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Article

SYNTHESIS AND CHARACTERISATION OF A NOVEL PHENANTHROLINE-BASED LIGAND FUNCTIONALIZED WITH AMPHIPILIC GROUPS AND ITS LUMINESCENT Zn(II) COMPLEX

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Abstract: *The present paper reports the synthesis of a new phenanthroline-based ligand (phenIm_1) and its Zn(II) coordination compound (phenIm_Zn). Their chemical structure was determined by a combination of FT-IR and NMR spectroscopy, while their purity was confirmed by elemental analysis. Their photophysical properties were investigated in freshly prepared dichloromethane solutions.*

Keywords: heterocycles; N-donor ligand; hydrophilic ligand; coordination compounds

INTRODUCTION

Since antiquity, metals have been used to treat various infectious and chronic conditions, especially when plant-based remedies were not efficient (Abdallah *et al.* 2022, Ma *et al.* 2020). Although conventionally most of the synthetic drugs were based initially on organic molecules, the discovery of *cis*-platin anticancer properties by Rosenberg (Rosenberg et al. 1965, Rosenberg et al. 1969) shifted the researchers focus on the design of new drugs based on *d*-block metal complexes (*d*-MCs). Nowadays, *d*-MCs are currently finding applications in biomedical fields as advanced drugs (Arun *et al.* 2025, Sodhi and Paul 2019, Zhang and Sadler 2017), while specific properties deriving from the metal centre like luminescence are relevant for applications in different fields like photodynamic therapy, sensing and imaging (Monro *et al.* 2019, Mari *et al.* 2015). While *d*-MCs are known for excellent therapeutic and luminescence efficiencies, several limitations like the poor solubility, lack of targeted and local delivery still need to be overcome (Zhuo *et al.* 2024, Sanz Mendiguchia *et al.* 2013).

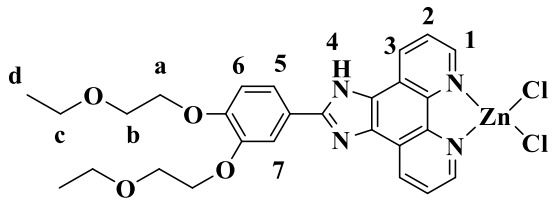
Varying the ligand structure and its functionalisation is a key strategy for enhancing the solubility and, consequently, the bioavailability of *d*-MCs (Wani *et al.* 2016). It has been demonstrated that neutral lipophilic *d*-MCs can passively diffuse through the lipid bilayer of biological membranes (Zhao *et al.* 2016).

Nowadays, non-platinum metallodrugs containing 3*d* metals like Cu and Zn are being investigated because of their biocompatibility and because they exhibit favourable toxicity profiles alongside potent DNA-binding, cleavage, and cytotoxic responses under physiological conditions (Khursheed *et al.* 2022). Among the biocompatible metals, zinc serves a wide range of essential biochemical and physiological functions, playing critical catalytic, structural, and regulatory roles in biological systems (Roohani *et al.* 2013), therefore making it a promising metal centre ion in medicinal bio-inorganic chemistry (Yoshikawa and Yasui 2012).

Due to their low toxicity and minimal side effects, Zn(II) complexes are widely investigated as DNA-binding agents, tumour photosensitizers, and antimicrobial therapeutics (Porchia et al. 2020). Moreover, N-donor ligands are the key structural components in

biologically active Zn(II) complexes (Porchia *et al.* 2020).

Therefore, in this present paper, we report the synthesis and characterization of a new ligand functionalized with amphiphilic chains, phenIm_1 (2-(3,4-bis(2-ethoxyethoxy)phenyl)-3b,7a-dihydro-1H-imidazo[4,5-f][1,10]phenanthroline) obtained by the reaction of 1,10-phenanthroline-5,6-dione with a functionalized aromatic aldehyde and ammonium acetate (See experimental section) and its zinc coordination compound (*phenIm_Zn*, **Figure 1**).



phenIm_Zn

Figure 1. The proposed chemical structure of *phenIm_Zn* and the proton labelling.

The structure of the intermediates and the phenanthroline derivative (*phenIm_1*) were assessed by No-D ^{1}H-NMR spectroscopy, whereas the purity of the *phenIm_1* ligand was confirmed by elemental analysis. The *phenIm_Zn*, complex was characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopies. Moreover, the photophysical properties were investigated for the phenanthroline ligand and its corresponding zinc complex.

