

Naphtyl-imidazo-anthraquinones as novel colorimetric and fluorimetric chemosensors for ion sensing

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Abstract

Novel colorimetric and fluorimetric chemosensors for F^- and CN^- containing anthraquinone and imidazole as signalling/binding sites have been synthesised and characterised. Upon addition of F^- , CN^- and OH^- to acetonitrile solutions of compounds **1-2**, a marked colour change from yellow to pink was observed and the fluorescence emission of **1** was switched “on”, whereas for **2** there was a fluorescence quenching. Considering recognition in organic aqueous mixture, it was found that selectivity for CN^- was achieved for both receptors, with an easily detectable colour change from yellow to orange. Compounds **1-2** in their deprotonated form, after fluoride addition, were studied as metal ion chemosensors and displayed a drastic change from pink to yellow after metal ion complexation giving a yellow-pink-yellow, reversible colorimetric reaction and a “on-off-on” fluorescence in acetonitrile. The binding stoichiometry between the receptors and the anions and cations was found to be 1:1 and 2:1 respectively. The binding process was also followed by 1H NMR titrations which corroborated the previous findings.

Keywords

Imidazole; Anthraquinone; Colorimetric and fluorimetric chemosensors; Fluoride, Cyanide; Aqueous media; Naked-eye detection.

1. Introduction

Artificial receptors that can recognise ionic species with high selectivity are of great interest and imperative for areas such as biological, clinical, environmental, and waste management applications. Recently many new systems based on fluorophores were synthesized and reported as anion and/or cation chemosensors. A fluorescent chemosensor for metal cations should be able to interact effectively with the metal ion in solution and to signal the recognition event by a change in the fluorescence properties such as the wavelength or intensity, as well as by the appearance of a

new fluorescence band. The classic design for a metal ion chemosensor consists of one or more fluorophores/chromophores linked to a coordinating unit through a spacer. The coordinating unit may be tailored by a proper choice of donor functions, able to coordinate a metal ion, and the molecular framework is determined by the target metal ion. Considering colorimetric/fluorimetric sensors, a colorimetric sensor has the advantage of the straightforward and rapid naked-eye detection through colour change without the need for expensive instrumentation [1-2].

For the recognition of certain anions (e.g., fluoride and cyanide), anthraquinone derivatives have been reported as suitable systems for colorimetric sensing since they are an example of electron acceptor groups that can be electronically connected with recognition units [1g,3]. There has been a renewal of interest over the past ten years in the 9,10-anthraquinone signalling unit due to its chemosensor ability for several cations such as copper, cobalt and nickel ions [4].

Imidazole derivatives can be used for several optical applications in materials and medicinal chemistry due to the versatility concerning its structure and photophysical properties [5]. This heterocycle acts as an excellent hydrogen bond donor group in anion receptor systems, and the acidity of the NH proton can be tuned by changing the electronic properties of the substituents at the ring. The presence of a donor pyridine-like nitrogen atom within the ring, capable of selectively binding cationic species also converts imidazoles into excellent metal ion sensors. Additionally, the binding properties of the imidazole core may be modulated by linear or angular annulation to other systems such as anthraquinone leading to expanded imidazole derivatives bearing several binding or signalling sites [6-7]. Although several imidazo-anthraquinones have been reported, up till now naphthyl-imidazo derivatives are still unknown. Given the known fluorescence of naphthalene and imidazole derivatives, it was envisaged that the introduction of an additional naphthalene system to the imidazo-anthraquinone core could impart interesting photophysical properties.

In recent years, considerable efforts have been dedicated to fluoride ion sensing via UV-vis, fluorescence, or other methods [1b-d,1f,3], since the development of a chemosensor for fluoride is of great relevance for environment and human health care [8].

Due to the toxicity of the cyanide anion, highly harmful to the environment and human health, there is an interest to develop new and more selective chemosensors for this analyte. Cyanide compounds are largely applied in several areas such as the polymer industry and in gold extraction process and as such, its use is not possible to avoid [9a-b]. A large number of systems have been reported till the present date but, nevertheless, they suffer from several drawbacks such as difficult synthesis, poor selectivity (especially in the presence of fluoride or acetate ions), only work in an organic media and the use of instrumentation is required. However, in recent years, several fluorimetric and/or colorimetric chemosensors as well as several dosimeters were reported for the cyanide ion detection in aqueous media [9c-q].

Therefore, chemosensors capable of selective colorimetric sensing of the cyanide anion can be extremely advantageous, especially for use in aqueous media.

Bearing the above facts in mind, the synthesis and sensing properties of two new receptors in which a naphthyl-imidazole system is annulated to the anthraquinone core is now reported. Starting from commercially available reagents, naphthyl-imidazoanthraquinones **1-2** were easily obtained by using a straightforward synthetic protocol. The spectrophotometric titrations of compounds **1-2** with several representative anions confirmed a selective “naked eye” detection of cyanide in an aqueous mixture with an organic solvent.

2. Experimental

2.1. Synthesis general

Reaction progress was monitored by thin layer chromatography (0.25 mm thick precoated silica plates: Merck Fertigplatten Kieselgel 60 F254). NMR spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz for ^1H and 100.6 MHz for ^{13}C using the solvent peak as internal reference. The solvents are indicated in parenthesis before the chemical shift values (δ relative to TMS and given in ppm). Mps were determined on a Gallenkamp apparatus. Infrared spectra were recorded on a BOMEM MB 104 spectrophotometer. Mass spectrometry analyses were performed at the “C.A.C.T.I. -Unidad de Espectrometria de Masas” at the University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. UV-visible absorption spectra (200 – 700 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. Fluorescence quantum yields were measured using a solution of quinine sulphate in sulphuric acid (0.5M) as standard ($\Phi_{\text{F}} = 0.54$ [10]) and corrected for the refraction index of the solvents.

2.2. General procedure for the synthesis of compounds **1-2**

The respective aldehyde (0.20 mmol) and 1,2-diaminoanthraquinone (0.24 mmol) were dissolved separately in ethanol (4 mL/mmol). Formic acid was added to the solution of aldehyde (0.04 mL/mmol of aldehyde) and the resulting solution was added to the solution of 1,2-diaminoanthraquinone and heated at reflux overnight. After cooling, the ethanol was evaporated and the crude imine was dissolved in a small volume of acetic acid (5 mL/mmol of imine). Lead tetraacetate was added (0.20 mmol) and the mixture was stirred overnight at room temperature. Addition of water to the reaction mixture gave a solid which was isolated by filtration and purified by recrystallisation from diethyl ether/chloroform.

2.2.1. 2-(4-Methoxynaphthalen-1-yl)-1H-anthra[1,2-d]imidazole-6,11-dione (1).

Dark yellow solid (60%). Mp: 315.1-317.8 °C. UV (acetonitrile): λ_{\max} nm (log ϵ) 422 (4.01). IR (KBr 1%) ν = 3426, 3081, 3003, 2931, 1663, 1582, 1518, 1487, 1461, 1441, 1397, 1375, 1326, 1289, 1247, 1157, 1094, 1007, 838, 766 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 4.07 (s, 3H, OCH_3), 7.14 (d, 1H, J = 8.0 Hz, H3'), 7.57-7.67 (m, 2H, H6' and H7'), 7.91 (broad s, 2H, H8 and H9), 8.07-8.30 (m, 6H, H2', H5', H4, H5, H7 and H10), 8.88 (d, 1H, J = 8.4 Hz, H8'), 13.22 (s, 1H, NH). ^{13}C NMR (100.6 MHz, $\text{DMSO-}d_6$) δ = 55.9 (OCH_3), 103.8 (C3'), 118.3 (C11a), 120.7 (C4), 124.8 (C5'), 124.9 (C5), 125.7 (C6'), 125.9 (C8'), 126.2 (C7'), 126.7 (C10), 127.6 (C7), 127.7 (C11b and C1'), 131.0 (C4a' and C8a'), 131.6 (C2'), 132.2 (C5a), 132.9 (C10a), 133.1 (C6a), 134.2 (C9), 134.4 (C8), 149.4 (C3a), 156.9 (C4'), 158.1 (C2), 182.4 (C=O), 183.1 (C=O). MS (FAB) m/z (%): 405 ($[\text{M}+\text{H}]^+$, 100), 404 (M^+ , 39), 307 (18), 155 (18), 154 (58). HRMS (FAB) for $\text{C}_{26}\text{H}_{17}\text{N}_2\text{O}_3$: calcd 405.1239, found 405.1240.

2.2.2. 2-(4-(*N,N*-Dimethylamino)naphthalen-1-yl)-1H-anthra[1,2-d]imidazole-6,11-dione (2).

