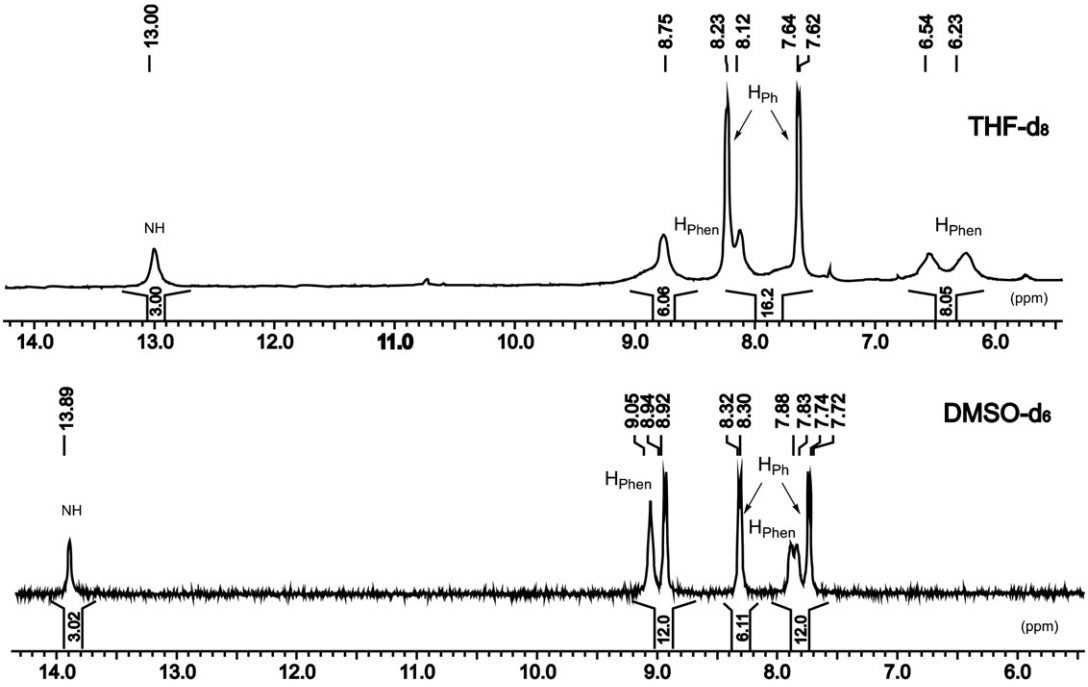


Fig. 5. ^{1}H NMR (400 MHz) of europium(III) complex **5** in different deuterated solvents.

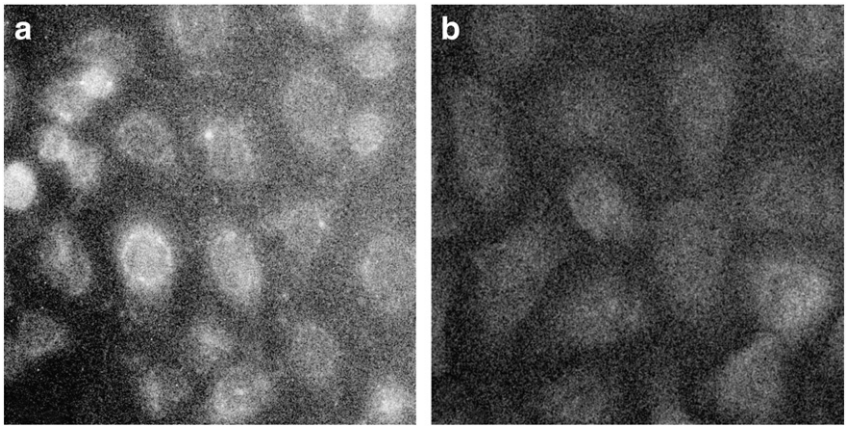


Fig. 7. In cell images of complexes **5** (a) and **7** (b) in OE21-fixed cells after 1 h incubation (irradiation at 405 nm and detection at 535 nm).

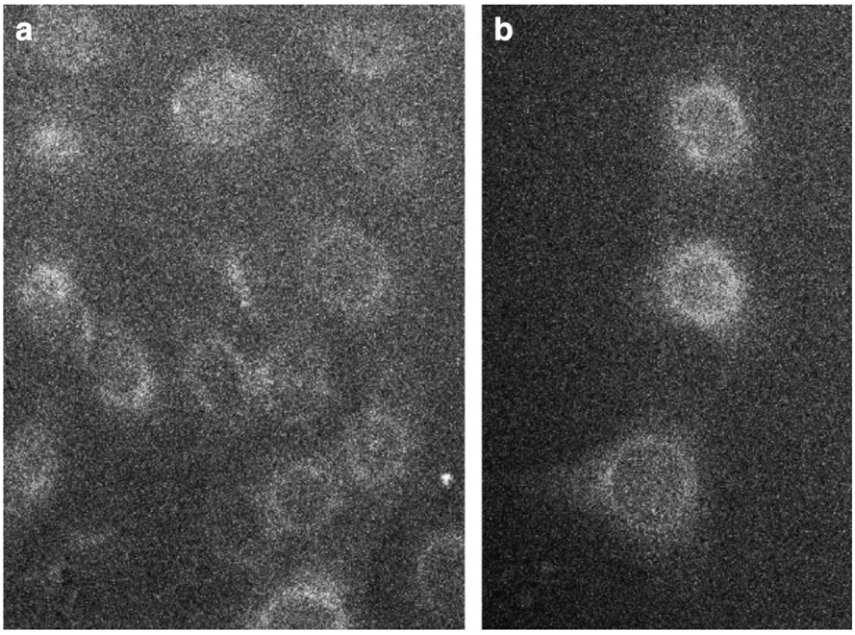


Fig. 8. In cell images of the complexes **8** (a) and **9** (b) in EtOH–PBS pH 7.4 for OE21-fixed cells after 2 h incubation (irradiation at 405 nm and detection at 535 nm).

in solvents such as THF and D_2O. However, quenching of the emission is observed in DMSO. Moreover, NMR analysis has also confirmed that DMSO-d_6 solutions contain only the free ligand for compounds **5** and **6**. Therefore, materials **5** and **6** are prone to dissociation in media such as DMSO. Interestingly, complex **7** is still present in the D_2O–DMSO-d_6 mixture. Analysis of complexes **1**–**3** in EtOH–PBS aqueous buffer revealed an intense emission of the lanthanide ion for **5** and **7**; however, **6** exhibits a spectrum similar to the one in DMSO. No spectral changes were observed for the zinc(II) complexes, they were shown to be stable in the media. Fluorescence cell images were recorded between 20 min to 6 h incubation times using complexes **5**–**9**. The europium samples show a good intensity after 1 h incubation and are photostable over 6 h. In contrast, the zinc complexes required longer incubation times and are less photostable. Furthermore, a decrease in fluorescence over time occurred as a result of photobleaching.

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