MATERIALS AND METHODS

All commercially available starting materials were used as received without further purification: 2-ethoxyethan-1-ol, 4-methylbenzenesulfonyl chloride (p-TsCl), 3,4-dihydroxybenzaldehyde, 1,10-phenanthroline hydrate (phen), $ZnCl_2$, $KBrO_3$, K_2CO_3, CH_3COONH_4, were purchased from Sigma Aldrich. NaOH, Na_2SO_4, H_2SO_4, Na_2CO_3, CH_3COOH were from Chimreactiv. The solvents used for the current study were: *N,N*-Dimethylformamide (DMF), ethyl acetate (AcOEt), tetrahydrofuran (THF), diethyl ether (Et_2O), dichloromethane (DCM), acetonitrile (AcN) and were purchased from Honeywell.

Infrared spectra (KBr pellets) were recorded in the range 4000-400 cm^{-1} on a Cary 630 FT-IR spectrophotometer.

The formation of the ligand and the intermediates was assessed on a Fourier 80 MHz benchtop by Bruker using No-Deuterium Proton-NMR in DCM (CH_2Cl_2) as solvent, by solvent suppression technique.

For the zinc complex, the ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance III HD – 500 MHz spectrometer in DMSO-d_6.

In order to confirm the purity of the samples, elemental analyses (C, H, N) were employed on an Elementar UNICUBE CHNS analyser with helium as the carrier gas.

Molar electrical conductivity was measured with a Mettler Toledo FiveEasy plus (FP30) conductivity meter equipped with a Lab conductivity sensor LE740.

Spectrofluorimetric grade DCM from VWR was used for photophysical investigations in the solution without further purification.

Absorption spectra were recorded using an Agilent Cary 60 spectrophotometer. Emission spectra were recorded on a Perkin Elmer LS 55 fluorimeter. For all measurements quartz cuvettes of a 1 cm path length were used.

The luminescence quantum yields of the samples (*phenIm_1* and *phenIm_Zn*) in solution were determined by the optical dilution method (Demas and Crosby 1971) using 2 - aminopyridine in DCM solution as a reference standard ($\Phi = 0.33$, Rusakowicz and Testa 1968) for the ligand, and $[Ru(bpy)_3]^{2+}$ in water ($\Phi = 0.04$, Suzuki et al. 2009) for the zinc complex.

Absorbance values used for quantum-yield measurements are 0.157 for Zn complex and for the ligand 0.140. The slits used for both excitation and emission were 4 nm and 4 nm, respectively speed of acquisition is 100nm/min. Integration ranges for ligand were 350 – 680 nm and for Zn complex 320 – 580 nm, nr of cycles

is 1. The refractive indexes for DCM is 1.4240, and for water is 1.3325.

EXPERIMENTAL SECTION

Synthesis of 2-ethoxyethyl 4-methylbenzenesulfonate, ***1***

Compound 1 was obtained following a reported procedure for similar derivatives (Zhang *et al.* 2022):

2-ethoxyethan-1-ol (2g, 0.0222 mol) was added over 10 mL NaOH (1.62 g, 0.0406 mol) solution in THF/H_2O (V/V=1/1), cooled on an ice bath under inert atmosphere. The mixture was stirred for 15 min, then 10 mL of THF solution containing 4-methylbenzenesulfonyl chloride (4.23 g, 0.0222 mol) was added dropwise. The reaction mixture was allowed to reach room temperature, and stirred for 2 hours. Then THF was evaporated under reduced pressure and the aqueos phase was extracted with 50 mL Et_2O (3x), the combined organic phase was dried over anhydrous Na_2SO_4, filtered and taken to dryness, to yield **1** as a colourless liquid. The compound was characterized by No-D NMR spectroscopy.

(1): colourless liquid (3.7 g, 0.01514 mol, η=68%).

No-D ^{1}H-NMR (80 MHz, CH_2Cl_2) δ-ppm 7.83 (d, *J* = 7.9 Hz, 2H), 7.41 (d, *J* = 8.0 Hz, 2H), 4.40 – 3.94 (m, 4H), 3.85 – 3.21 (m, 4H), 2.49 (s, 3H), 1.16 (t, *J* = 6.9 Hz, 3H).