Dark red solid (64%). Mp: 214.4-216.9 °C. UV (acetonitrile): λ_{\max} nm (log ϵ) 435 (4.06). IR (KBr 1%) ν = 3418, 2925, 2852, 1667, 1627, 1582, 1524, 1460, 1440, 1388, 1328, 1292, 1200, 1138, 1094, 1058, 1007, 938, 888, 836, 714 cm^{-1} . ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 2.94 (s, 6H, $\text{N}(\text{CH}_3)_2$), 7.23 (d, 1H, J = 8.0 Hz, H3'), 7.57-7.63 (m, 2H, H6' and H7'), 7.91-7.94 (m, 2H, H8 and H9), 8.05 (d, 1H, J = 8.0 Hz, H2'), 8.11 (d, 1H, H4), 8.19-8.26 (m, 4H, H5', H5, H7 and H10), 8.84-8.86 (m, 1H, H8'), 13.25 (s, 1H, NH). ^{13}C NMR (100.6 MHz, $\text{DMSO-}d_6$): δ = 44.6 ($\text{N}(\text{CH}_3)_2$), 112.2 (C3'), 118.4 (C11a), 120.2 (C4), 124.5 (C5'), 124.8 (C5), 125.3 (C6'), 126.2 (C10), 126.3 (C8'), 126.4 (C7), 126.9 (C7'), 127.7 (C1'), 127.8 (C11b), 130.3 (C2'), 132.2 (C4a'), 132.3 (C8a'), 133.0 (C10a), 133.2 (C6a), 133.3 (C5a), 134.2 (C9), 134.4 (C8), 149.5 (C3a), 153.0 (C4'), 158.3 (C2), 182.4 (C=O), 183.1 (C=O). MS (FAB) m/z (%): 418 ($[\text{M}+\text{H}]^+$, 100), 417 (M^+ , 58), 281 (17), 219 (39), 191 (26), 155 (41), 154 (68). HRMS (FAB) for $\text{C}_{27}\text{H}_{20}\text{N}_3\text{S}_2$: calcd 418.1556, found 418.1552.

2.3. Spectrophotometric and spectrofluorimetric titrations of imidazo-anthraquinones 1-2

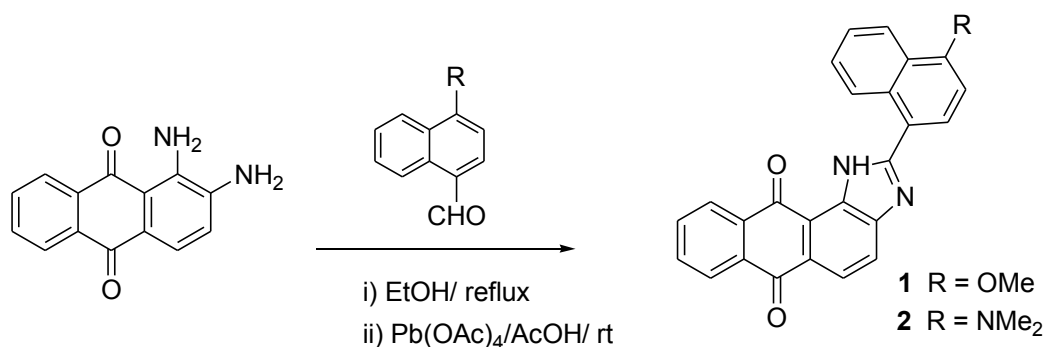
Solutions of naphthyl-imidazo-anthraquinones **1-2** (ca. 1.0×10^{-4} to 1.7×10^{-5} M) and of the anions/cations under study (1.0×10^{-2} M) were prepared in acetonitrile or acetonitrile/water (9:1) (in the form of tetrabutylammonium salts for F^- , Cl^- , Br^- , I^- , CN^- , OH^- , AcO^- , BzO^- , NO_3^- , ClO_4^- , HSO_4^- , and H_2PO_4^- , perchlorate salts for Cd^{2+} , Ca^{2+} , Na^+ , Cr^{3+} , Zn^{2+} , Hg^{2+} , Fe^{2+} and Fe^{3+} and hexahydrated tetrafluoroborate salts for Cu^{2+} , Co^{2+} , Ni^{2+} and Pd^{2+}). Titration of the ligands with the

several ions was performed by the sequential addition of ion solution to the naphthylimidazo-anthraquinones solution, in a 10 mm path length quartz cuvette and absorption and emission spectra were measured at 298 K. The binding stoichiometry of the naphthyl-imidazo-anthraquinones was determined by using Job's plots. The association constants were obtained with Hyperquad Software.

3. Results and discussion

3.1. Synthesis and characterisation

The reaction between 1,2-diaminoanthraquinone and the appropriate naphthaldehyde under heating at reflux for 12 h in ethanol, with formic acid as catalyst, resulted in the corresponding imines which were cyclised to the imidazo-anthraquinones **1-2** by using lead tetraacetate in acetic acid at room temperature (Scheme 1) [11]. The crude products were obtained as solids and recrystallised from diethyl ether and chloroform to give the pure compounds **1-2** in good yields (60-64%), which were completely characterised by ^1H and ^{13}C NMR, IR and HRMS. The ^1H NMR signal of the NH proton, in $\text{DMSO-}d_6$ at 25 °C, was seen downfield at 13.22 and 13.25 ppm, for **1** and **2** respectively, indicating high acidity and strong hydrogen-bonding ability (Table 1).



Scheme 1. Synthesis of naphthyl-imidazo-anthraquinones **1-2**.

Table 1 - Yields and spectroscopic data for compounds **1-2** in acetonitrile solution.

Cpd	R	Yield (%)	NMR δ_{H} (ppm) ^a	IR ν (cm^{-1}) ^b	UV-vis				
					Absorption		Fluorescence		
					λ_{abs} (nm)	$\log \epsilon$	λ_{em} (nm)	$\Delta\lambda$ (nm)	Φ_{F}
1	OMe	60	13.22	3426, 1663	422	4.01	650	228	0.005
2	NMe ₂	64	13.25	3418, 1667	435	4.06	566	131	0.084

^a For the NH in $\text{DMSO-}d_6$ (400 MHz). ^b For the NH and C=O stretching bands (in KBr disc).

An intense lowest energy charge-transfer absorption band in the visible region was seen in the UV-visible absorption spectra of compounds **1-2** in acetonitrile solutions, its position being influenced by the electronic nature of the substituents at the naphthyl moiety (OMe or NMe₂) (Table 1). This charge transfer band arises from electronic interaction between the donor substituted naphthyl group functionalized with strong electron donor groups and the acceptor imidazo-anthraquinone moiety.

3.2. Spectrophotometric and spectrofluorimetric titrations of compounds **1-2** with anions in acetonitrile and acetonitrile/water

Compounds **1-2** were evaluated as chemosensors by spectrophotometric and spectrofluorimetric titrations in the presence of several anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, AcO⁻, BzO⁻, NO₃⁻, ClO₄⁻, HSO₄⁻, H₂PO₄⁻ and OH⁻) in acetonitrile. A preliminary test in the presence of 100 equivalents of the anions revealed that compounds **1-2** responded significantly to the presence of F⁻, CN⁻ and OH⁻ with a distinct colour change from yellow to pink.

In the UV-vis spectra of compound **1** (10⁻⁵ M in acetonitrile) with fluoride, cyanide and hydroxide it was seen an intensity decrease and a small bathochromic shift of the absorption band together with the simultaneous growth of a new red-shifted band. As for the spectrofluorimetric titration with the same compound, the response to the presence of these anions was seen by an increase in the fluorescence intensity and a small bathochromic shift of the emission band (Figure 1).

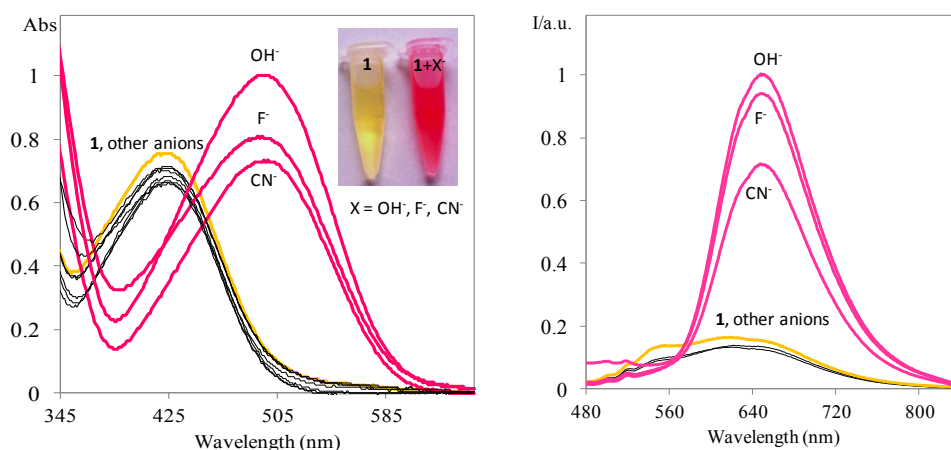


Figure 1. Normalised absorption and emission spectra of naphthyl-imidazo-anthraquinone **1** in the absence and presence of 100 equivalents of several anions (F⁻, Cl⁻, Br⁻, I⁻, CN⁻, AcO⁻, BzO⁻, NO₃⁻, ClO₄⁻, HSO₄⁻, H₂PO₄⁻ and OH⁻) in acetonitrile ([**1**] = 1.7 × 10⁻⁵ M).

