Non-canonical amino acids bearing thiophene and bithiophene: synthesis by an Ugi multicomponent reaction and studies on ion recognition ability

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Abstract: Novel thienyl and bithienyl amino acids with different substituents were obtained by a multicomponent Ugi reaction between a heterocyclic aldehyde, an amine, an acid and an isocyanide. Due to the presence of the sulphur heterocycle at the side chain, these unnatural amino acids are highly emissive and bear extra electron donating atoms so they were tested for their ability to act as fluorescent probes and chemosensors in the recognition of biomedically relevant ions in acetonitrile and acetonitrile/water solutions. The results obtained from spectrophotometric/spectrofluorimeric titrations in the presence of organic and inorganic anions, and alkaline, alkaline-earth and transition metal cations indicated that the bithienyl amino acid bearing a methoxy group is a selective colorimetric chemosensor for Cu^{2+}, while the other (bi)thienyl amino acids act as fluorimetric chemosensors with high sensitivity towards Fe^{3+} and Cu^{2+} in a metal–ligand complex with 1:2 stoichiometry. The photophysical and ion sensing properties of these amino acids confirm their potential as fluorescent probes suitable for incorporation into peptidic frameworks with chemosensory ability.

Introduction

Non-canonical amino acids of synthetic origin are useful for the preparation of functional peptides with tailored properties for varied applications such as increased fluorescence, conformational rigidity, and metal complexation ability, among other properties. Recent reports include the application of such amino acids in studies of molecular flexibility and protein folding, substrate binding activity of proteins, antigenicity or enzymatic activity, targeting peptides for molecular imaging, peptidomimetics biological activity and protein engineering (Kajihara et al. 2006; Hennig et al. 2007; Katritzki and Narindoshvili 2009; Lee et al. 2010; Wang et al 2012; Pless and Ahern 2013; Niu and Guo, 2013; Liu et al. 2015, Zhou et al 2016).

Many biochemical processes rely on the coordinating ability that amino acids and peptides display towards metal ions because they possess electron donor atoms like nitrogen, oxygen and sulphur at

the main and side chains (Zheng et al. 2003; Shimazaki et al. 2009). Therefore, the insertion of suitable heterocycles at the side chain of natural amino acids, along increasing the number of binding sites, can provide increased UV absorption and fluorescence, which can be valuable for biochemistry, cellular biology and cellular imaging applications. Fluorescent probes are indispensable tools for monitoring ions and biomolecules with high sensitivity in cells and tissues, as they present distinct advantages in fluorescence detection in terms of sensitivity, selectivity, response time and local observation, etc. There are various examples of fluorescent unnatural amino acids, displaying better photophysical properties than tryptophan, that have been inserted in peptide and protein frameworks in order to afford fluorescently labelled entities (Katritzki and Narindoshvili 2009; Cheruku et al. 2015).

Thiophene and its derivatives exhibit interesting optical properties that have led to their application as sensors and fluorescent reporters (Capobianco et al. 2012). Oligomers of thiophene present improved luminescent properties are more readily soluble in organic solvents, improves absorption efficiency and thermal stability of the resultant molecule without reducing fluorescence (Pina et al. 2010).

Selective recognition of anions is also a very dynamic topic due to their importance in medicinal and environmental areas. Especially, the development of colorimetric and fluorimetric chemosensors for anions has been widely investigated due to the relevance of several anions in biological processes (Veale and Gunnlaugsson 2010; Moragues et al. 2011; Santos-Figueroa et al. 2013).

Bearing the above facts in mind, there is a practical interest on the design of unnatural amino acids and our research group has been engaged on the synthesis of heterocyclic amino acids and their application as fluorescent markers and fluorimetric probes for metal ions (Batista et al. 2012; Costa et al. 2007, 2008a, 2008b; Esteves et al. 2009, 2010, 2011, 2016; Oliveira et al. 2011). We now report the synthesis and characterization of novel non-canonical amino acids bearing thiophene and bithiophene moieties, by an Ugi multicomponent reaction between a heterocyclic aldehyde, an amine, an acid and an isocyanide. This reaction is a straightforward method for the synthesis of α- and α,α-substituted glycines that allows the introduction of a variety of groups and functionalities at the side chain (Dömling 2006). The thiophene coordinating/reporting unit was linked with different substituents to tune the photophysical properties of the new probes and optimize the recognition of target analytes through greater fluorescence sensitivity. The recognition ability of these non-canonical amino acids toward different ions of analytical and biological relevance was evaluated by UV–vis absorption and fluorescence spectroscopy. Spectrophotometric and spectrofluorimetric titrations were made to assess their potential to act as fluorescent probes suitable for incorporation into peptidic frameworks with chemosensory ability.

Experimental Section

General

All melting points were measured on a Stuart SMP3 melting point apparatus. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light. Chromatography on silica gel was carried out on Merck Kieselgel (230-240 mesh). IR spectra were determined on a BOMEM MB 104 spectrophotometer. NMR spectra were obtained on a Varian Unity Plus Spectrometer at an operating frequency of 300 MHz for ^{1}H and 75.4 MHz for ^{13}C or a Bruker Avance III 400 at an operating frequency of 400 MHz for ^{1}H and 100.6 MHz for ^{13}C using the solvent peak as internal reference at 25 ºC. All chemical shifts are given in ppm using tetramethylsilane as reference and *J* values are given in Hz. Assignments were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectrometry analyses were performed at the "C.A.C.T.I. - Unidad de Espectrometria de Masas", at University of Vigo, Spain. Fluorescence spectra were collected using a FluoroMax-4 spectrofluorometer. UV-visible absorption spectra (200 – 600 nm) were obtained using a Shimadzu UV/2501PC spectrophotometer. All reagents were commercially available and used as received.

General procedure for the synthesis of thienyl amino acid derivatives 2a-j by an Ugi multicomponent reaction

The appropriate aldehyde **1a-j** (1 equiv) and 4-methoxybenzylamine (1 equiv) were dissolved in dry methanol (5 mL/mmol of aldehyde) and stirred for 1 hour at 50 ºC, to form the corresponding imine. Acetic acid (1 equiv) was added to the previous mixture and stirred for 15 minutes at room temperature. Then, cyclohexyl isocyanide was added (1 equiv) and the mixture was left stirring at room temperature for 24 h. The solvent was evaporated and the crude was chromatographed through a silica gel column with dichloromethane-hexane (2:1) (to elute any unreacted isocyanide), followed by dichloromethane (to elute any unreacted aldehyde) and dichloromethane-methanol (90:1) (to elute the desired product). The fractions containing the product were evaporated to dryness in a rotary evaporator.

The synthetic details and characterization for compounds **2a** and **2h**, considered as models for the thienyl and bithienyl set of amino acids, respectively, are given below. The synthetic details and characterization for compounds **2b-g**,**i-j** are given in the Supplementary Material.

***N*-Cyclohexyl-2-(*N*-(4'-methoxybenzyl)acetamido)-2-(thiophen-2-yl)acetamide 2a.** Starting from 2-formylthiophene **1a** (0.156 g, 1.39×10^{-3} mol), 4-methoxybenzylamine (0.18 mL, 1.39×10^{-3} mol), acetic acid (0.08 mL, 1.39×10^{-3} mol) and cyclohexyl isocyanide (0.17 mL, 1.39×10^{-3} mol), compound **2a** was obtained as an orange oil (0.184 g, 4.6×10^{-4} mol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.05-1.13 (m, 3H, 3 × *H*-cHex), 1.24-1.33 (m, 2H, 2 × *H*-cHex), 1.50-1.62 (m, 3H, 3 × *H*-cHex), 1.77-1.86 (m, 2H, 3 × *H*-cHex), 2.01 (s, 3H, CH_3CO), 3.70 (s, 4H, OCH_3 and *H1*-cHex), 4.58 (s, 2H, NCH_2), 6.07 (s, 1H, α-H), 6.23 (d, *J* 8.0 Hz, 1H, NH), 6.72 (d, *J* 8.4 Hz, 2H, H3' and H5'), 6.87 (dd, *J* 3.4 and 5.0 Hz, 1H, H4), 6.95 (d, *J* 8.4 Hz, 2H, H2' and H6'), 7.05 (d, *J* 3.4 Hz, 1H, H3), 7.21 (d, *J* 5.0 Hz, 1H, H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.15 (*C*H_3CO), 24.43 (*C*-cHex), 24.48 (*C*-cHex), 25.24 (*C*-cHex), 32.38 (2 × *C*-cHex), 48.32 (*C1*-cHex), 50.07 (NCH_2), 54.96 (OCH_3), 57.52 (α-CH), 113.63 (C3' and C5'), 126.26 (C4), 127.14 (C5), 127.31 (C2' and C6'), 129.02 (C3), 129.30 (C1'), 136.91 (C2), 158.42 (C4'), 167.62 (C=O amide), 171.95 (CH_3*C*O); IR (liquid film, cm^{-1}): ν = 3306, 3070, 2933, 2855, 1633, 1586, 1542, 1513, 1464, 1451, 1409, 1364, 1350, 1289, 1247, 1207, 1176, 1111, 1093, 1036, 979, 912, 892, 840, 811, 735, 699, 665, 543. UV/Vis (ethanol, nm): λ_{max} (log ε) = 275 (4.19). MS: *m/z* (ESI, %) 401 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{22}H_{29}N_2O_3S$ 401.18934, found 401.18900.

2-([2,2'-Bithiophen]-5-yl)-*N*-cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)acetamide 2h. Starting from 5-formyl-2,2'-bithiophene **1h** (0.210 g, 1.08×10^{-3} mol), 4-methoxybenzylamine (0.14 mL, 1.08×10^{-3} mol), acetic acid (0.06 mL, 1.08×10^{-3} mol) and cyclohexyl isocyanide (0.13 mL, 1.08×10^{-3} mol), compound **2h** was obtained as an orange oil (0.215 g, 4.45×10^{-4} mol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.06-1.17 (m, 3H, 3 × *H*-cHex), 1.27-1.36 (m, 2H, 2 × *H*-cHex), 1.54-1.66 (m, 3H, 3 × *H*-cHex), 1.80-1.92 (m, 2H, 2 × *H*-cHex), 2.08 (s, 3H, CH_3CO), 3.73 (s, 4H, OCH_3 and *H1*-cHex), 4.56-4.67 (m, 2H, NCH_2), 5.91 (s, 1H, α-H), 6.23 (d, *J* 7.2 Hz, 1H, NH), 6.77 (d, *J* 8.8 Hz, 2H, H3'' and H5''), 6.92 (d, *J* 3.6 Hz, 1H, H3), 6.95 (d, *J* 3.6 Hz, 1H, H4), 6.96-6.98 (m, 1H, H4'), 7.06 (d, *J* 8.8 Hz, 2H, H2'' and H6''), 7.12 (d, *J* 2.8 Hz, 1H, H5'), 7.18 (d, *J* 5.2 Hz, 1H, H3'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.21 (*C*H_3CO), 24.53 (*C*-cHex), 24.60 (*C*-cHex), 25.32 (*C*-cHex), 32.48 (*C*-cHex), 32.53 (*C*-cHex), 48.51 (*C1*-cHex), 50.49 (NCH_2), 55.08 (OCH_3), 58.25 (α-CH), 113.81 (C3'' and C5''), 122.60 (C4), 123.75 (C5'), 124.50 (C3'), 127.59 (C2'' and C6''), 127.69 (C4'), 128.83 (C1''), 129.92 (C3), 135.73 (C2), 135.73 (C2'), 139.28 (C5), 158.65 (C4''), 167.41 (C=O amide), 172.01 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3297, 3069, 2932, 2854, 1654, 1586, 1542, 1513, 1451, 1409, 1351, 1303, 1248, 1209, 1177, 1111, 1093, 1035, 979, 956, 918, 892,

840, 811, 735, 699, 665, 543. UV/Vis (ethanol, nm): λ_{max} (log ε) = 310 (4.23). MS: *m/z* (ESI, %) 483 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{26}H_{31}N_2O_3S_2$ 483.17706, found 483.17668.