Synthesis of 3,4-bis(2-ethoxyethoxy)benzaldehyde, ***2***

Compound 2 was obtained following a Williamson ether synthesis procedure (Percec *et al.* 2009):

0.514 g of 3,4-dihydroxybenzaldehyde (0.00372 mol) and 2.26 g of K_2CO_3 (0.01634 mol) in 25 mL of DMF were added to a 100 mL single neck round-bottom flask; the reaction mixture was then stirred at 60°C for approximately 30 minutes, then compound 1 (2 g, 0.008186 mol) dissolved in DMF was added. The temperature was raised to 80°C, and the reaction mixture is kept under stirring for 24 hours. Following, the reaction mixture was cooled to room temperature, filtered through Celite and washed thoroughly with DCM; the mother liquor was then taken to dryness using a rotary evaporator. The residue was dissolved in DCM and washed with brine. The organic phase was then dried over anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator. The pure compound was obtained by column chromatography (SiO_2; Hexane:Ethyl acetate = 7:4).

(2): colorless oil (0.652 g, 0.002310 mol, 62.1%)

The compound was characterized through No-D NMR.

No-D ^{1}H-NMR (80 MHz, CH_2Cl_2) δ-ppm: 9.84 (s, 1H), 7.47 (overlapped peaks, 2H), 7.22 – 6.84 (m, 1H), 4.24 – 3.92 (m, 4H), 3.90 – 3.73 (m, 4H), 3.69 – 3.36 (m, 4H), 1.23 (t, *J* = 7.0 Hz, 6H).

Synthesis of 1,10-phenanthroline-5,6-dione, ***3***

1,10-phenanthroline-5,6-dione was obtained following a reported procedure (Zheng *et al.* 2010):

10 g of 1,10-phenanthroline hydrate (0.0505 mol) was added to a solution of 32 mL H_2SO_4 60%, cooled on an ice bath, then $KBrO_3$ (11 g, 0.0658 mol) was added in portions avoiding the rise of temperature above 20°C. After the addition of $KBrO_3$ the reaction mixture was stirred at room temperature overnight, then, the mixture was poured over ice and was carefully neutralized to pH= 5.5 using a saturated solution of sodium carbonate (Na_2CO_3). The formed precipitate was filtered out and the mother liquor was extracted with 100 mL DCM (2x). The combined organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and taken to dryness. 1,10-phenanthroline-5,6-dione was isolated after recrystallizations from hot EtOH as a yellow powder (2 g, 0.00952 mol, η=18.9 %). The compound was characterized through ^{1}H-NMR NMR.

^{1}H-NMR (80 MHz, DMSO-d_6) δ-ppm: 8.99 (dd, *J* = 4.7, 1.8 Hz, 2H), 8.39 (dd, *J* = 7.8, 1.8 Hz, 2H), 7.67 (dd, *J* = 7.8, 4.7 Hz, 2H).

Synthesis of 2-(3,4-bis(2-ethoxyethoxy)phenyl)-3b,7a-dihydro-1H-imidazo[4,5-f][1,10]phenanthroline, phenIm_1

The ligand was obtained adapting a procedure reported for similar phenanthroline-imidazole compounds (Cardinaels *et al.* 2005)

0.310 g of 1,10-phenanthroline-5,6-dione (compound **3**, 0.001475 mol) and 0.909 g of ammonium acetate (0.0118 mol) were added to

a single neck 100 mL round-bottom flask containing 40 mL of glacial acetic acid, and the reaction mixture was brought to reflux. Initially, the dione (**3**) did not appear to dissolve completely, but upon reaching 100°C, it formed a clear, yellow solution. Once the reaction mixture reached 100°C and the dione was fully dissolved, the aldehyde (**2**, 0.500 g, 0.00177 mol) dissolved in 15 mL of warm acetic acid was added dropwise to the reaction mixture. The pale orange reaction mixture was stirred overnight at 125°C. Following, the reaction mixture was cooled to room temperature and poured into a large Berzelius flask containing ice; the pH was adjusted to 7 with ammonia solution and then extracted with DCM (4x). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The final product was recrystallized by DCM/Et_2O, and isolated as a pale orange powder. The compound was characterized by FT-IR and No-D NMR spectroscopy and its purity was confirmed by elemental analysis.

(*phenIm_1*): pale orange powder (0.389 g, 0.000820 mol, η=56%).