Compound **1** exhibited an absorption band at 422 nm and a yellow colour in acetonitrile solution. Upon addition of increasing amount of fluoride, this band progressively decreased while a new absorption band at 493 nm increased in intensity ($\Delta\lambda= 71$ nm) (Figure 2A). A clearly visible colour modulation from yellow to pink was observed, suggesting the deprotonation of the receptor. This process can be related with the appearance of a new absorption band at longer wavelengths, whereas the formation of hydrogen-bonding complexes can be reflected by a small shift of the absorption band due to coordination of the anions with the receptor [12]. The same effect was seen with cyanide and hydroxide ions (Figure 2C and 2E).

Spectrofluorimetric titrations showed different emission behaviour according to the donor group attached to the naphthyl moiety. Compounds **1-2** were excited in the pseudo-isosbestic points observed in the course of UV-vis titrations and upon addition of fluoride, cyanide and hydroxide to the solution of compound **1** an increase in fluorescence intensity was observed (the emission was switched “on”), accompanied by a 26 nm bathochromic shift of λ_{em} to 650 nm. The fluorescence enhancement (CHEF effect) observed for compound **1** was less effective upon addition of cyanide, which required almost 60 equivalents to reach a plateau, while only 6 equivalents were necessary for the fluoride, probably due to the lower basicity of cyanide in acetonitrile comparatively to the fluoride anion (Figure 2B, D and F).

On the other hand, compound **2** revealed a $\sim 50\%$ quenching of the initial emission intensity upon addition of increasing amounts of fluoride, cyanide and hydroxide (Figures S1-S3 in supporting information). Since compounds **1-2** showed a similar UV-vis absorption with these anions, the strong difference in emission properties should most likely be attributed to the presence of methoxy and *N,N*-dimethylamino donor groups in compounds **1** and **2**, respectively. The quenching effect observed for compound **2** can be understood as a photoinduced electron transfer (PET) effect from the lone electron pair located at the nitrogen of the *N,N*-dimethylamino donor group, due to lower electronegativity of nitrogen compared to the oxygen. The lone electron pair is more available to promote PET and, consequently, the quenching of the emission. As in the case of the absorption spectra, the small red-shift of the fluorescence band can be related with the formation of the deprotonated species [13].

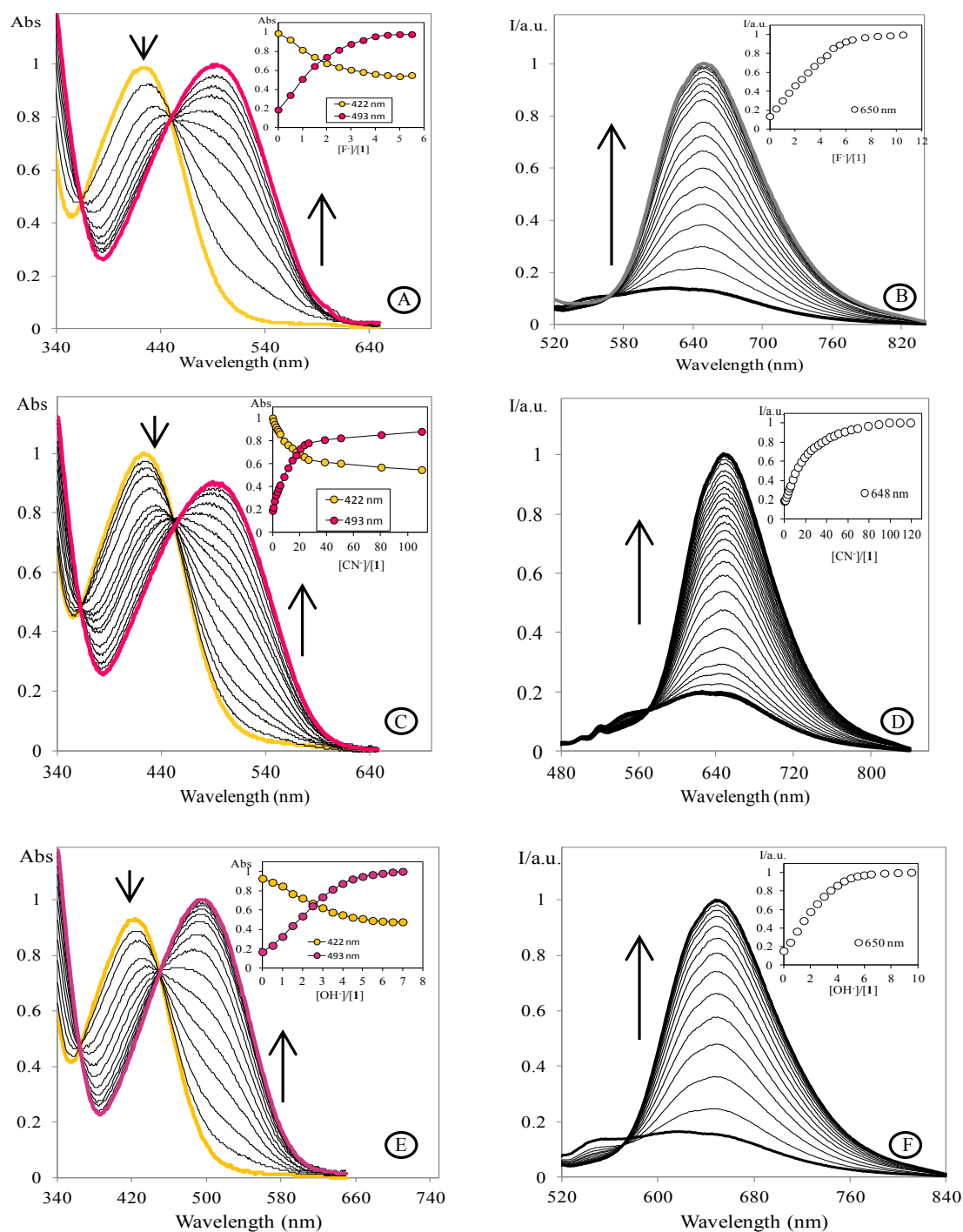


Fig. 2. Spectrophotometric (A, C and E) and spectrofluorimetric titrations (B, D and F) of compound **1** with F^- , CN^- and OH^- in acetonitrile ($[1] = 1.7 \times 10^{-5}$ M, $T = 298$ K, $\lambda_{exc} = 422$ nm). The insets represent the maximum of absorption and emission bands.

Coordination to metal ions by 9,10-anthraquinone derivatives can be achieved through the lone electron pairs of the oxygen from the carbonyl groups resulting in the metal ion complexes [4]. A great advantage presented by the deprotonated form of compounds **1-2** is that they are emissive and at the same time provide an additional binding site for recognition purposes formed by the deprotonated N from the imidazole and the O from the carbonyl group of the anthraquinone. The

behaviour of compounds **1-2** in the presence of several alkaline, alkaline-earth and transition metal cations (Na^+ , Cd^{2+} , Ca^{2+} , Cr^{3+} , Zn^{2+} , Hg^{2+} , Fe^{2+} , Fe^{3+} , Cu^{2+} , Co^{2+} , Ni^{2+} and Pd^{2+}) was investigated after addition of fluoride ion (5 equiv), to ensure complete deprotonation of the imidazoanthraquinone system. In all cases, the original absorption band centred at 493-497 nm, related to the deprotonated compound, was blue-shifted and the original band centred at 422-435 nm, related to the free ligand, was restored, suggesting the formation of metal complexes in solution.

The interaction between the deprotonated system **1** and Cu^{2+} is presented as a representative example (Figure 3). A plateau was achieved after addition of 8 equivalents of metal ion and in all cases, the pink colour of the solution, observed upon fluoride addition, changed to yellow upon metal complexation giving a yellow-pink-yellow, reversible colorimetric reaction. The stability constants of the complexes of **1-2** with the anions and Cu^{2+} were calculated and are presented in Table 2, and in general, the strongest interaction was observed for system **1**.