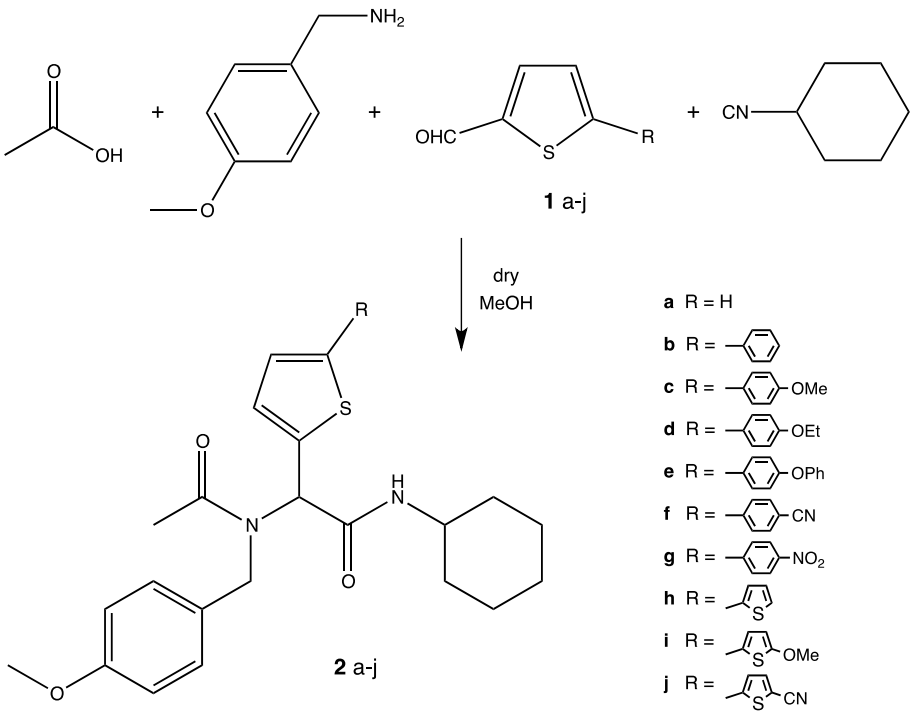
Spectrophotometric titrations and chemosensing studies for thienyl amino acids 2a-j

Solutions of compounds **2a-j** (1.0×10^{-5} to 1.0×10^{-6} M) and of the ions under study (1.0×10^{-1} to 1.0×10^{-3} M) were prepared in UV-grade acetonitrile (in the form of hydrated tetrafluorborate salts for Cu^+, Ag^+, Pd^{2+} and Co^{2+}, hydrated perchlorate salts for K^+, Cd^{2+}, Ca^{2+}, Fe^{3+}, Fe^{2+}, Cr^{3+}, Cu^{2+}, Ni^{2+}, Cs^+, Na^+, Hg^{2+}, Pb^{2+}, Zn^{2+} and hydrated tetrabutylammonium salts for CH_3COO^-, F^-, I^-, ClO_4^-, CN^-, NO_3^-, BzO^-, Cl^-, Br^- and OH^-). Titration of the compounds with the several ions was performed by the sequential addition of ion to the compound solution, in a 10 mm path length quartz cuvette and emission spectra were measured by excitation at the wavelength of maximum absorption for each compound, indicated in Table 2, with a 2 nm slit. The linearity of the absorption *versus* concentration was checked within the used concentration. The binding stoichiometry of the thienyl amino acids with the ions was determined by Job's plots. The association constants were obtained with HypSpec program.

Results and Discussion

Synthesis

New non-canonical amino acids bearing thiophene and bithiopene units with substituents of different electronic character as side chains were obtained by an Ugi reaction. This reaction is an isocyanide-based four-component reaction proposed in 1959 by Ivar Ugi as an alternative to the classical methods for amino acid synthesis, by reacting an acid, an amine, an isocyanide and a carbonyl compound. Following the original application of the Ugi reaction, it can be used for the synthesis of α-amino acids (if an aldehyde is used as the carbonyl component) and α,α-dialkylamino acids (if a ketone is used as the carbonyl component) (Dömling 2006; Costa et al. 2003). In this work, acetic acid, 4-methoxybenzylamine and cyclohexyl isocyanide were used, along with a series of thiophene and bithiophene aldehydes **1a-j** bearing different substituents. The protected amino acids **2a-j** were prepared in fair to moderate yields (15-60%) (Scheme 1, Table 1). These new compounds were fully characterised by the usual spectroscopic techniques.



Scheme 1. Synthesis of (bi)thienyl amino acid derivatives **2a-j**.

Table 1. Yields, UV-visible absorption and fluorescence data for amino acids **2a-j** in absolute ethanol.

Cpd.	Yield (%)	UV/Vis absorption		Fluorescence			
		λ_{abs}	log ε	λ_{em}	Stokes' shift (cm^{-1})	Stokes' shift (nm)	Φ_F
2a	33	276	3.60	303	3627	27	0.005
2b	60	286	4.01	353	6636	67	0.089
2c	25	298	3.55	362	5933	64	0.293
2d	26	299	3.54	362	5821	63	0.284
2e	21	299	4.25	358	5512	59	0.221
2f	27	317	4.22	386	5639	69	0.039
2g	34	349	4.21	511	9084	162	0.002
2h	41	310	3.49	375	5591	65	0.420
2i	41	327	4.31	402	5705	75	0.037
2j	15	336	4.21	415	5666	79	0.124

The acid, amine and isocyanide components were chosen considering previous work that ensures straightforward removal of the groups at the *N*- and *C*-terminal by acidolysis to afford the free non canonical amino acids for subsequent use in peptide synthesis (Costa et al. 2003; Castro et al. 2016).

Photophysical study of (bi)thienyl amino acid derivatives 2a-j

The electron donor or acceptor character of the substituents envisaged the modulation of the photophysical and the recognition properties of the resulting compounds. Therefore, the absorption and emission spectra of (bi)thienyl amino acids derivatives **2a-j** were measured in absolute ethanol (10^{-6}-10^{-5} M solution) (Table 1). The nature of the substituent had a clear influence on the absorption and emission bands of compounds **2a-j** (Figures 1 and 2).

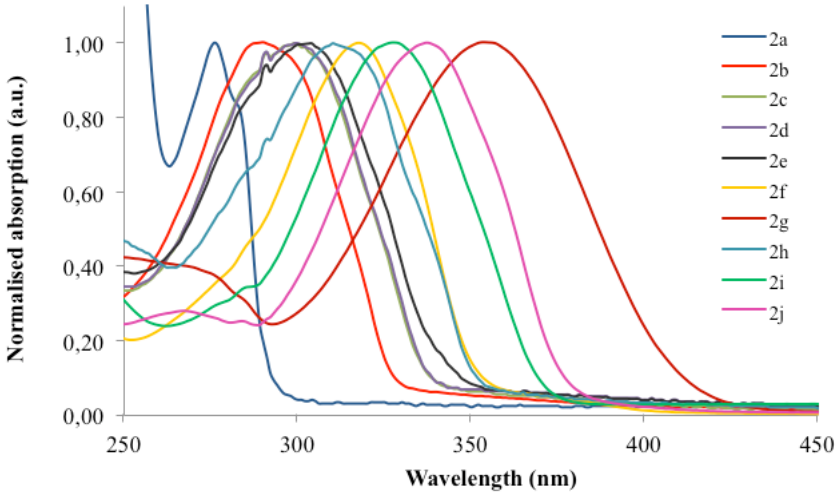


Figure 1. Normalised UV-visible absorption spectra of (bi)thienyl amino acids **2a-j** in ACN at T = 298 K.

By comparison to compound **2a**, as the parent compound, the presence of a phenyl ring bearing and electron donor group (as in **2c**-**e**) lead to an expected bathochromic shift (*ca*. 20 nm) of the maximum wavelength of absorption (λ_{abs}). The bathochromic shift was more pronounced when electron acceptor groups were present: a 41 nm shift with the cyano group (for **2f**) and a 73 nm shift with the nitro group (for **2g**). The same trend was seen in the fluorescence spectra, with larger bathochromic shifts (especially for the nitro derivative with a 208 nm shift).

Comparison of the electronic absorption and emission spectra of compound **2b** (R = phenyl) with compound **2h** (R = thiophene), compound **2c** (R = methoxyphenyl), with **2i** (R = methoxythiophene), as well as comparison of derivative **2f** (R = cyanophenyl), with **2j** (R = cyanothiophene) revealed that the substitution of an aryl group by a thiophene caused a red shift of the maximum absorption

(between 19-29 nm) and emission (between 22-40 nm) wavelengths. This observation clearly indicates that the incorporation of thiophene units enhances the charge-transfer properties of the overall system and the optical data obtained can be largely explained by the bathochromic effect of sulphur and also the increase of the π-overlap between the thiophene units.

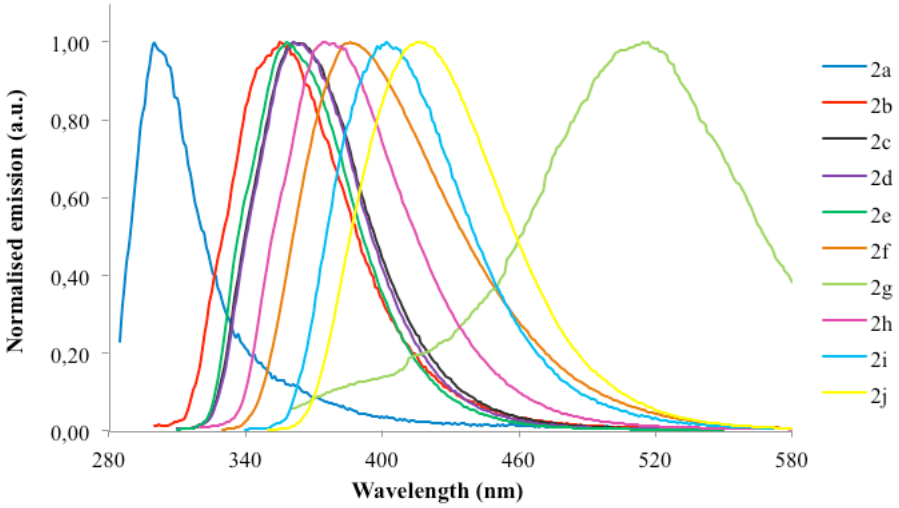


Figure 2. Normalised fluorescence spectra of (bi)thienyl amino acids **2a-j** in ACN at T = 298 K ($\lambda_{exc} = \lambda_{abs}$ for each compound).

The synthesized compounds showed moderate to large Stokes' shifts (the lowest being 3009 cm^{-1} for **2a** and the highest 9084 cm^{-1} for **2g**). A large Stokes' shift is an interesting characteristic for a fluorescent probe, when using fluorescence based techniques, that allows an improved separation of the light inherent to the matrix and the light dispersed by the sample (Holler et al. 2002).

The relative fluorescence quantum yields of the ethanolic solutions of compounds **2a-j** were determined using a 10^{-6} M solution of 9,10-diphenylanthracene in ethanol as standard (Φ_F = 0.95) (Morris et al. 1976). It was found that the thienyl amino acids **2c-e** (bearing donor groups) and bithienyl amino acids **2h**,**j** were the most emissive ($0.124 \leq \phi_F \leq 0.420$). The most fluorescent derivative was compound **2h** (bithiophene) and the presence of the nitro group resulted in an expected fluorescence quenching, with compound **2g** being practically non-emissive in ethanol.

For the subsequent chemosensing study towards different ions, the absorption and emission spectra of (bi)thienyl amino acids **2a-j** were also measured in acetonitrile (10^{-6}-10^{-5} M solution) and its mixture with water (9:1) (Table 2). It was found that the presence of water did not influence the fluorescence quantum yields but the character of the solvent did, as the quantum yield was lower in ethanol (a protic solvent) when compared to acetonitrile (an aprotic solvent).

Table 2. UV-visible absorption and fluorescence data for amino acids **2a-j** in ACN, ACN/H_2O (9:1) and EtOH//H_2O (9:1).