FT-IR (KBr, cm^{-1}): 2969, 2934, 2869 ν(C-H);1 607, 1559 (ν (C=C)), (ν (C=N)); 1123 ν(C-O-C);

No-D ^{1}H-NMR (80 MHz, CH_2Cl_2) δ-ppm: 9.56 – 8.36 (broad, 4H), 7.88 (d, *J* = 9.9 Hz, 2H), 7.34 (broad, 2H), 6.81 (d, *J* = 7.9 Hz, 1H), 4.14 – 3.93 (m, 8H), 3.44 (ddt, *J* = 17.7, 13.9, 6.3 Hz, 8H), 1.09 (dt, *J* = 8.8, 6.9 Hz, 6H, Hd).

Anal. Calcd. for $C_{27}H_{30}N_4O_4$ (474.561 g·mol^{-1}): C, 68.34; H, 6.37; N, 11.81%. Found: C, 68.81; H, 6.08; N, 12.27%

Synthesis of phenIm_Zn

A clear solution of $ZnCl_2$ (0.129 g, 0.0009523 mol) in 10 mL of MeOH was added to an orange solution of *phenIm_1* (0.300 g, 0.0006349 mol) in 25 mL of MeOH. The resulting orange reaction mixture was stirred at room temperature for 4 hours. Following, the solvent was evaporated and the pure produce was obtained after precipitation from DCM/Et_2O, followed by filtration and washings with small volumes of Et_2O and AcOEt.

(*phenIm_Zn*): pale yellow solid (0.290 g, 0.000476 mmol, 75%)

FT-IR (KBr, cm^{-1}): 2969, 2934, 2869 ν(C-H); 1607, 1581 (ν (C=C)) and (ν (C=N)); 1123 ν(C-O-C); 427 ν(Zn-N)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 14.00 (s, 1H, H^4), 9.16 (d, *J* = 33.3 Hz, 2H, H^1), 8.77 (broad, 2H, H^3), 8.04 (broad, 2H, H^2), 7.86 (d, *J* = 30.8 Hz, 2H, H^5,H^7), 7.18 (d, *J* = 7.9 Hz, 1H, H^6), 4.22 (dt, *J* = 35.1, 4.0 Hz, 4H, H^a), 3.76 (dt, *J* = 17.9, 4.7 Hz, 4H, H^b), 3.55 (dq, *J* = 10.6, 7.0 Hz, 4H, H^c), 1.15 (td, *J* = 6.9, 5.3 Hz, 6H, H^d).

^{13}C NMR (126 MHz, DMSO-d_6) δ 152.30, 150.72 148.93, 133.49, 125.90, 125.75, 120.53, 114.30, 112.74, 69.18, 68.88, 68.78, 66.28, 40.56, 40.39, 40.22, 40.06, 39.89, 15.61, 15.59.