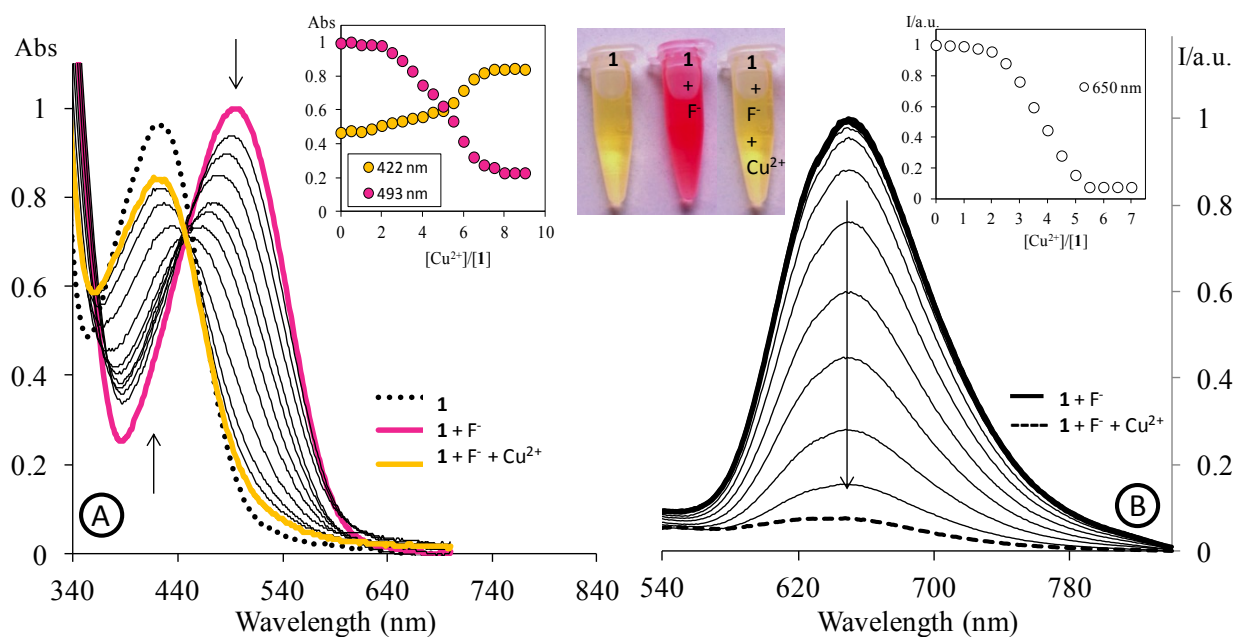


Fig. 3. Spectrophotometric (A) and spectrofluorimetric (B) titration of **1** in acetonitrile after addition of F^- (5 equiv) with increasing amount of Cu^{2+} ($T = 298\text{K}$, $[\mathbf{1}] = 1.7 \times 10^{-5}\text{ M}$, $\lambda_{\text{exc}} = 450\text{ nm}$). The inset represents the normalised absorption at 422 and 493 nm (A) and the normalised emission at 650 nm (B).

Regarding the fluorimetric response, for all metals studied a CHEQ effect in the fluorescence intensity was observed (see Figures S7-S9 in supporting information for the other metal cations). The quenching effect in the presence of Cu^{2+} (Figure 3) can be attributed to an energy transfer quenching of the π^* emissive state through low-lying metal-centred unfilled d-orbitals [14]. This

result suggests the involvement of the metal ion with both donor atoms, the O from the anthraquinone carbonyl group and the N from the imidazole ring through two units of the ligand.

Table 2 - Association constants for compounds **1-2** in the presence of F^- , CN^- and Cu^{2+} in CH_3CN and CH_3CN/H_2O (9:1, v/v). For all interactions, the stoichiometry suggested from Hyperquad software was 1:1 (L:A) or 2:1 (L:M).

Compound	Ion	$\log K_{ass}$	
		CH_3CN	CH_3CN/H_2O (9:1)
1	F^-	4.143 ± 0.003	^a
	CN^-	3.608 ± 0.001	3.689 ± 0.017
	Cu^{2+}	8.940 ± 0.034	---
2	F^-	3.914 ± 0.005	^a
	CN^-	3.063 ± 0.002	2.893 ± 0.020
	Cu^{2+}	8.256 ± 0.043	---

^a No reliable results were obtained.

A significant gain in sensitivity was achieved upon previous deprotonation with fluoride as can be understood from the fluorescence intensity data presented in Figure 4. Imidazo-anthraquinone **1** was scarcely fluorescent with a fluorescence quantum yield of 0.005; deprotonation of **1** with 5 equivalents of fluoride ion lead to a more emissive species with a four-fold increase of the fluorescence intensity and a fluorescence quantum yield of 0.022. Complexation of deprotonated **1** with Cu^{2+} resulted in a decrease of fluorescence (quantum yield 0.002), whereas the interaction of imidazo-anthraquinone **1** with Cu^{2+} also resulted in a non-emissive complex (quantum yield 0.003).

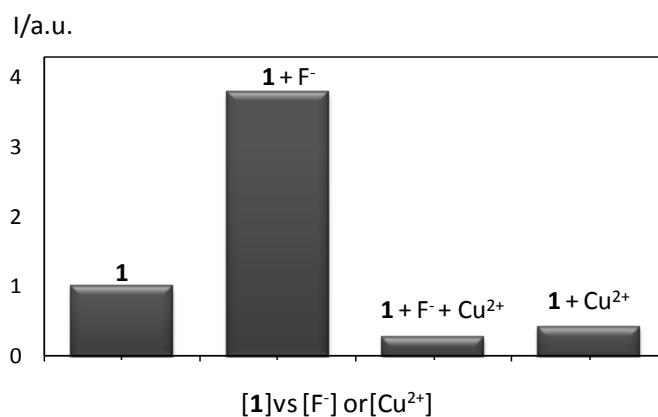


Fig. 4. Relative fluorescence intensity of imidazo-anthraquinone **1** in the presence of F^- and Cu^{2+} in acetonitrile.

Most of the sensing systems reported so far as chemosensors for fluoride or cyanide can only be used in organic media, so the search for effective systems for use in aqueous media is still a great challenge. Bearing this fact in mind, an anion selectivity assay in an organic solvent-water system $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (9:1, v/v) was carried out. In figures 5 and 6 the comparison of the sensory study of compound **1** in organic (acetonitrile) and aqueous media is presented, showing that its poor selectivity in acetonitrile was largely improved in aqueous media. In the aqueous mixture, it was possible to distinguish cyanide from fluoride since a change in colour from yellow to orange was only observed for the cyanide.

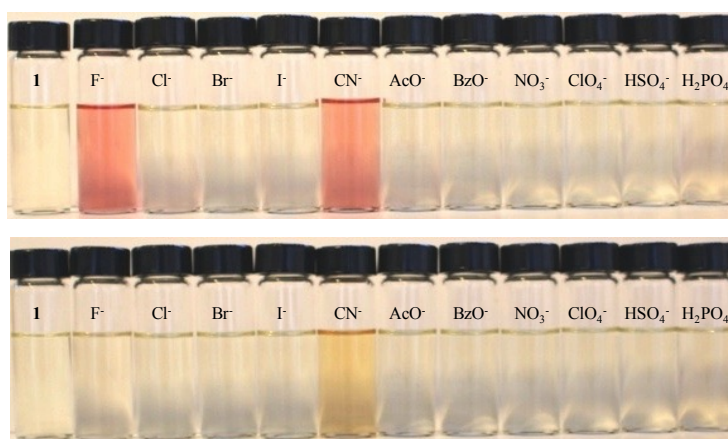


Fig. 5. Color changes of compound **1** (5×10^{-4} M in CH_3CN and $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (9:1)) in the presence of 100 equiv. of F⁻, Cl⁻, Br⁻, I⁻, CN⁻, AcO⁻, BzO⁻, NO₃⁻, ClO₄⁻, HSO₄⁻ and H₂PO₄⁻.