Cpd.	Solvent	UV/Vis		Fluorescence			
		λ_{max}	log ε	λ_{em}	Stokes' shift (cm^{-1})	Stokes' shift (nm)	Φ_F
2a	ACN	276	3.48	301	3009	25	0.005
	ACN/H_2O (9:1)	276	3.61	301	3009	25	0.004
	EtOH	276	3.60	303	3627	27	0.005
	EtOH/H_2O (9:1)	276	3.48	304	3337	28	0.005
2b	ACN	290	3.99	358	6550	68	0.099
	ACN/H_2O (9:1)	290	3.99	357	6472	67	0.094
	EtOH	286	4.01	353	6636	67	0.089
	EtOH/H_2O (9:1)	286	3.97	354	6716	68	0.088
2c	ACN	298	3.53	362	5933	64	0.306
	ACN/H_2O (9:1)	299	3.56	363	5897	64	0.285
	EtOH	299	3.55	362	5821	63	0.293
	EtOH/H_2O (9:1)	299	3.59	362	5821	63	0.273
2d	ACN	299	3.48	362	5821	63	0.288
	ACN/H_2O (9:1)	299	3.49	363	5897	64	0.290
	EtOH	300	3.54	362	5709	62	0.284
	EtOH/H_2O (9:1)	299	3.55	364	5972	65	0.294
2e	ACN	298	4.26	358	5624	60	0.250
	ACN/H_2O (9:1)	299	4.27	358	5512	59	0.221
	EtOH	299	4.25	357	5434	58	0.221
	EtOH/H_2O (9:1)	299	4.24	358	5512	59	0.219
2f	ACN	318	4.22	386	5540	68	0.043
	ACN/H_2O (9:1)	318	4.21	389	5740	71	0.042
	EtOH	317	4.22	386	5639	69	0.039

	EtOH/H_2O (9:1)	318	4.20	387	5607	69	0.038
2g	ACN	353	4.20	511	8759	158	0.006
	ACN/H_2O (9:1)	353	4.21	511	8759	158	0.004
	EtOH	349	4.21	511	9084	162	0.002
	EtOH/H_2O (9:1)	352	4.18	511	8840	159	0.002
2h	ACN	310	3.51	375	5591	65	0.420
	ACN/H_2O (9:1)	309	3.47	375	5696	64	0.402
	EtOH	310	3.49	375	5591	65	0.397
	EtOH/H_2O (9:1)	310	3.51	375	5591	65	0.374
2i	ACN	327	4.31	403	5767	76	0.043
	ACN/H_2O (9:1)	328	4.31	405	5796	77	0.042
	EtOH	327	4.31	402	5705	75	0.037
	EtOH/H_2O (9:1)	328	4.31	401	5550	73	0.039
2j	ACN	337	4.20	417	5693	80	0.176
	ACN/H_2O (9:1)	337	4.21	419	5807	82	0.175
	EtOH	336	4.21	415	5666	79	0.124
	EtOH/H_2O (9:1)	337	4.20	415	5577	78	0.143

Spectrophotometric and spectrofluorimetric titrations with ions

The new (bi)thienyl amino acids **2a-j** were tested for their ability to act as fluorescent chemosensors in the recognition of biomedically relevant ions by performing spectrophotometric and spectrofluorimetric titrations in ACN and ACN/H_2O (9:1), in the presence of relevant organic and inorganic anions (AcO^-, F^-, Cl^-, Br^-, I^-, ClO_4^-, CN^-, NO_3^-, BzO^-, OH^-, $H_2PO_4^-$ and HSO_4^-) and of alkaline, alkaline-earth and transition metal cations (Na^+, K^+, Cs^+, Ag^+, Cu^+, Cu^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Pd^{2+}, Ni^{2+}, Hg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+} and Cr^{3+}). As stated previously, the introduction of a UV-active and fluorescent heterocyclic unit at the side chain of the amino acid is expected to provide additional binding sites for a variety of ions.

A preliminary evaluation of the chemosensing ability was performed by addition of 100 equiv of each cation/anion to solutions of amino acids **2a-j** in acetonitrile and the changes in the intensity of the UV-vis absorption and fluorescence spectra were recorded.

In the UV-vis absorption spectra of the various amino acids in the presence of each tested ion, no changes were seen in the bands corresponding to the maximum wavelength of absorption, except for methoxybithienyl amino acid **2i** in the presence of Cu^{2+}. It was found that this amino acid is a very sensitive and selective colorimetric chemosensor for Cu^{2+} as it displayed a marked colour change from pale yellow to pink. Among all the other cations tested, only Cu^{+} induced a minor pink coloration (Figure 3, top) that was negligible compared to that of Cu^{2+}. The spectrophotometric titration with Cu^{2+} revealed that, upon addition of increasing amounts of the cation, the band at 327 nm decreased, accompanied by the appearance and increase of a new red-shifted band at 529 nm (Figure 3, bottom).

The same preliminary test was carried out in order to assess the changes (band shift and/or intensity) in the fluorescence spectra of the various amino acids in the presence of each tested ion. The nitro derivative **2g** was not tested since it was practically non-fluorescent. This test revealed the ability of compounds **2a-f,h-j** to interact especially with the more basic anions F^{-} and OH^{-} and with Cu^{2+} and Fe^{3+}, with different sensitivity (the amount of ion necessary to induce changes in the fluorescence spectra depending on the compound). The sensing ability for anions was lower (requiring more equivalents for a significant fluorescence quenching, *ca.* 80-90%) than for cations (which required less equivalents for a complete quenching).

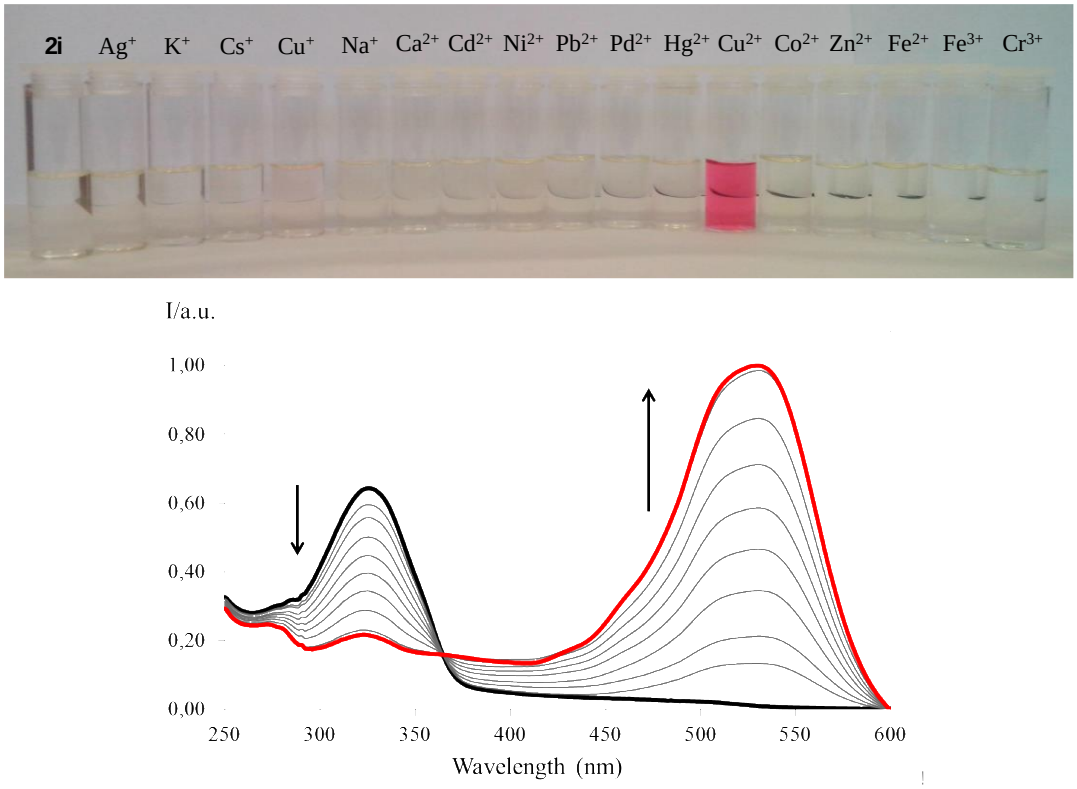


Figure 3. (top) Colour changes of bithienyl amino acid **2i** in acetonitrile (1.0×10^{-4} mol dm^{-3}) in the presence of 10 equiv of the various metal cations; (bottom) Spectrophotometric titration with Cu^{2+} (up to 5 equiv) in acetonitrile.

In the case of methoxybithienyl amino acid **2i**, chosen as representative example, in the spectrofluorimetric titrations with F^- and OH^-, upon addition of the anion it was visible the appearance and increase of a new band at 484 nm suggesting the formation of the deprotonated form of the amino acid due to the basicity of the anions. In the spectrofluorimetric titrations with Cu^{2+} and Fe^{3+}, a considerable decrease of the fluorescence intensity was observed for (bi)thienyl amino acids **2a-f**,**h-j**, with a small number of metal equivalents being necessary to completely quench fluorescence (Figure 4 for the titration of **2i** with F^-, OH^-, Cu^{2+} and Fe^{3+}). Also, for some amino acids the addition of much larger amounts (more than 100 equiv) of Hg^{2+} (**2h**) and Pd^{2+} (**2b-f** and **2h**) induced considerable but incomplete quenching.

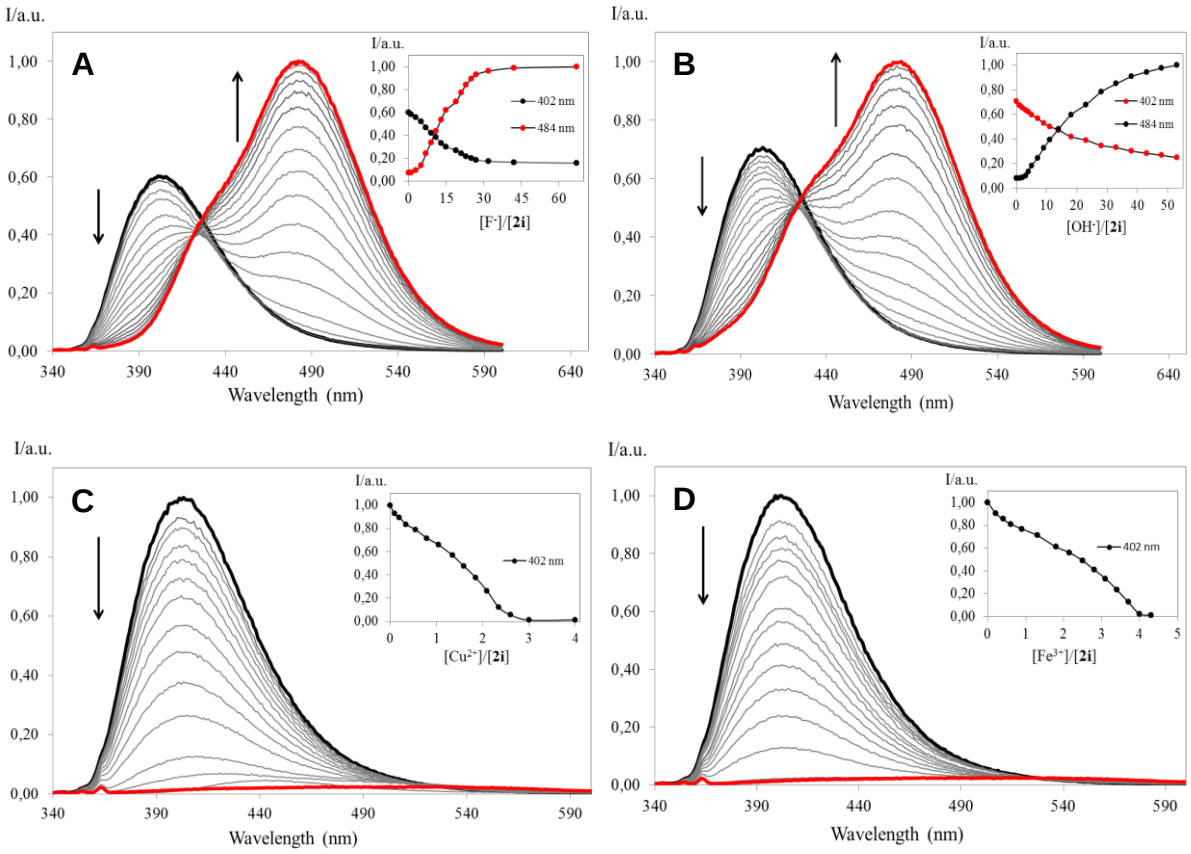


Figure 4. Fluorimetric titrations of bithienyl amino acid **2i** with F^- (A), OH^- (B), Cu^{2+} (C) and Fe^{3+} (D), in acetonitrile [λ_{exc} = 327 nm]. Inset: normalised emission at 402 nm and 484 nm, as a function of added ion equivalents.