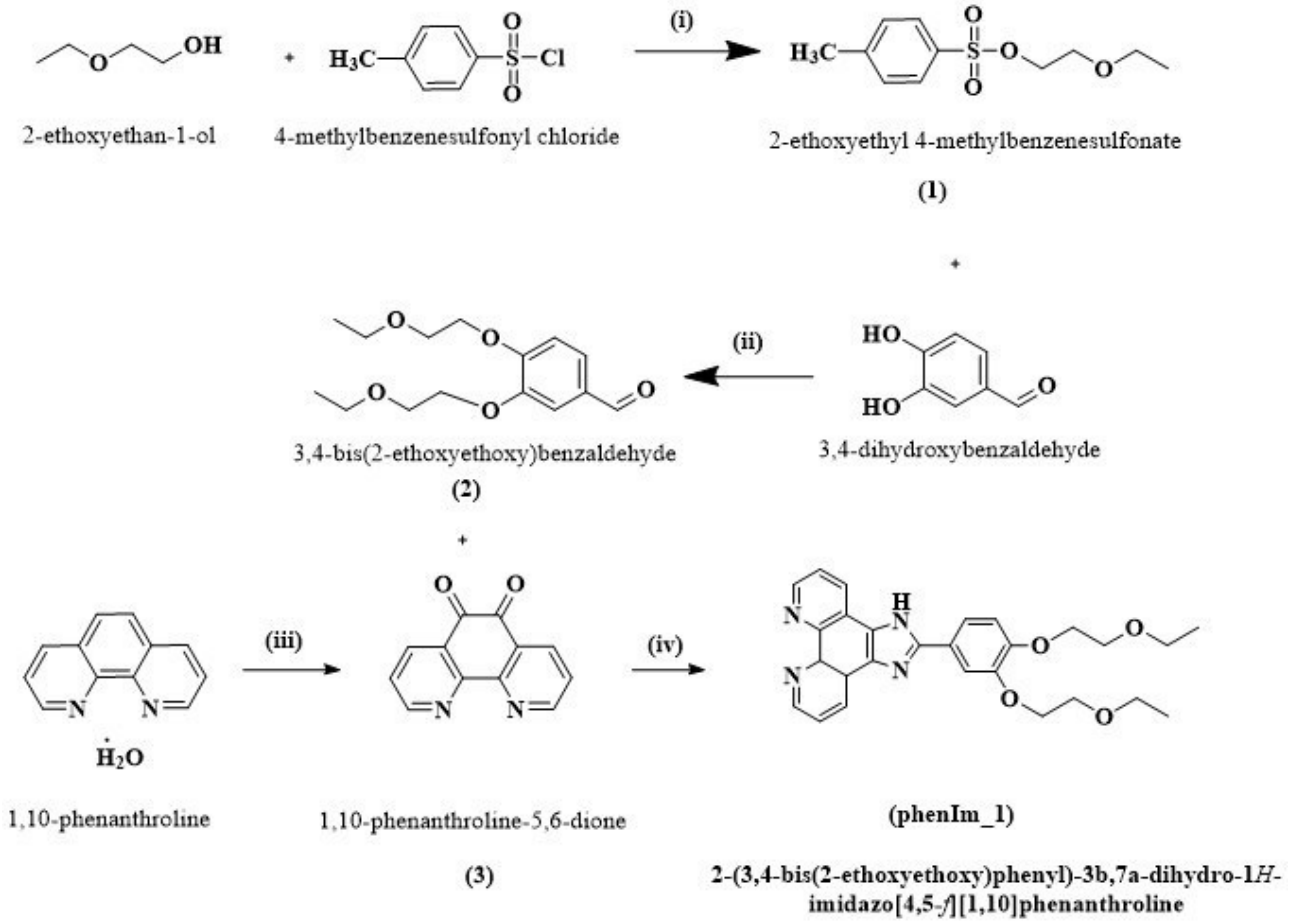
Anal. Calcd. for $C_{27}H_{30}Cl_2N_4O_4Zn$ (610.841 g·mol^{-1}): C, 53.09; H, 4.95; N, 9.17%. Found: C, 53.68; H, 4.80; N, 9.28%

RESULTS AND DISCUSSIONS

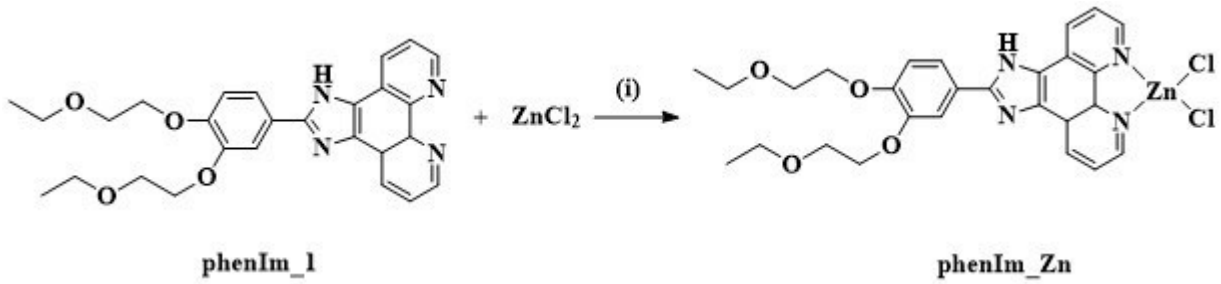
To induce solubility in polar solvents for the targeted complex we have designed a 2-phenylimidazo [4,5-*f*],[1,10]phenanthroline (phenIm_1) ligand functionalized with ethoxyethoxy chains as depicted in the reaction **Scheme 1**.

In order to obtain the phenanthroline derivative *phenIm_1*, firstly we isolated 3,4-bis(2-ethoxyethoxy)benzaldehyde (compound 2 in Scheme 1) in two steps. First, we converted the hydroxyl group of ethoxyethanol to a tosylate. Then, a Williamson etherification reaction between the isolated tosylate and 3,4-dihydroxybenzaldehyde was carried out (Percec *et al.* 2009). 1,10-phenanthroline-5,6-dione (compound **3**) was obtained under mild reaction conditions at r.t. from 1,10-phenanthroline hydrate using potassium bromate ($KBrO_3$) and 60% sulfuric acid (H_2SO_4) as oxidant, according to a published procedure (Zheng *et al.* 2010).