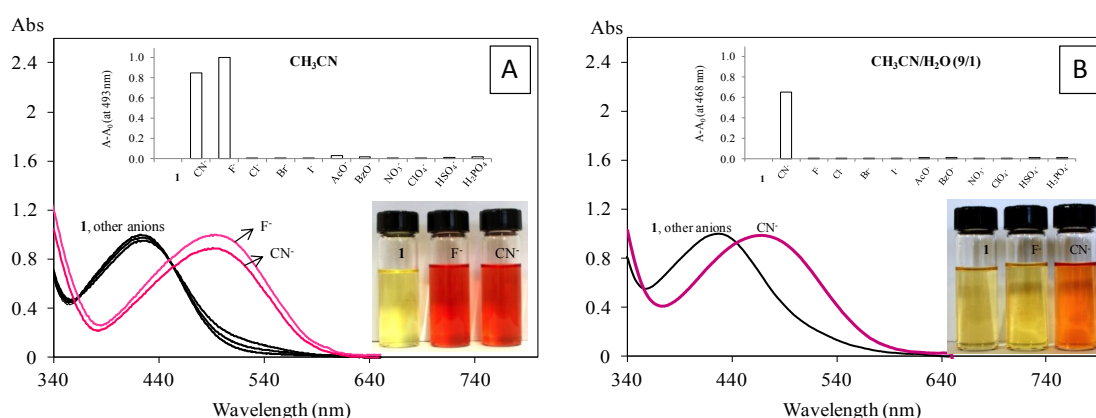


Fig. 6. UV-vis spectral responses of **1** (1.0×10^{-4} M) in (A) CH_3CN and (B) $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (9:1) in the presence of 100 equiv of anions. In the inset are represented the normalised changes in absorbance at 493 nm and 468 nm after addition of anions.

During titration of compound **1** with CN⁻ in CH₃CN/H₂O (9:1, v/v) a small red shift of the ICT band to $\lambda = 427$ nm was observed when compared to the titration in acetonitrile solution ($\Delta\lambda = 5$ nm). On the other hand, the band of the complex formed was blue-shifted to $\lambda = 468$ nm ($\Delta\lambda = 25$ nm) (see Figures S4-S6 in supporting information for the titration of compound **1** with fluoride and of compound **2** with fluoride and cyanide in CH₃CN/H₂O (9:1, v/v)).

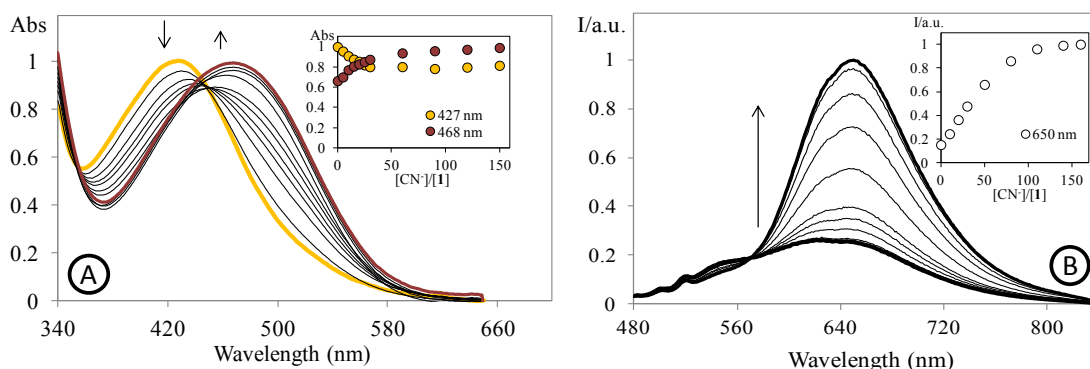


Fig. 7. Spectrophotometric (A) and fluorimetric titrations (B) of **1** in CH₃CN/H₂O (9:1, v/v) solution ($[1] = 1.7 \times 10^{-5}$ M, $\lambda_{\text{exc}} = 427$ nm, T = 298 K) upon addition of increasing amount of CN⁻. The insets represent the maximum of absorption and emission bands.

The association constants were also obtained for titrations in CH₃CN/H₂O (9:1, v/v), but it was not possible to obtain reliable results for the K_{ass} with fluoride for both receptors (see Table 2). The best results were obtained for compound **1** ($\log K_{\text{ass}} = 3.689 \pm 0.017$), and in order to explore the applicability of this sensor, the limits of detection (LOD) and quantification (LOQ) at 427 nm were determined in CH₃CN/H₂O (9:1, v/v). Thus, the values were of 4.76 ± 0.03 mM (LOD) and of 15.9 ± 0.07 mM (LOQ) (Table 3). Earlier several researchers reported the limits of detection of the cyanide ion which are in the range of mM till μM [9c-d, 9l-q].

Table 3 - Limit of detection (LOD) and limit of quantification (LOQ) for the cyanide ion in CH₃CN and CH₃CN/H₂O (9:1, v/v).

Solvent	LOD [mM]	LOQ [mM]
CH ₃ CN	1.23	4.12
CH ₃ CN-H ₂ O (9/1, v/v)	4.76	15.9

3.3. ^1H NMR titrations

The sensory behaviour observed by the spectrophotometric and spectrofluorimetric titrations was also confirmed by performing ^1H NMR titrations but due to the limited solubility of compounds **1-2** in deuterated acetonitrile, the titrations were carried out with the fluoride ion in $\text{DMSO-}d_6$ at room temperature.

The signal of the imidazole NH appearing downfield suggested high acidity and strong hydrogen-bonding ability. Upon addition of 2 equivalents of F^- , this peak became broader, indicating a hydrogen-bonding interaction between the fluoride and the NH proton (Figure 8). After addition of 4 equivalents of F^- , one triplet signal started developing at ~ 16 ppm, which was clearly visible after addition of 6 equivalents of F^- . This triplet can be ascribed to the formation of HF_2^- , thus confirming the imidazole NH deprotonation. This fact was also accompanied by the upfield shifts of the H4 and H5 protons ($\Delta\delta \sim 0.3$ ppm) of the anthraquinone moiety, adjacent to imidazole ring, which should be due to the increasing electron density on the ring owing to through-bond effects. On the other hand, significant large downfield shifts were observed for H2' and H8', by $\Delta\delta \sim 0.3$ ppm and ~ 1.3 ppm, respectively, due to through-space effects, which polarise the C-H bonds in proximity to the hydrogen bond, creating a partial positive charge at H2' and most significantly at H8'.

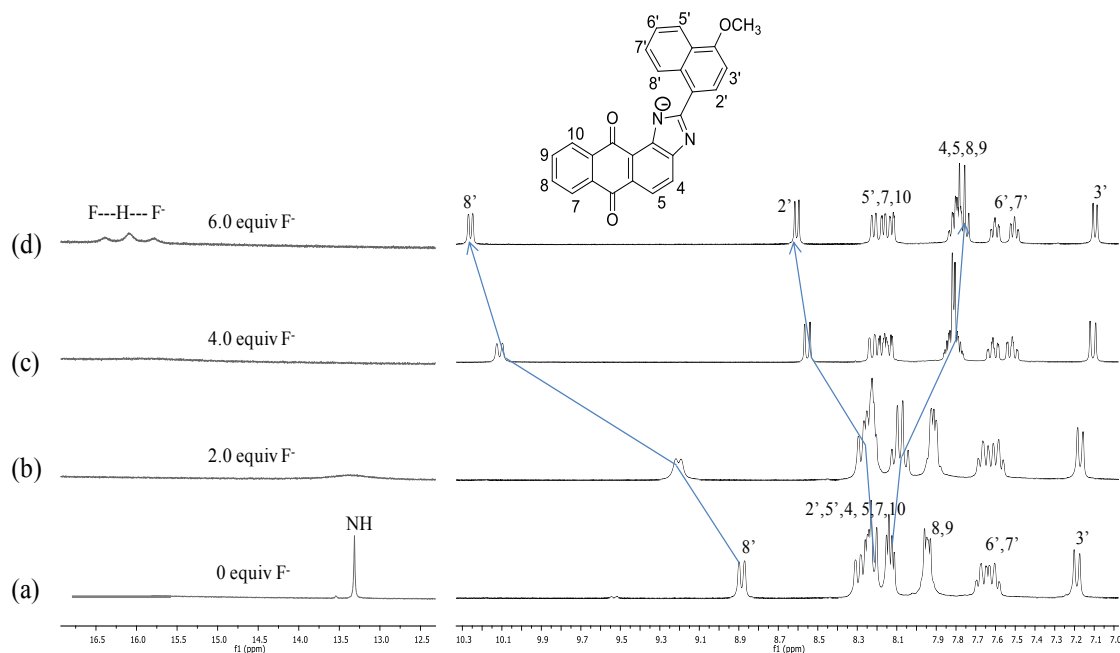


Fig. 8. Partial ^1H NMR spectra of imidazo-anthraquinone **1** (1.2×10^{-2} M) in $\text{DMSO-}d_6$ in: (a) absence and the presence of (b) 2, (c) 4, and (d) 6 equivalents of F^- .