Association constants (K_{ass}) between several amino acids and some selected ions were calculated from the spectrofluorimetric titration data with HypSpec program. The results suggested the formation of a ligand-metal(anion) complex with 2:1 stoichiometry (which was confirmed with Job's plots) and it was found that the new amino acids bind preferentially to Fe^{3+} and Cu^{2+} (Table 3 for anions and table 4 for cations). Although it cannot be stated that the new amino acids are selective for any cation, they display higher sensitivity for iron and copper as seen by the larger association constants. Moreover, the K_{ass} obtained for the bithienyl amino acids **2i** and **2j** are higher than the corresponding thienyl amino acids **2c** (bearing a methoxy group) and **2f** (bearing a cyano group), showing the effect of the additional sulphur donor atom on the coordination ability. Previous studies on other heterocyclic amino acids have shown that free carboxylic and amino terminals did not influence significantly the coordination process, which should preferably occur through the heteroatoms at the side chain of the amino acids (Esteves et al. 2010).

Table 3. Logarithm of association constants (log K_{ass}) for the interaction of (bi)thienyl amino acids **2c-f,h-j** with several anions in acetonitrile (ligand:anion stoichiometry 2:1).

Cpd \ Anion	CN^-	F^-	OH^-
2c	---	8.04 ± 0.03	10.39 ± 0.05
2d	---	8.02 ± 0.04	10.28 ± 0.07
2e	---	8.55 ± 0.04	---
2f	11.47 ± 0.07	11.05 ± 0.06	11.09 ± 0.06
2h	8.64 ± 0.04	8.48 ± 0.02	10.27 ± 0.05
2i	8.61 ± 0.03	8.78 ± 0.02	7.1 ± 0.2
2j	12.06 ± 0.04	12.44 ± 0.05	12.04 ± 0.06

Table 4. Logarithm of association constants (log K_{ass}) for the interaction of (bi)thienyl amino acids **2b-f,h-j** with several cations in acetonitrile (ligand:metal stoichiometry 2:1).

Cpd \ Cation	Cu^{2+}	Fe^{3+}	Hg^{2+}	Pd^{2+}
2b	7.232 ± 0.006	7.233 ± 0.007	---	7.247 ± 0.005
2c	12.29 ± 0.31	11.33 ± 0.29	---	11.09 ± 0.07
2d	11.24 ± 0.23	11.35 ± 0.24	---	11.07 ± 0.08
2e	12.54 ± 0.04	13.38 ± 0.05	---	12.15 ± 0.06
2f	12.80 ± 0.07	12.00 ± 0.06	---	---
2h	11.42 ± 0.20	11.40 ± 0.19	11.07 ± 0.08	11.11 ± 0.05
2i	13.33 ± 0.10	13.80 ± 0.15	11.97 ± 0.07	12.20 ± 0.07
2j	13.21 ± 0.05	13.32 ± 0.05	---	12.79 ± 0.06

Conclusions

New heterocyclic amino acids **2** containing thiophene and bithiophene units as side chain were synthesised and evaluated as fluorescent chemosensors based on an amino acid core for a series of biomedically relevant ions. From the spectrofluorimetric titrations in acetonitrile, it was found that the (bi)thienyl amino acids were more sensitive towards Fe^{3+} and Cu^{2+}, when compared to the other tested ions, as a very low number of metal equivalents was enough to obtain a complete fluorescence quenching. The results indicated that there is a strong interaction with Fe^{3+} and Cu^{2+} through the donor N, O and S atoms at the side chain of the various amino acids and they can act as fluorimetric chemosensors. Interestingly, the methoxybithienyl amino acid **2i** was found to be a very sensitive and selective colorimetric chemosensor for Cu^{2+} as it displayed a marked colour change from pale yellow to pink.

Due to their emissive properties and their recognition ability, these heterocyclic amino acids could find application as fluorescent building blocks for the preparation of peptides with chemosensory ability. Further studies will be undertaken in order to clarify aspects which are important for the pratical biomedical application of the synthesized compounds, such as the behaviour of these probes in the complex mixture of compounds in biological systems and their performance in the presence of ions and probes at physiological concentrations.

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Supplementary material

1. Synthetic details and characterization for compounds **2b**-**g**,**i**-**j**

***N*-Cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)-2-(5-phenylthiophen-2-yl)acetamide 2b.** Starting from 2-(4'-formylphenyl)thiophene **1b** (0.132 g, 7.03 × 10^{-4} mol), 4-methoxybenzylamine (0.09 mL, 7.03 × 10^{-4} mol), acetic acid (0.04 mL, 7.03 × 10^{-4} mol) and cyclohexyl isocyanide (0.09 mL, 7.03 × 10^{-4} mol), compound **2b** was obtained as an orange oil (0.201 g, 4.22 × 10^{-4} mol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.10-1.19 (m, 3H, 3 × *H*-cHex), 1.28-1.38 (m, 2H, 2 × *H*-cHex), 1.56-1.69 (m, 3H, 3 × *H*-cHex), 1.83-1.92 (m, 2H, 2 × *H*-cHex), 2.11 (s, 3H, CH_3CO), 3.75 (s, 4H, OCH_3 and *H1*-cHex), 4.63 (d, *J* 8.0Hz, 2H, NCH_2), 5.89 (s, 1H, α-H), 6.01 (d, *J* 7.6 Hz, 1H, NH), 6.79 (d, *J* 8.8 Hz, 2H, H3'' and H5''), 7.02 (d, *J* 3.6 Hz, 1H, H3), 7.08 (d, *J* 8.6 Hz, 2H, H2'' and H6''), 7.11 (d, *J* 3.6 Hz, 1H, H4), 7.29 (d, *J* 7.2 Hz, 1H, H4'), 7.36 (t, *J* 7.2 Hz, 2H, H3' and H5'), 7.56 (d, *J* 7.2Hz, 2H, H2' and H6'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.37 (*C*H_3CO), 24.68 (*C*-cHex), 24.74 (*C*-cHex), 25.48 (*C*-cHex), 32.69 (*C*-cHex), 32.73 (*C*-cHex), 48.68 (*C1*-cHex), 50.78 (NCH_2), 55.26 (OCH_3), 58.73 (α-CH), 113.97 (C3'' and C5''), 122.30 (C4), 125.79 (C2' and C6'), 127.70 (C2'',C4' and C-6''), 128.90 (C3' and C5'), 129.25 (C1''), 130.42 (C3), 134.02 (C1'), 136.28 (C2), 146.36 (C5), 158.82 (C4''), 167.65 (C=O amide), 172.17 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3301, 3060, 2931, 2854, 1649, 1585, 1544, 1513, 1462, 1450, 1407, 1364, 1350, 1303, 1289, 1247, 1209, 1176, 1110, 1093, 1074, 1033, 978, 956, 917, 892, 813, 757, 701, 665, 543. UV/Vis (ethanol, nm): λ_{max} (log ε) = 286 (4.01). MS: *m/z* (ESI, %) 477 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{28}H_{33}N_2O_3S$ 477.22064, found 477.22014.

***N*-Cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)-2-(5-(4'-methoxyphenyl)thiophen-2-yl)acetamide 2c.** Starting from 2-formyl-5-(4'-methoxyphenyl)thiophene **1c** (0.086 g, 3.95 × 10^{-4} mol), 4-methoxybenzylamine (0.05 mL, 3.95 × 10^{-4} mol), acetic acid (0.02 mL, 3.95 × 10^{-4} mol) and cyclohexyl isocyanide (0.05 mL, 3.95 × 10^{-4} mol), compound **2c** was obtained as an orange oil (0.050 g, 9.87 × 10^{-5} mol, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.07-1.18 (m, 3H, 3 × *H*-cHex), 1.26-1.38 (m, 2H, 2 × *H*-cHex), 1.55-1.70 (m, 3H, 3 × *H*-cHex), 1.82-1.94 (m, 2H, 2x × *H*-cHex), 2.10 (s, 3H, CH_3CO), 3.75 (s, 4H, 4''-OCH_3 and *H1*-cHex), 3.83 (s, 3H, 4'-OCH_3), 4.57-4.68 (m, 2H, NCH_2), 5.87 (s, 1H, α-H), 6.04 (d, *J* 8.0 Hz, 1H, NH), 6.78 (d, *J* 8.4 Hz, 2H, H3'' and H5''), 6.89 (d, *J* 8.8 Hz, 2H, H3' and H5'), 6.99 (s, 2H, H3 and H4), 7.08 (d, *J* 8.4 Hz, 2H, H2'' and H6''), 7.48 (d, *J* 8.8 Hz, 2H, H2' and H6'). ^{13}C

NMR (100.6 MHz, $CDCl_3$): δ = 22.31 (*C*H_3CO), 24.63 (*C*-cHex), 24.69 (*C*-cHex), 25.42 (*C*-cHex), 32.62 (*C*-cHex), 32.66 (*C*-cHex), 48.59 (*C1*-cHex), 50.67 (NCH_2), 55.19 (4''-O*C*H_3), 55.29 (4'-O*C*H_3), 58.69 (α-CH), 113.89 (C3'' and C5''), 114.22 (C3' and C5'), 121.18 (C4), 126.81 (C1'), 127.00 (C2' and C6'), 127.70 (C2'' and C6''), 129.01 (C1''), 130.32 (C3), 135.05 (C2), 146.24 (C5), 158.72 (C4''), 159.35 (C4'), 167.67 (C=O amide), 172.08 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3410, 3297, 3066, 3001, 2932, 2854, 1650, 1632, 1612, 1586, 1544, 1513, 1463, 1409, 1364, 1351, 1287, 1249, 1177, 1111, 1090, 1033, 979, 957, 917, 892, 830, 805. UV/Vis (ethanol, nm): λ_{max} (log ε) = 299 (4.21). MS: *m/z* (ESI, %) 507 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{29}H_{35}N_2O_4S$ 507.23120, found 507.23085.

***N*-Cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)-2-(5-(4'-ethoxyphenyl)thiophen-2-yl)acetamide 2d.** Starting from 2-formyl-5-(4'-ethoxyphenyl)thiophene **1d** (0.187 g, 8.05×10^{-4} mol), 4-methoxybenzylamine (0.11 mL, 8.05×10^{-4} mol), acetic acid (0.05 mL, 8.05×10^{-4} mol) and cyclohexyl isocyanide (0.10 mL, 8.05×10^{-4} mol), compound **2d** was obtained as an orange oil (0.109 g, 2.09×10^{-4} mol, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08-1.17 (m, 3H, 3 × *H*-cHex), 1.26-1.37 (m, 2H, 2 × *H*-cHex), 1.40 (t, *J* 6.8 Hz, 3H, OCH_2CH_3), 1.54-1.67 (m, 3H, 3 × *H*-cHex), 1.81-1.93 (m, 2H, 2 × *H*-cHex), 2.07 (s, 3H, CH_3CO), 3.72 (s, 4H, OCH_3 and *H1*-cHex), 4.02 (q, *J* 6.8 Hz, 2H, OCH_2CH_3), 4.56-4.67 (m, 2H, NCH_2), 5.90 (s, 1H, α-H), 6.18 (d, *J* 2.4 Hz, 1H, NH), 6.79 (d, *J* 8.6 Hz, 2H, H3'' and H5''), 6.86 (d, *J* 8.8 Hz, 2H, H3' and H5'), 6.97 (s, 2H, H3 and H4), 7.06 (d, *J* 8.6 Hz, 2H, H2'' and H6''), 7.44 (d, *J* 8.8Hz, 2H, H2' and H6'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 14.64 (OCH_2CH_3), 22.23 (CH_3CO), 24.55 (*C*-cHex), 24.61 (*C*-cHex), 25.50 (*C*-cHex), 32.50 (*C*-cHex), 32.53 (*C*-cHex), 48.50 (*C1*-cHex), 50.52 (NCH_2), 55.07 (OCH_3), 58.48 (α-CH), 63.39 (OCH_2CH_3), 113.78 (C3'' and C5''), 114.67 (C3' and C5'), 121.03 (C4), 126.86 (C2' and C6'), 127.59 (C2'' and C6''), 129.01 (C1''), 130.19 (C3), 131.86 (C1'), 135.05 (C2), 146.15 (C5), 158.60 (C4' and C4''), 167.62 (C=O amide), 172.02 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3410, 3297, 3062, 2979, 2930, 2855, 1652, 1611, 1586, 1572, 1544, 1513, 1463, 1408, 1364, 1351, 1303, 1287, 1248, 1177, 1116, 1090, 1039, 979, 958, 921, 892, 825, 803. UV/Vis (ethanol, nm): λ_{max} (log ε) = 299 (4.21). MS: *m/z* (ESI, %) 342 (M^+-178, 100), 521 (M^+, 89). HMRS: *m/z* (ESI) calc. for $C_{30}H_{37}N_2O_4S$ 521.24685, found 521.24652.