Then, the *phenIm_1* ligand was obtained through a Debus-Radziszewski reaction in a single step from three components: compound 2, compound 3 and CH_3COONH_4 in acetic acid (Cardinaels *et al.* 2005).



Scheme 1. Reaction pathway for the synthesis of *phenIm_1*. Reagents and conditions: (i) NaOH, THF/H_2O, r.t, 2h, inert atmosphere; (ii) K_2CO_3, DMF, 80°C, 24 h, inert atmosphere; (iii) H_2SO_4 60%, $KBrO_3$, r.t, overnight; (iv) CH_3COONH_4, CH_3COOH, 125°C, overnight.



Scheme 2. Reaction pathway for the synthesis of *phenIm_Zn.* Reagents and conditions: (i) MeOH, r.t, 4 h.

The reaction of the *phenIm_1* with an excess of $ZnCl_2$ favoured the formation of the neutral complex *phenIm_Zn* (**Scheme 2**) with the metal centre tetracoordinated by the bidentate phenanthroline derivative and two monodentate chlorine ligands.

The targeted complex resulted to be scarcely soluble in water, but presented a good solubility in polar solvents (DMSO, methanol, acetonitrile) and non-polar solvents (dichloromethane, tetrahydrofuran).

Figure 2 presents the FT-IR spectra of the complex *phenIm_Zn* plotted against *phenIm_1* ligand. The FT-IR spectra of the ligand and its corresponding complex presents the characteristic absorption bands related to the functional groups present in the molecule. Thus, the absorption bands at 2969, 2934 and 2869 cm^{-1} are characteristic for the C-H bond stretching vibration while $\nu_{C\text{-}O\text{-}C}$ bonds, originating from the ethoxyethoxy chains are observed at 1125 cm^{-1} (Chen *et al* 2021).

The successful coordination of the ligand to the metal centre is proven by the shift with about 20 cm^{-1} of the $\nu_{C=N}$ characteristic band (Burger *et al.* 2001). The formation of the Zn(II) complex is further supported by the presence of the absorption band at 427 cm^{-1} which is characteristic for metal-nitrogen stretching (Pook 2023).

The ^{1}H-NMR spectra of complex *phenIm_Zn*, recorded in DMSO-d_6 revealed the presence of the protons corresponding to the phenanthroline ligand. The purity of the compounds is confirmed by elemental analysis (see Experimental Section).

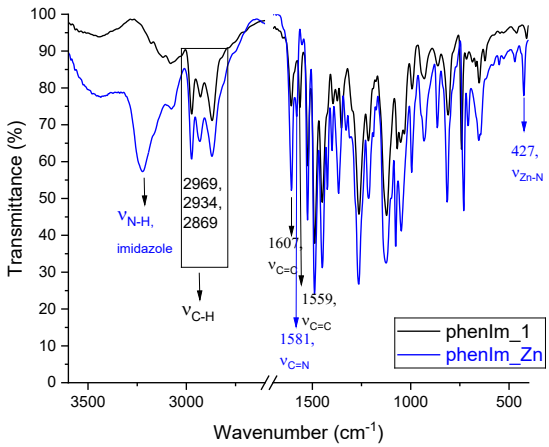


Figure 2. FT-IR spectra of Zn(II) (*phenIm_Zn* – blue) complex plotted against the phenanthroline ligand (*phenIm_1* – black)

The experimental conductivity value for *phenIm_Zn* measured for 10^{-3} mol·L^{-1} solution in acetonitrile at room temperature, falls in the range for non-electrolytes, confirming the neutral character of the complex (Geary 1971).

The photophysical studies (absorption and emission spectra) for the ligand and the Zn(II) complex were carried out. The spectra were recorded in diluted DCM solutions, at a concentration of $5\cdot10^{-6}$ mol·L^{-1} in order to minimize concentration-dependent artifacts.

Figures 3 and **4** present the absorption and emission spectra of the analysed samples, while their data is summarized in **Table 1**.