The results obtained through UV-vis and ^1H NMR titrations were found to be in agreement: upon addition of 2 equivalents of fluoride ion, the imidazo-anthraquinone **1** formed a hydrogen-bond complex, from the ^1H NMR titration data, whereas drastic changes were seen at the same time in the UV-vis spectra. With the increase of the fluoride concentration, a new deprotonated species was formed and the corresponding CT band in the UV-vis spectra was clearly visible, accompanied by a pronounced colour change from yellow to pink. In the NMR spectra, the triplet due to the HF_2^- ion confirmed the deprotonation of the imidazole NH, along with downfield and upfield shifts of several protons of the naphthyl-imidazo-anthraquinone. Based on these concurring observations, it may be suggested that the interaction between the ligand and fluoride is a two-step process, firstly by hydrogen binding at low fluoride concentration, followed by interaction of the excess fluoride with the ligand-fluoride complex and thus inducing deprotonation of the ligand [3e].

4. Conclusions

Novel naphthyl-imidazo-anthraquinones **1-2** were synthesized in good yields and completely characterised. Their ability as colorimetric and fluorimetric sensors towards anions was studied in acetonitrile and in a mixture of acetonitrile and water (in a 9:1 proportion). Through spectrophotometric and spectrofluorimetric titrations of both anthraquinones with various anions, higher sensitivity for fluoride and cyanide ions was observed, but with different fluorogenic behaviour: imidazo-anthraquinone **1** displayed a CHEF effect whereas anthraquinone **2** responded with a CHEQ effect. As for the colorimetric behaviour, straightforward naked-eye detection from yellow to pink was possible after addition of these anions. By using a mixture of acetonitrile and water (9:1), a very significant increase in selectivity towards CN^- was achieved.

Moreover, addition of excess fluoride ion to both ligands resulted in the deprotonation of the imidazole NH, and the fluorescent deprotonated form could be used for fluorimetric sensing of metal ions such as Cu^{2+} , Pd^{2+} and Hg^{2+} , suggesting the formation of 2:1 L:M complexes. The binding process was also followed by ^1H NMR titrations which corroborated the previous findings.

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Supporting Information

1. Spectrophotometric and spectrofluorimetric titrations of imidazo-anthraquinone **2** with cyanide, fluoride and hydroxide in acetonitrile and acetonitrile/water solution (9:1) 19
2. Spectrophotometric and spectrofluorimetric titration of imidazo-anthraquinones **1-2** with Cu^{2+} , Pd^{2+} , and Hg^{2+} , after fluoride ion addition. 2

1. Spectrophotometric and spectrofluorimetric titration of imidzo-anthraquinone 2 with cyanide, fluoride and hydroxide in acetonitrile and acetonitrile/water solution (9:1)

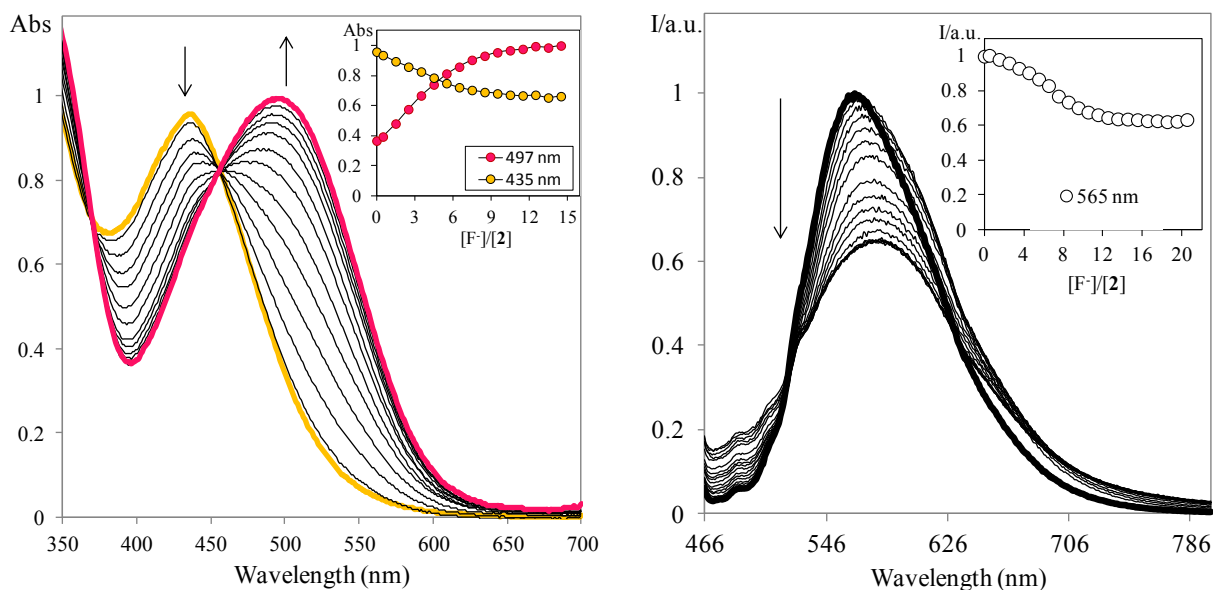


Fig. S1. Spectrophotometric (A) and spectrofluorimetric (B) titrations of **2** in acetonitrile with F⁻ ([2] = 1.7×10^{-5} M, T = 298 K). The inset represents the normalised absorption at 435 and 497 nm (A) and the normalised emission at 566 nm (B).

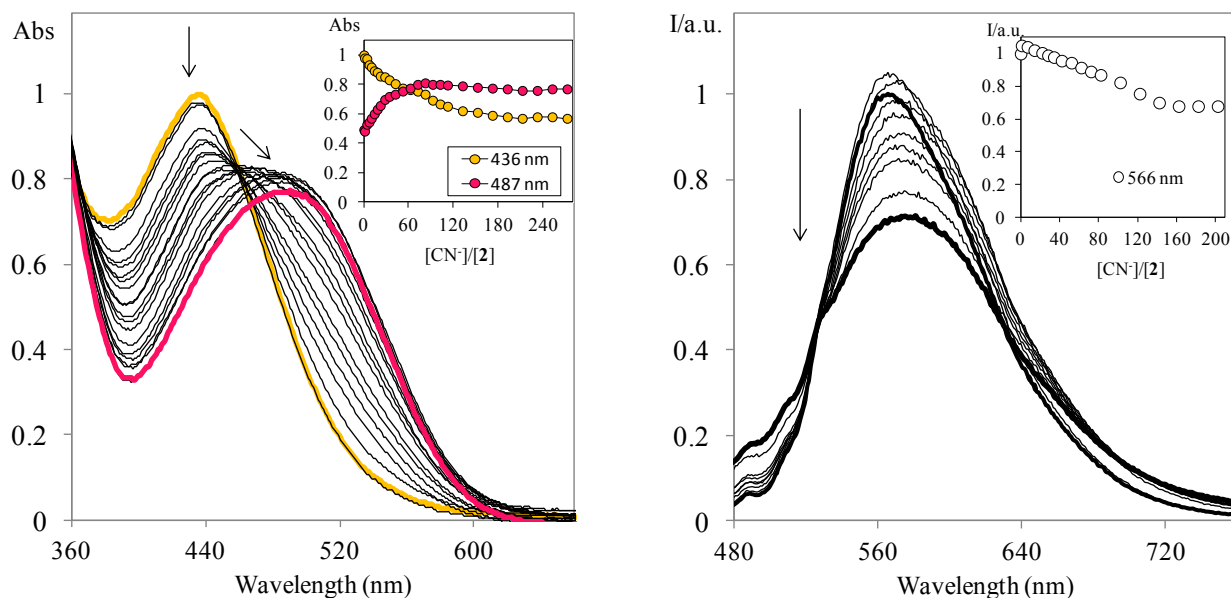


Figure S2: Spectrophotometric and spectrofluorimetric titration of **2** in acetonitrile solution ([2] = 1.7×10^{-5} M, $\lambda_{\text{exc}} = 433$ nm, T = 298 K) upon addition of increasing amount of CN⁻. The insets represent the maximums of absorption and emission bands.