***N*-Cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)-2-(5-(4'-phenoxyphenyl)thiophen-2-yl)acetamide 2e.** Starting from 2-formyl-5-(4'-phenoxyphenyl)thiophene **1e** (0.188 g, 6.70×10^{-4} mol), 4-methoxybenzylamine (0.09 mL, 6.70×10^{-4} mol), acetic acid (0.04 mL, 6.70×10^{-4} mol) and

cyclohexyl isocyanide (0.08 mL, 6.70 × 10^{-4} mol), compound **2e** was obtained as an orange oil (0.080 g, 1.41 × 10^{-4} mmol, 21%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.10-1.19 (m, 3H, 3 × *H*-cHex), 1.26-1.40 (m, 2H, 2 × *H*-cHex), 1.55-1.70 (m, 3H, 3 × *H*-cHex), 1.83-1.95 (m, 2H, 2 × *H*-cHex), 2.11 (s, 3H, CH_3CO), 3.75 (s, 4H, OCH_3 and *H1*-cHex), 4.63 (d, *J* 4.8Hz, 2H, NCH_2), 5.89 (br s, 1H, α-H), 6.04 (d, *J* 7.8 Hz, 1H, NH), 6.79 (d, *J* 8.7 Hz, 2H, H3''' and H5'''), 7.08 (d, *J* 8.7 Hz, 2H, H2''' and H6'''), 6.98-7.05 (m, 6H, H3, H4, H2'', H3', H5' and H6''), 7.13 (dt, J 6.0 and 0.9 Hz, 1H, H4''), 7.36 (dt, *J* 6.0 and 0.9 Hz, 2H, H3'' and H5''), 7.51 (d, *J* 8.7 Hz, 2H, H2' and H6'). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 22.33 (*C*H_3CO), 24.63 (*C*-cHex), 24.69 (*C*-cHex), 25.42 (*C*-cHex), 32.63 (*C*-cHex), 32.68 (*C*-cHex), 48.61 (*C1*-cHex), 50.68 (NCH_2), 55.20 (OCH_3), 58.63 (α-CH), 113.90 (C3''' and C5'''), 118.98 (C2'' and C6''), 119.02 (C3' and C5'), 121.80 (C4), 123.52 (C4''), 127.17 (C2' and C6'), 127.71 (C2''' and C6'''), 129.14 (C1'''), 129.78 (C3'' and C5''), 130.38 (C3), 135.79 (C2), 140.09 (C1'), 145.76 (C5), 156.79 (C1''), 157.08 (C4'), 158.75 (C4'''), 167.61 (C=O amide), 172.11 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3301, 3060, 2931, 2854, 1649, 1585, 1544, 1513, 1462, 1450, 1407, 1364, 1350, 1303, 1289, 1247, 1209, 1176, 1110, 1093, 1074, 1033, 978, 956, 917, 892, 813, 757, 701, 665, 543. UV/Vis (ethanol, nm): λ_{max} (log ε) = 299 (4.15). MS: *m/z* (ESI, %) 390 (M^+-178, 100), 569 (M^+, 96). HMRS: *m/z* (ESI) calc. for $C_{34}H_{37}N_2O_4S$ 569.24685, found 569.24654.

2-(5-(4'-Cyanophenyl)thiophen-2-yl)-*N*-cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)acetamide 2f. Starting from 2-formyl-5-(4'-cyanophenyl)thiophene **1f** (0.148 g, 6.97 × 10^{-4} mol), 4-methoxybenzylamine (0.09 mL, 6.97 × 10^{-4} mol), acetic acid (0.04 mL, 6.97 × 10^{-4} mol) and cyclohexyl isocyanide (0.09 mL, 6.97 × 10^{-4} mol), compound **2f** was obtained as an orange oil (0.091 g, 1.88 × 10^{-4} mol, 27%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.09-1.16 (m, 3H, 3 × *H*-cHex), 1.24-1.37 (m, 2H, 2 × *H*-cHex), 1.54-1.67 (m, 3H, 3 × *H*-cHex), 1.79-1.92 (m, 2H, 2 × *H*-cHex), 2.11 (s, 3H, CH_3CO), 3.72 (s, 4H, OCH_3 and *H1*-cHex), 4.61 (d, *J* 3.0Hz, 2H, NCH_2), 5.97 (s, 1H, α-H), 6.22 (d, *J* 6.9 Hz, 1H, NH), 6.76 (d, *J* 7.2 Hz, 2H, H3'' and H5''), 7.03 (d, *J* 7.2 Hz, 2H, H2'' and H6''), 7.03 (d, *J* 3.9 Hz, 1H, H3), 7.19 (d, *J* 3.9 Hz, 1H, H4), 7.58-7.63 (m, 4H, H2', H3', H5' and H6'). ^{13}C NMR (75.4 MHz, $CDCl_3$): δ = 22.25 (*C*H_3CO), 24.54 (*C*-cHex), 24.59 (*C*-cHex), 25.30 (*C*-cHex), 32.49 (*C*-cHex), 32.54 (*C*-cHex), 48.61 (*C1*-cHex), 50.49 (NCH_2), 55.13 (OCH_3), 58.06 (α-CH), 110.59 (C4'), 113.85 (C3'' and C5''), 118.63 (CN), 124.13 (C4), 125.81 (C2' and C6'), 127.60 (C2'' and C6''), 128.63 (C1''), 130.51 (C3), 132.58 (C3' and C5'), 138.19 (C1'), 138.68 (C2), 143.62 (C5), 158.73 (C4''), 167.23 (C=O amide), 172.15 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3301, 3061, 2999, 2933, 2855, 2226, 1657, 1604 1586,

1538, 1513, 1463, 1451, 1409, 1364, 1350, 1303, 1289, 1248, 1208, 1177, 1111, 1092, 1034, 979, 958, 941, 918, 892, 838, 814, 736, 701, 665, 543, 514. UV/Vis (ethanol, nm): λ_{max} (log ε) = 317 (4.22). MS: *m/z* (ESI, %) 502 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{29}H_{32}N_3O_3S$ 502.21589, found 502.21535.

***N*-Cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)-2-(5-(4'-nitrophenyl)thiophen-2-yl)acetamide 2g.** Starting from 2-formyl-5-(4'-nitrophenyl)thiophene **1g** (0.186 g, 8.00 × 10^{-4} mol), 4-methoxybenzylamine (0.10 mL, 8.00 × 10^{-4} mol), acetic acid (0.05 mL, 8.00 × 10^{-4} mol) and cyclohexyl isocyanide (0.10 mL, 8.00 × 10^{-4} mol), compound **2g** was obtained as a yellow oil (0.137 g, 2.72 × 10^{-4} mol, 34%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.09-1.16 (m, 3H, 3 × *H*-cHex), 1.27-1.32 (m, 2H, 2 × *H*-cHex), 1.61-1.66 (m, 3H, 3 × *H*-cHex), 1.82-1.93 (m, 2H, 2 × *H*-cHex), 2.10 (s, 3H, CH_3CO), 3.71 (s, 4H, OCH_3 and *H1*-cHex), 4.62 (d, *J* 8.0Hz, 2H, NCH_2), 6.03 (s, 1H, α-H), 6.33 (d, *J* 7.6 Hz, 1H, NH), 6.75 (d, *J* 8.8 Hz, 2H, H3'' and H5''), 7.03 (d, *J* 3.6 Hz, 1H, H3), 7.06 (d, *J* 8.6 Hz, 2H, H2'' and H6''), 7.23 (d, *J* 3.6 Hz, 1H, H4), 7.63 (t, *J* 7.2 Hz, 2H, H2' and H6'), 8.14 (d, *J* 7.2Hz, 2H, H3' and H5'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.22 (CH_3CO), 24.52 (*C*-cHex), 24.57 (*C*-cHex), 25.29 (*C*-cHex), 32.45 (*C*-cHex), 32.52 (*C*-cHex), 48.59 (*C1*-cHex), 50.39 (NCH_2), 55.09 (OCH_3), 57.89 (α-CH), 113.82 (C3'' and C5''), 124.18 (C3' and C5'), 124.68 (C4), 125.70 (C2' and C6'), 127.57 (C2'' and C6''), 128.64 (C1''), 130.52 (C3), 139.43 (C2), 140.09 (C1'), 143.00 (C5), 146.51 (C4'), 158.71 (C4''), 167.14 (C=O amide), 172.11 (CH_3CO). IR (liquid film, cm^{-1}): ν = 3301, 3066, 2931, 2854, 1737, 1648, 1630, 1588, 1543, 1512, 1489, 1463, 1408, 1364, 1350, 1303, 1288, 1243, 1202, 1171, 1108, 1071, 1035, 978, 958, 916, 891, 869, 836, 803, 751, 736, 660, 512, 503. UV/Vis (ethanol, nm): λ_{max} (log ε) = 349 (4.21). MS: *m/z* (ESI, %) 522 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{28}H_{32}N_3O_5S$ 522.20572, found 522.20509.

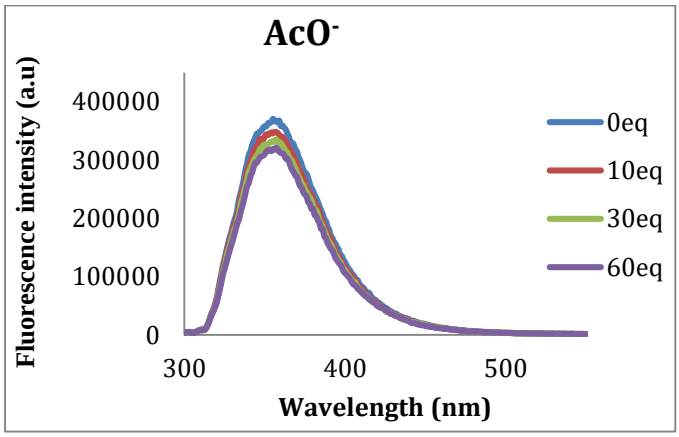
***N*-Cyclohexyl-2-(5'-methoxy-[2,2'-bithiophen]-5-yl)-2-(*N*-(4''-methoxybenzyl)acetamido)acetamide 2i.** Starting from 5-formyl-5'-methoxy-2,2'-bithiophene **1i** (0.142 g, 6.34 × 10^{-4} mol), 4-methoxybenzylamine (0.08 mL, 6.34 × 10^{-4} mol), acetic acid (0.04 mL, 6.34 × 10^{-4} mol) and cyclohexyl isocyanide (0.08 mL, 6.34 × 10^{-4} mol), compound **2i** was obtained as an orange oil (0.131 g, 2.60 × 10^{-4} mol, 41%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.08-1.16 (m, 3H, 3 × *H*-cHex), 1.26-1.32 (m, 2H, 2 × *H*-cHex), 1.53-1.66 (m, 3H, 3 × *H*-cHex), 1.79-1.90 (m, 2H, 2 × *H*-cHex), 2.06 (s, 3H, CH_3CO), 3.73 (s, 4H, 4''-OCH_3 and *H1*-cHex), 3.86 (s, 3H, 4'-OCH_3), 4.54-4.65 (m, 2H,

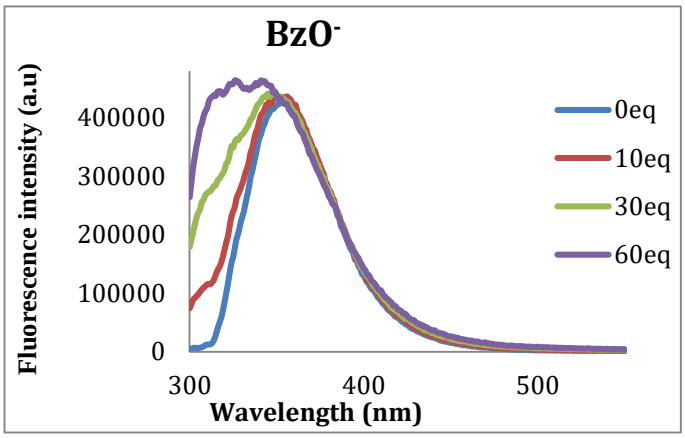
NCH_2), 5.86 (s, 1H, α-H), 6.08 (d, *J* 3.8 Hz, 1H, H4'), 6.21 (br s, 1H, NH), 6.74 (d, *J* 4.0 Hz, 2H, H3 and H4), 6.77 (d, *J* 8.4 Hz, 2H, H3'' and H5''), 6.87 (d, *J* 3.8 Hz, 1H, H3'), 7.05 (d, *J* 8.4 Hz, 2H, H2'' and H6''). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.22 (*C*H_3CO), 24.57 (*C*-cHex), 24.64 (*C*-cHex), 25.36 (*C*-cHex), 32.53 (*C*-cHex), 32.57 (*C*-cHex), 48.53 (*C1*-cHex), 50.58 (NCH_2), 55.12 (4''-O*C*H_3), 58.48 (α-CH), 60.14 (4'-O*C*H_3), 104.32 (C4'), 113.84 (C3'' and C5''), 121.07 (C4), 121.52 (C3), 123.23 (C5'), 127.65 (C2'' and C6''), 128.88 (C1''), 129.88 (C3'), 134.47 (C2), 140.04 (C5), 158.68 (C4''), 165.66 (C2'), 167.49 (C=O amide), 172.00 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3411, 3302, 3068, 3007, 2933, 2854, 1650, 1632, 1586, 1532, 1513, 1498, 1462, 1451, 1408, 1350, 1303, 1289, 1249, 1202, 1176, 1111, 1092, 1052, 1036, 993, 911, 892, 873, 803, 770, 732, 646, 510. UV/Vis (ethanol, nm): λ_{max} (log ε) = 329 (4.20). MS: *m/z* (ESI, %) 334 (M^+-178, 100), 513 (M^+, 49). HMRS: *m/z* (ESI) calc. for $C_{27}H_{33}N_2O_4S_2$ 513.18763, found 513.18750.