The absorption bands between 260 – 300 nm, present in the spectrum of both the ligand and the complex are characteristic for π – π* transitions (Zhong *et al.* 2016). Moreover, the absorption bands centred at 311 nm and 328 nm for *phenIm_1* and 307 nm for *phenIm_Zn* respectively, were assigned to an intramolecular charge transfer from the fused imidazole to the phenanthroline core (Devi *et al.* 2020, Zhong *et al.* 2016)

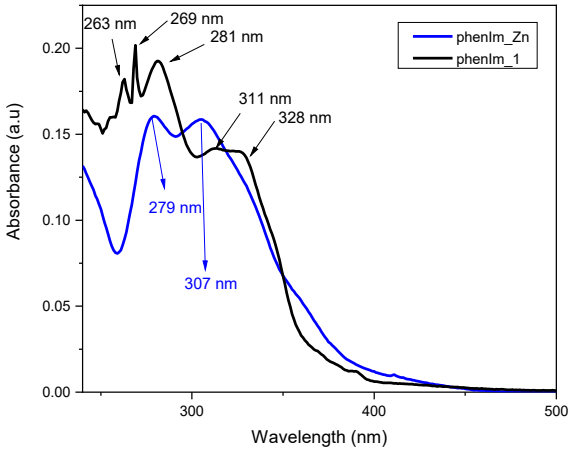


Figure 3. UV-Vis spectra of Zn(II) (*phenIm_Zn* – blue) complex plotted against the phenanthroline ligand (*phenIm_1* – black) recorded in DCM at $5\cdot10^{-6}$ mol·L^{-1}

Regarding the emission spectra (**Figure 4**, **Table 1**), *phenIm_1* presents an emission band centred at 439 nm, which originates from the π→π* singlet excited state, which upon complexation to *phenIm_Zn* suffers a bathochromic shift to 539 nm. Also, a large Stokes shift ($\Delta\lambda = \Delta\lambda_{max,em} - \Delta\lambda_{max,abs}$) of 232 nm is observed for Zn(II) complex.

The increase of the Zn(II) complex emission quantum yield with respect to the ligand is due to the stabilisation of the excited states of the ligand following coordination to the metal centre, suggested by the red shift of the emission maxima of the complex with respect to the emission maxima of the ligand. (Szerb *et al.* 2022)

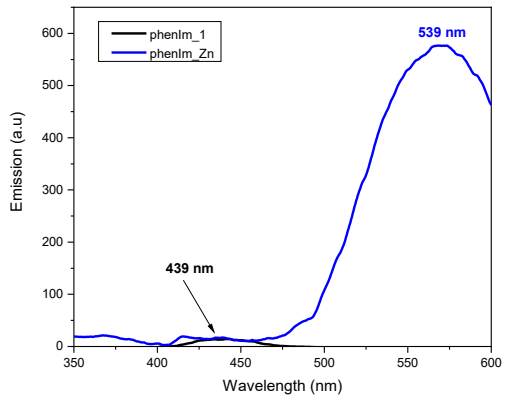


Figure 4. Emission spectra of Zn(II) (*phenIm_Zn* – blue) complex plotted against the phenanthroline ligand (*phenIm_1* – black) recorded in DCM at $5 \cdot 10^{-6}$ mol·L^{-1}

Table 1. Photophysical data of *phenIm_1* and *phenIm_Zn* complex in DCM at $5 \cdot 10^{-6}$ mol·L^{-1}.

Sample	**Abs, λ_{max}/nm (ε/M^{-1} cm^{-1})**	**Em, λ_{max}/nm**	QY **(%)**
phenIm_1	263(36400), 269(40600), 281(38600), 311(28400), 328(27600)	439 (λ_{ex}=325 nm)	1
phenIm_Zn	279(31800) 307(31600)	539 (λ_{ex}=305 nm)	24.13

CONCLUSIONS

A new ligand functionalised with lipophilic chains and its Zn(II) coordination complex were designed, synthesized and characterized.

Although insoluble in water, the complex resulted highly soluble in organic solvents, especially in DMSO which renders it proper for biological studies. Moreover, while the ligand emits modestly, with an emission centred at 439 nm, the coordination to Zn(II) centre stabilises the excited states of the ligand and thus 24 fold increase of the emission quantum yield is obtained. Also, the large Stokes shift value observed for the complex makes it suitable for bioimaging applications, where it is important to avoid the scattered light from the excitation beam overlapping with emission signal.

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