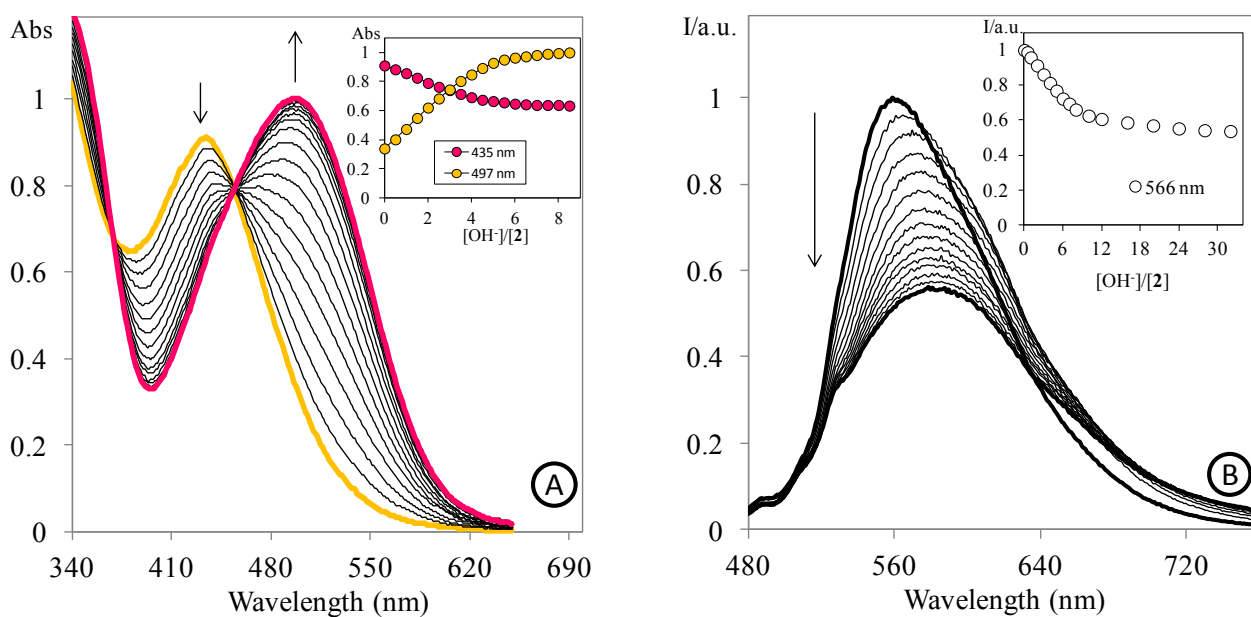


Figure S3. Spectrophotometric titration (A) and fluorimetric titration (B) of compound **2** with OH^- in acetonitrile ($[\mathbf{2}] = 1.7 \times 10^{-5} \text{ M}$, $T = 298 \text{ K}$, $\lambda_{\text{exc}} = 436 \text{ nm}$). The insets represent the maximums of absorption and emission bands.

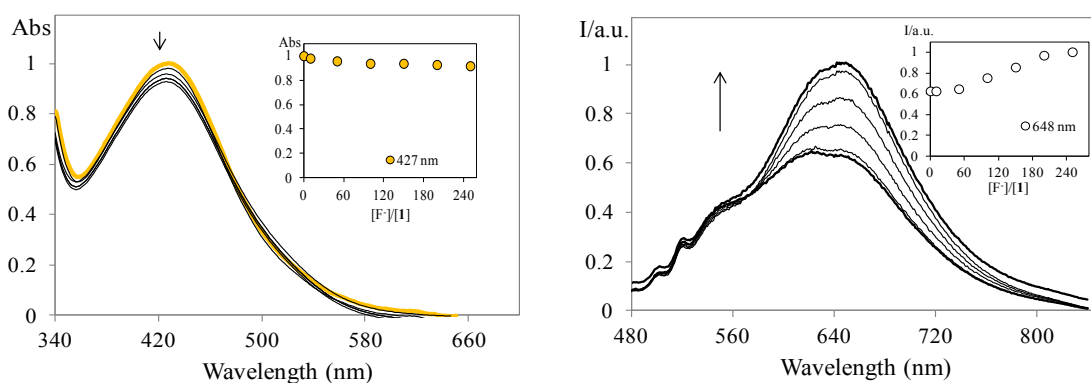


Figure S4: Spectrophotometric and spectrofluorimetric titration of **1** in acetonitrile/ H_2O (9/1) solution ($[\mathbf{1}] = 1.7 \times 10^{-5} \text{ M}$, $\lambda_{\text{exc}} = 427 \text{ nm}$, $T = 298 \text{ K}$) upon addition of increasing amount of F^- . The insets represent the maximums of absorption and emission bands.

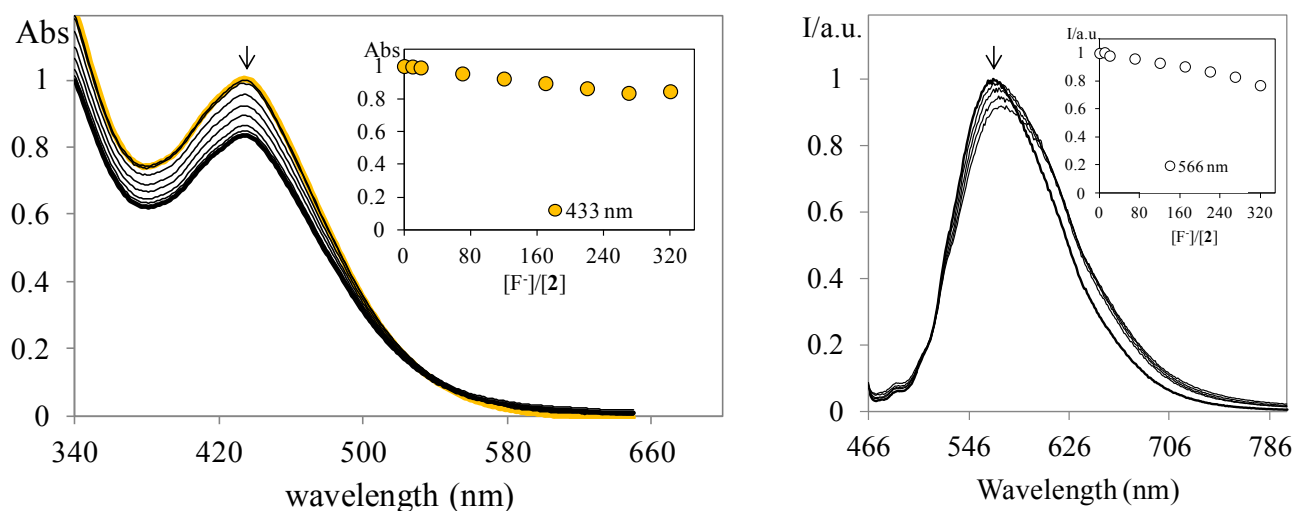


Figure S5: Spectrophotometric and spectrofluorimetric titration of **2** in acetonitrile/H₂O (9/1) solution ($[2] = 4.7 \times 10^{-5}$ M, $\lambda_{\text{exc}} = 433$ nm, T = 298 K) upon addition of increasing amount of F⁻. The insets represent the maximums of absorption and emission bands.

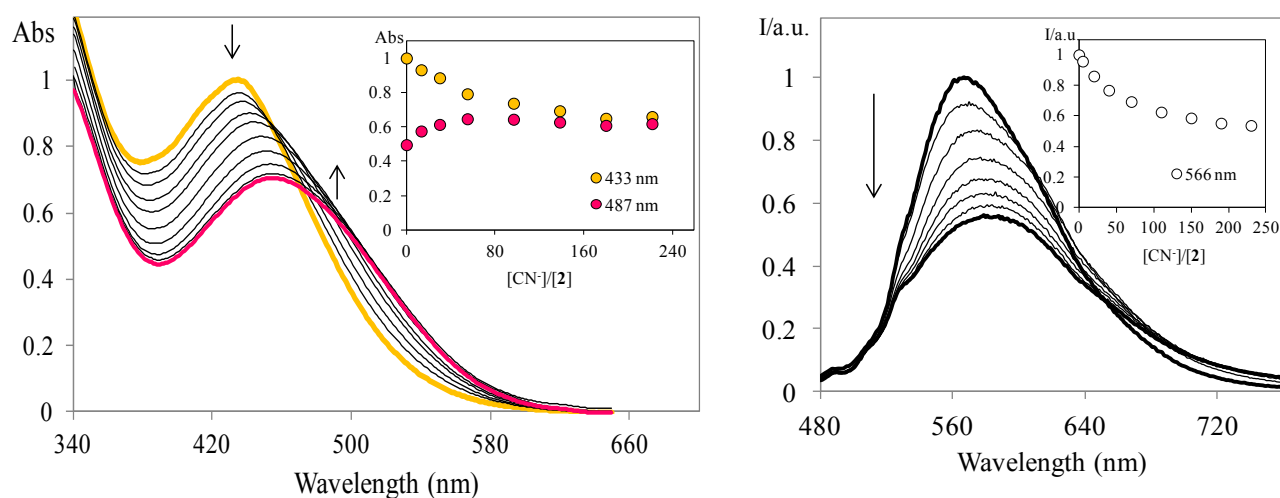


Figure S6: Spectrophotometric and spectrofluorimetric titration of **2** in acetonitrile/H₂O (9/1) solution ($[2] = 4.7 \times 10^{-5}$ M, $\lambda_{\text{exc}} = 433$ nm, T = 298 K) upon addition of increasing amount of CN⁻. The insets represent the maximums of absorption and emission bands.