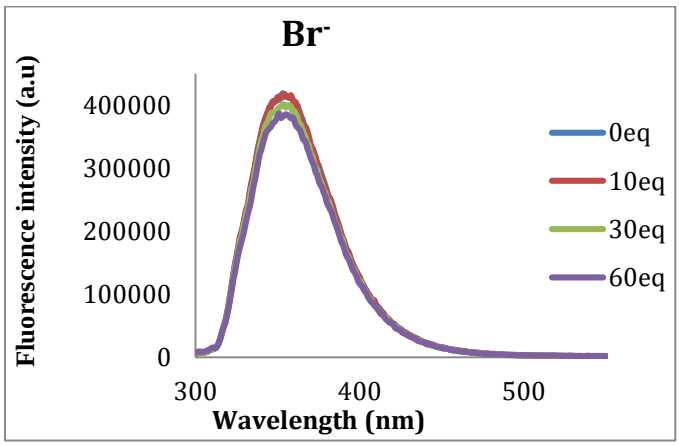
2-(5'-Cyano-[2,2'-bithiophen]-5-yl)-*N*-cyclohexyl-2-(*N*-(4''-methoxybenzyl)acetamido)acetamide

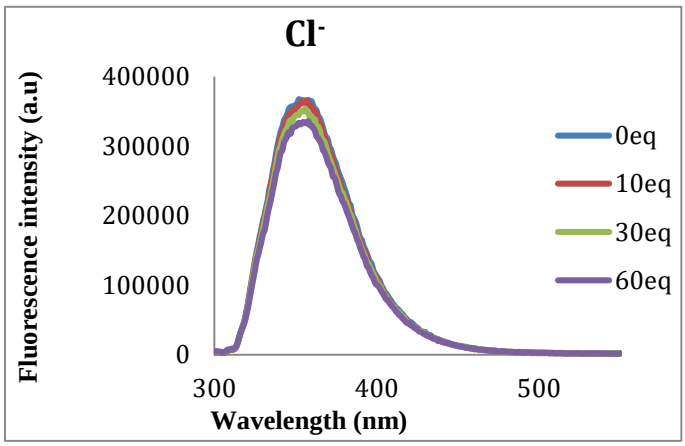
2j. Starting from 5'-cyano-5-formyl-2,2'-bithiophene **1j** (0.175 g, 8.00×10^{-4} mol), 4-methoxybenzylamine (0.10 mL, 8.00×10^{-4} mol), acetic acid (0.05 mL, 8.00×10^{-4} mol) and cyclohexyl isocyanide (0.10 mL, 8.00×10^{-4} mol), compound **2j** was obtained as an orange oil (0.052 g, 1.20×10^{-4} mol, 15%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.10-1.19 (m, 3H, 3 × *H*-cHex), 1.26-1.35 (m, 2H, 2 × *H*-cHex), 1.57-1.70 (m, 3H, 3 × *H*-cHex), 1.83-1.93 (m, 2H, 2 × *H*-cHex), 2.14 (s, 3H, CH_3CO), 3.76 (s, 4H, OCH_3 and *H1*-cHex), 4.60 (s, 2H, NCH_2), 5.93 (s, 1H, α-H), 6.11 (d, *J* 8.0 Hz, 1H, NH), 6.79 (d, *J* 8.4 Hz, 2H, H3'' and H5''), 6.95 (d, *J* 3.8 Hz, 1H, H3), 7.04 (d, *J* 8.4 Hz, 2H, H2'' and H6''), 7.06 (d, *J* 3.8 Hz, 1H, H4), 7.09 (d, *J* 3.8 Hz, 1H, H3'), 7.51 (d, *J* 3.8 Hz, 1H, H4'). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 22.28 (*C*H_3CO), 24.60 (*C*-cHex), 24.65 (*C*-cHex), 25.39 (*C*-cHex), 32.58 (*C*-cHex), 32.65 (*C*-cHex), 48.72 (*C1*-cHex), 50.62 (NCH_2), 55.25 (O*C*H_3), 58.10 (α-CH), 107.61 (C5'), 113.98 (C3'' and C5''), 114.12 (CN), 123.50 (C3'), 124.85 (C4), 127.74 (C2'' and C6''), 128.47 (C1''), 130.33 (C5'), 136.84 (C2'), 138.23 (C4'), 138.48 (C2), 144.29 (C5), 158.92 (C4''), 167.17 (C=O amide), 172.25 (CH_3*C*O). IR (liquid film, cm^{-1}): ν = 3302, 3070, 2998, 2932, 2854, 2217, 1652, 1586, 1532, 1513, 1464, 1451, 1436, 1407, 1363, 1351, 1303, 1292, 1248, 1207, 1177, 1112, 1093, 1035, 979, 963, 918, 805, 736, 709, 512. UV/Vis (ethanol, nm): λ_{max} (log ε) = 337 (4.21). MS: *m/z* (ESI, %) 508 (M^+, 100). HMRS: *m/z* (ESI) calc. for $C_{27}H_{30}N_3O_3S_2$ 508.17231, found 508.17183.

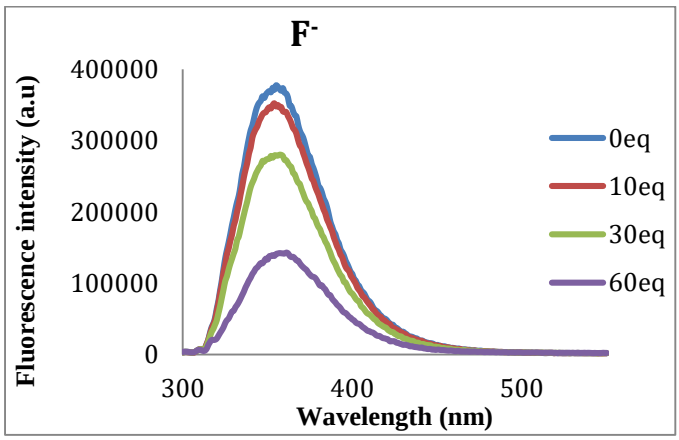
2. Spectrofluorimetric titrations of compound **2b** with all the tested ions (in acetonitrile)

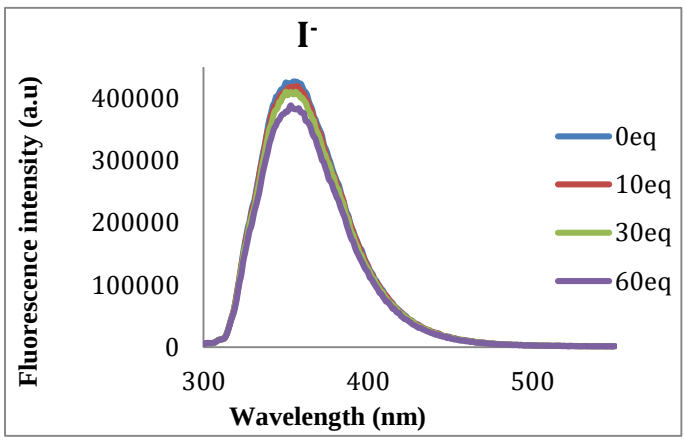


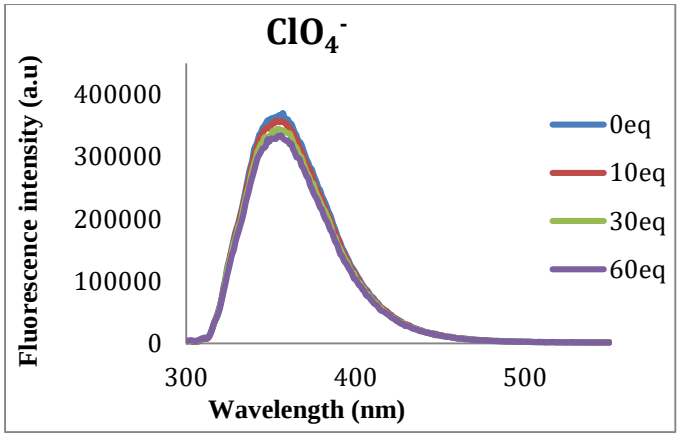








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300
400
500
Wavelength (nm)
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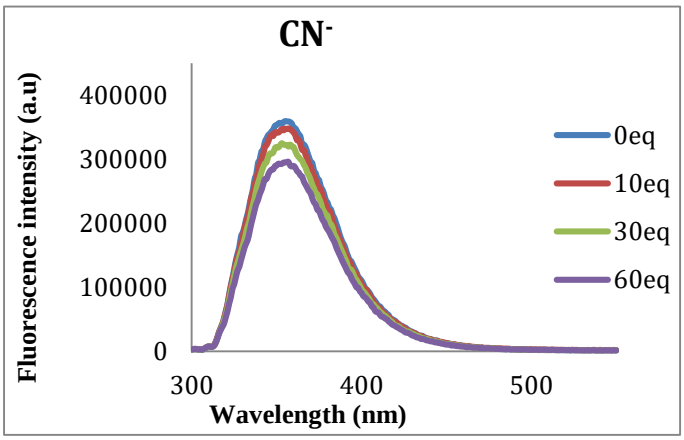
10eq
30eq
60eq