2: Spectrophotometric and spectrofluorimetric titration of imidzo-anthraquinones 1-2 with Cu^{2+} , Pd^{2+} , and Hg^{2+} , after fluoride ion addition.

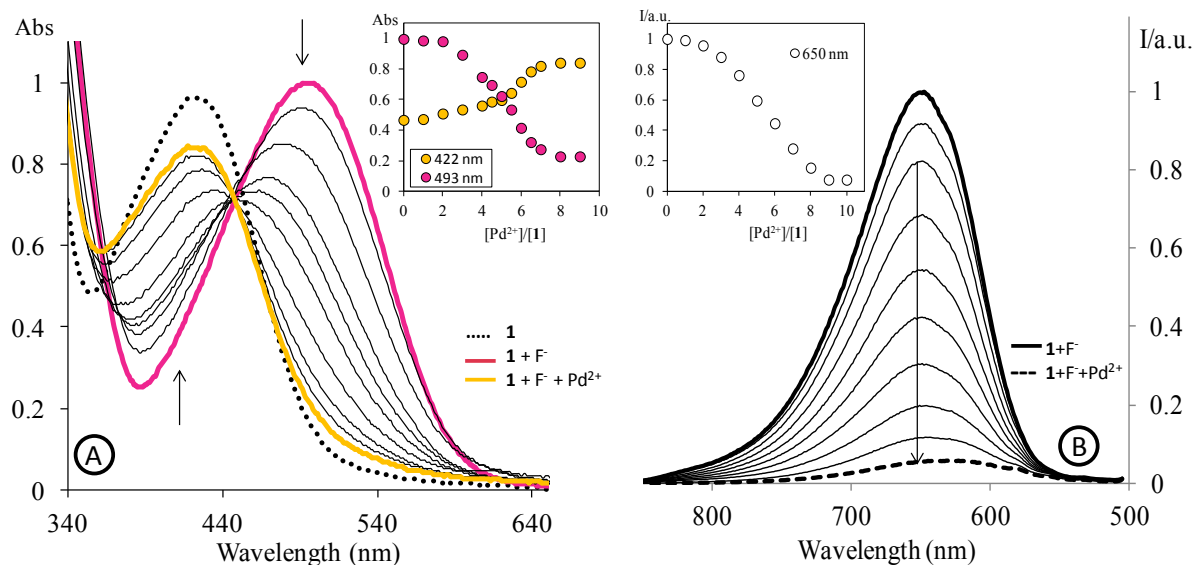


Figure S7: Spectrophotometric and spectrofluorimetric titration of **1** in acetonitrile solution with increasing addition of Pd^{2+} , after addition of 5 equiv of F^- ($[\text{1}] = 1.7 \times 10^{-5}$ M, $\lambda_{\text{exc}} = 450$ nm, $T = 298$ K).

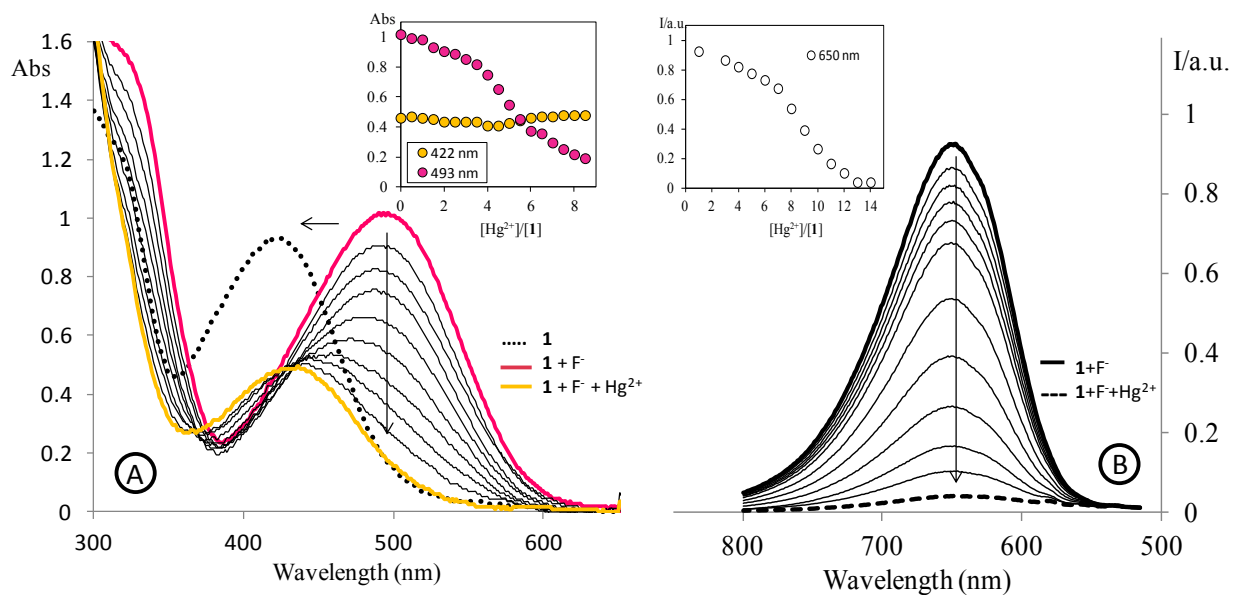


Figure S8: Spectrophotometric and spectrofluorimetric titration of **1** in acetonitrile solution with increasing addition of Hg^{2+} , after addition of 5 equiv of F^- ($[\text{1}] = 1.7 \times 10^{-5}$ M, $\lambda_{\text{exc}} = 450$ nm, $T = 298$ K).

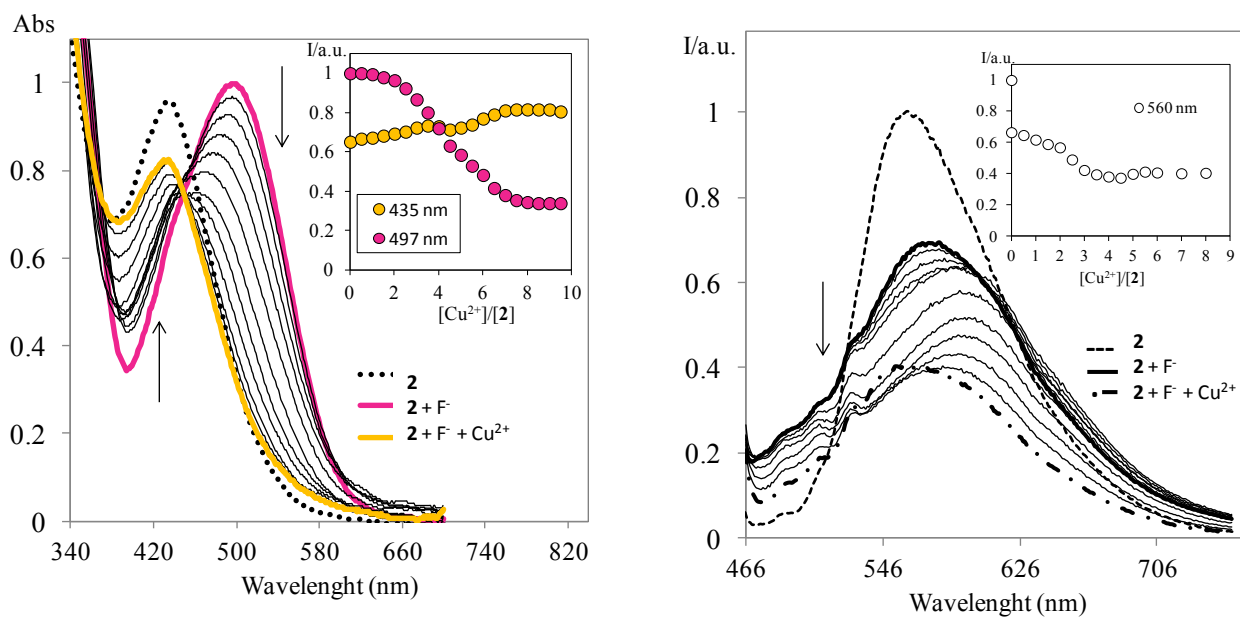


Figure S9: Spectrophotometric and spectrofluorimetric titration of **2** in acetonitrile solution with increasing addition of Cu²⁺, after addition of 5 equiv of F⁻ ([**2**] = 1.7 × 10⁻⁵ M, λ_{exc} = 456 nm, T = 298 K).