CN^-
Fluorescence intensity (a.u)
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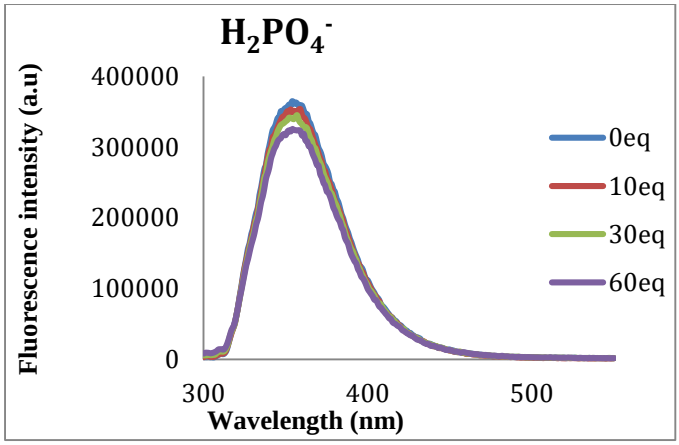
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Wavelength (nm)
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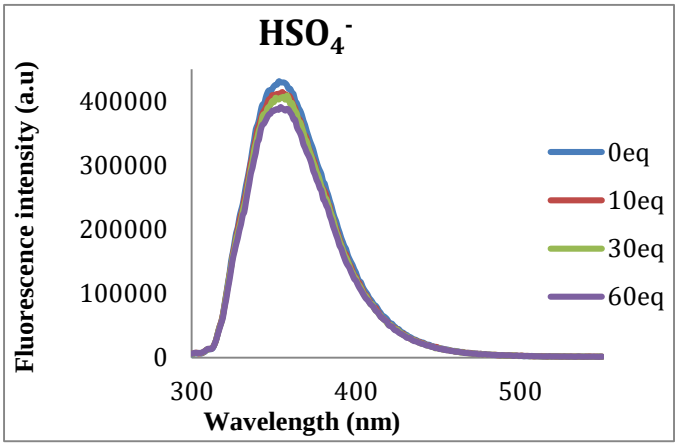
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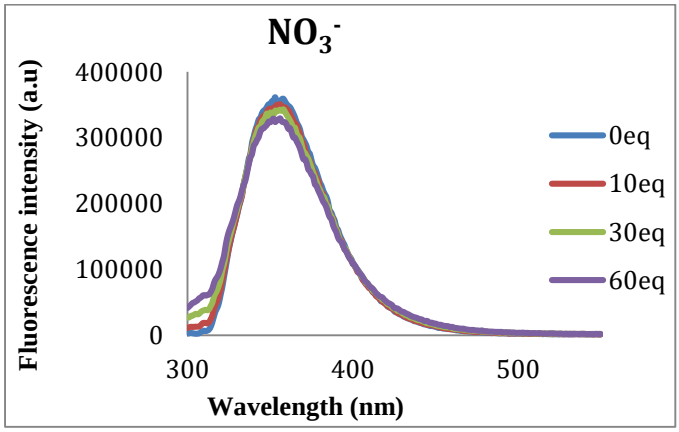
NO_3^-
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Wavelength (nm)
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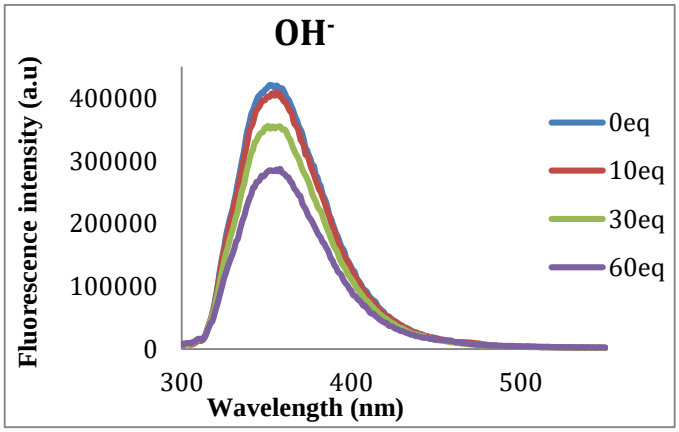
OH^-
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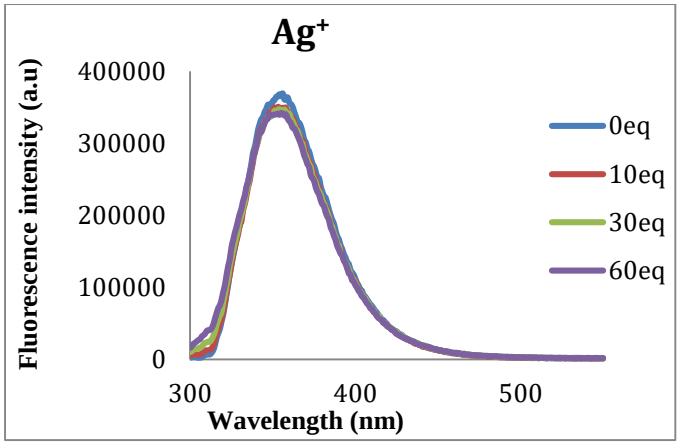
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Wavelength (nm)
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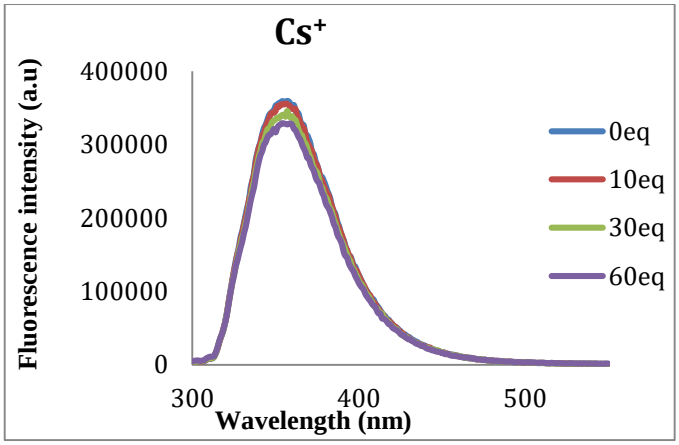
Cs+
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Wavelength (nm)
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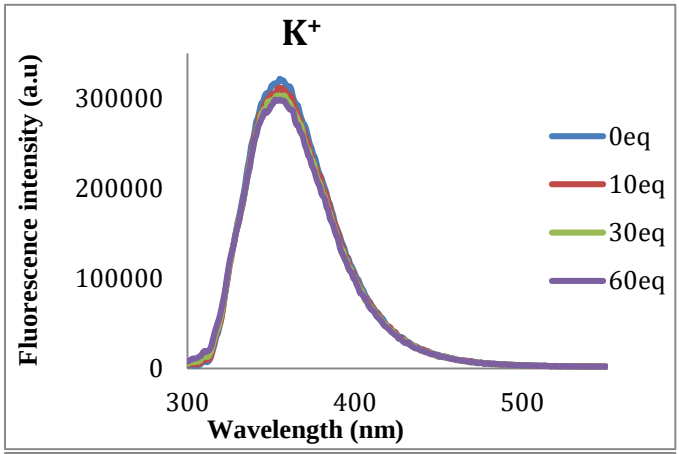
K+
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Wavelength (nm)
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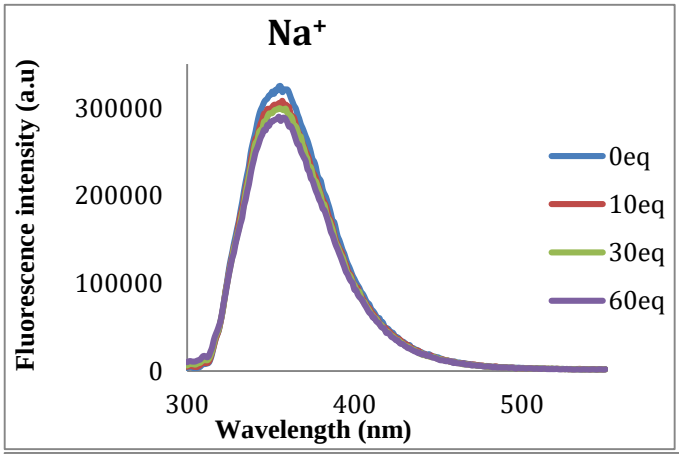
Na+
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Wavelength (nm)
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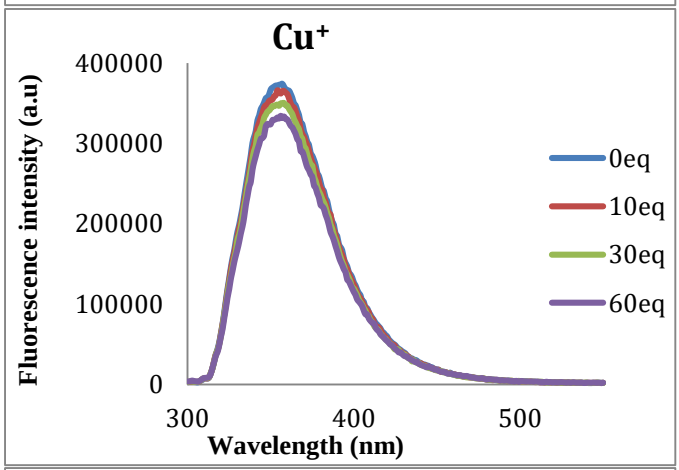
Cu+
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400
500
Wavelength (nm)
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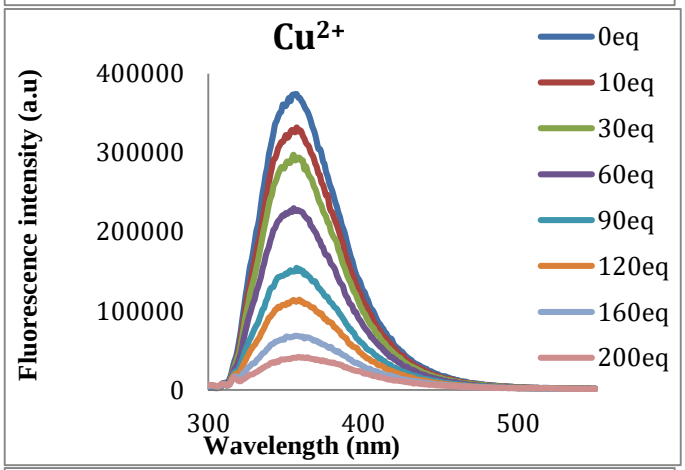
30eq
60eq


Cu2+
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100000
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400
500
Wavelength (nm)
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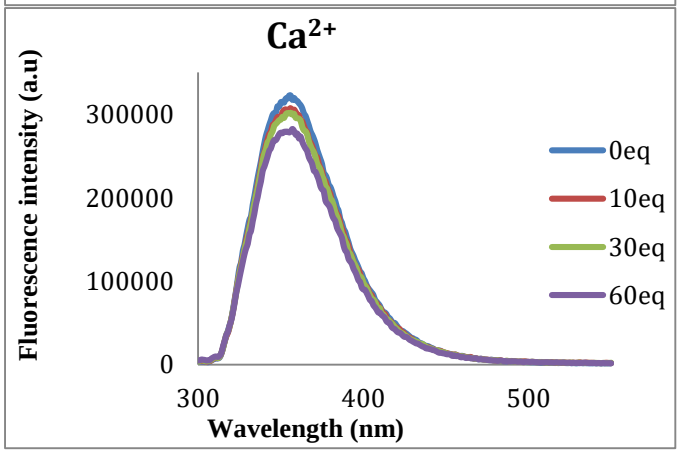
30eq
60eq
90eq
120eq
160eq
200eq


Ca2+
Fluorescence intensity (a.u)
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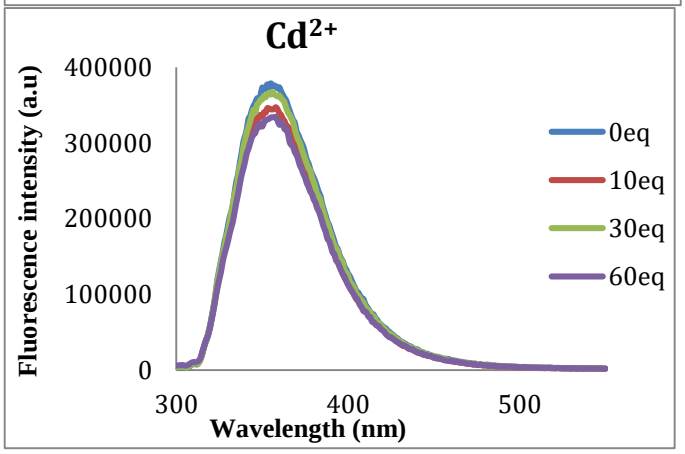
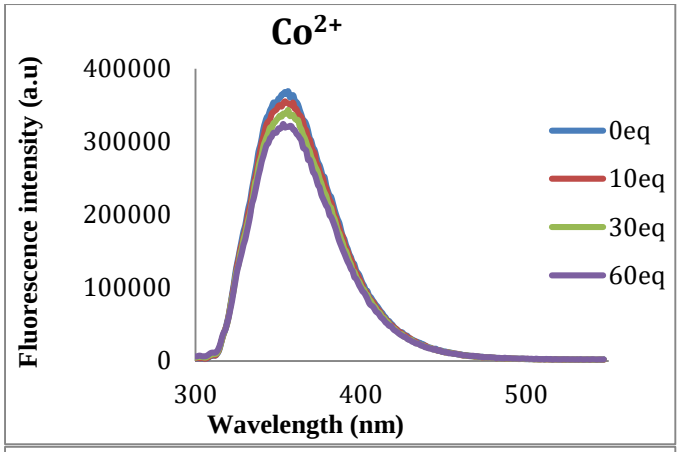
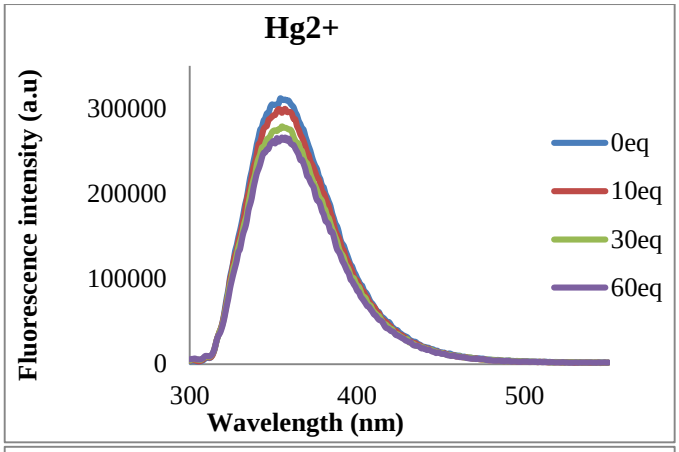
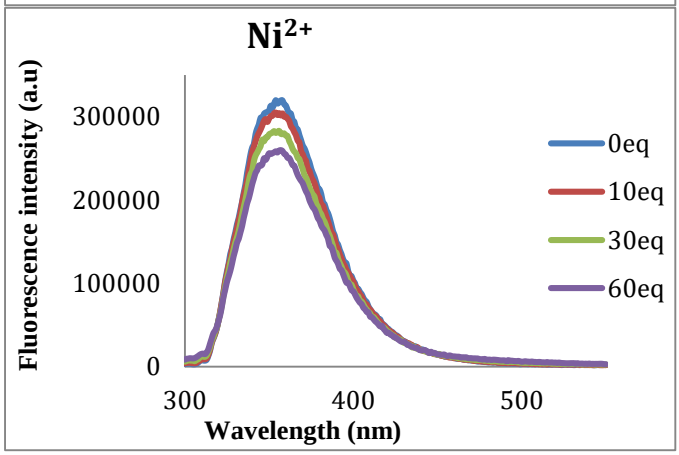
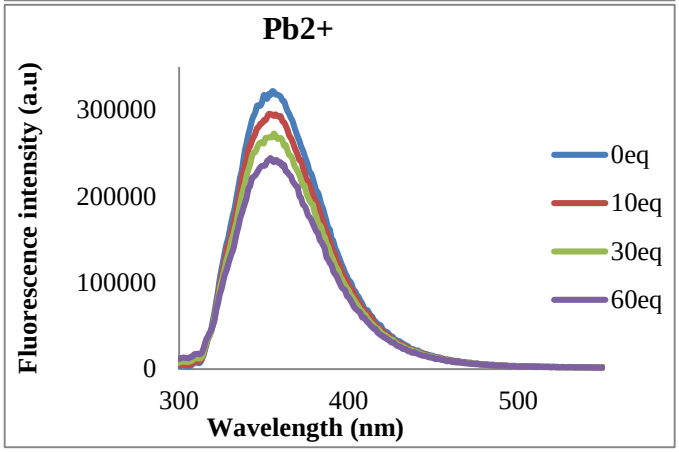
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

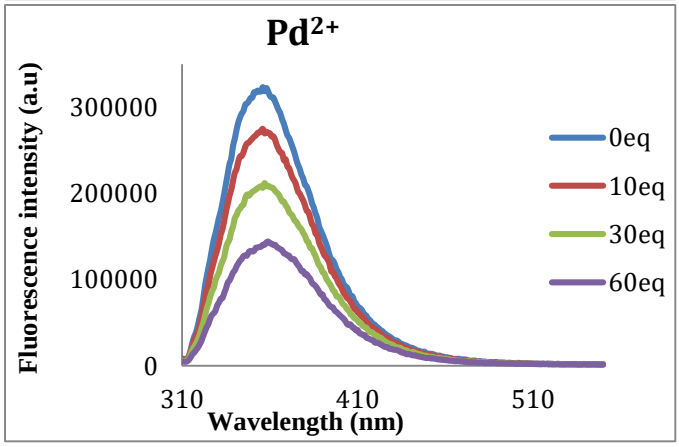

Cd2+
Fluorescence intensity (a.u)
400000
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

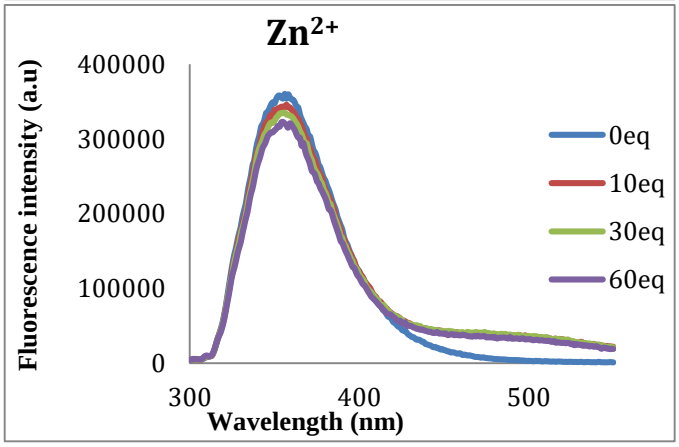

Co2+
Fluorescence intensity (a.u)
400000
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

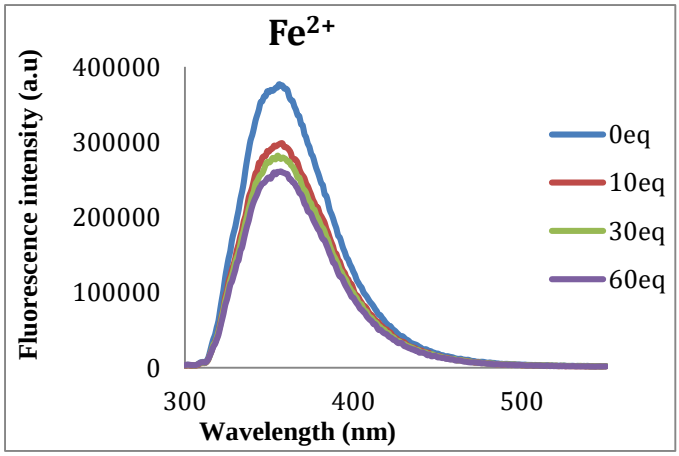

Hg2+
Fluorescence intensity (a.u)
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

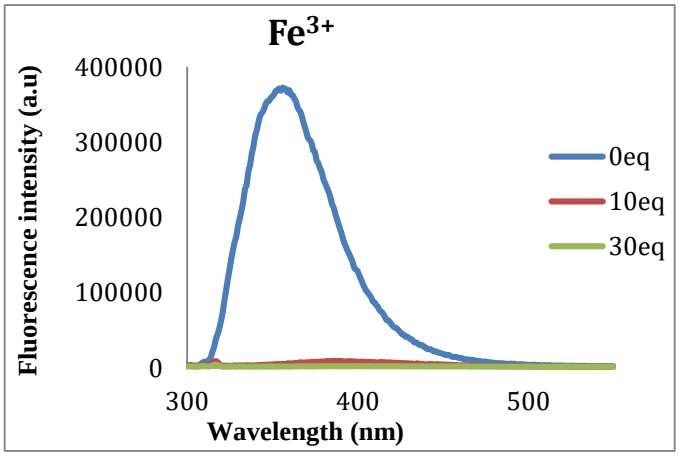

Ni2+
Fluorescence intensity (a.u)
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

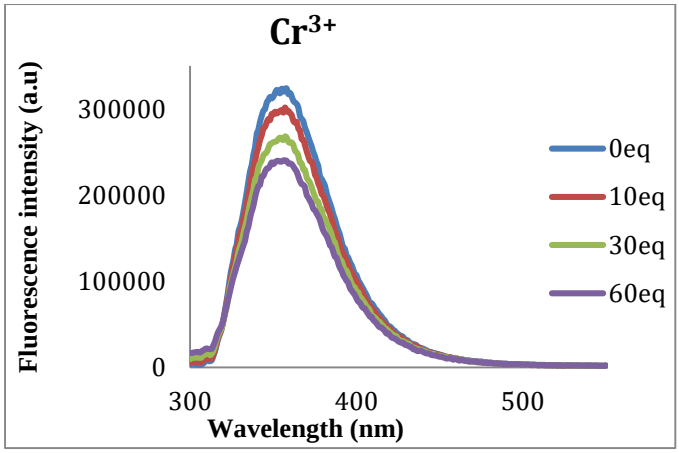

Pb2+
Fluorescence intensity (a.u)
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq


Pd2+
Fluorescence intensity (a.u)
300000
200000
100000
0
310
410
510
Wavelength (nm)
0eq
10eq
30eq
60eq

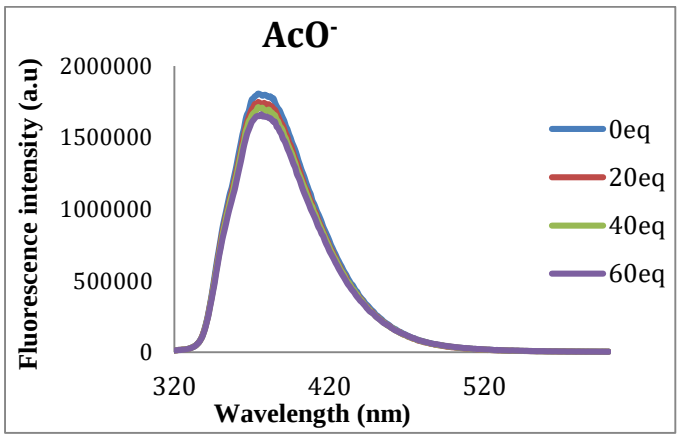

Zn2+
Fluorescence intensity (a.u)
400000
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

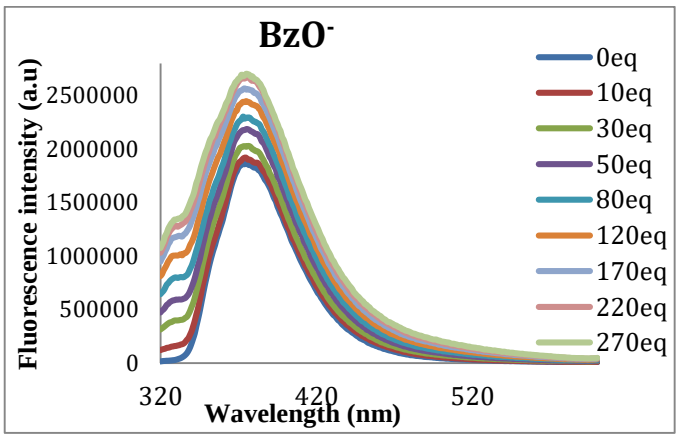

Fe2+
Fluorescence intensity (a.u)
400000
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq
60eq

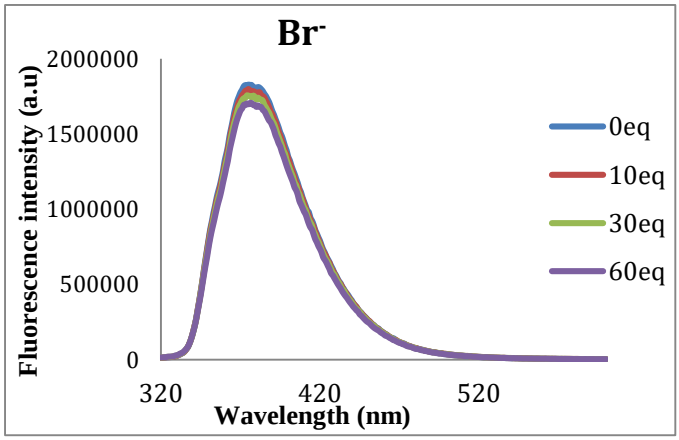

Fe3+
Fluorescence intensity (a.u)
400000
300000
200000
100000
0
300
400
500
Wavelength (nm)
0eq
10eq
30eq

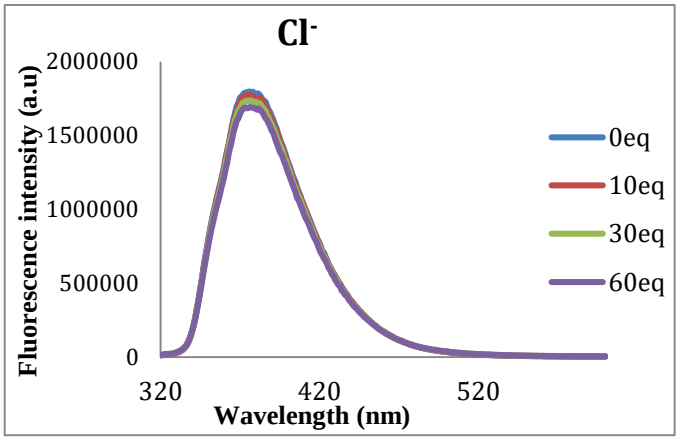


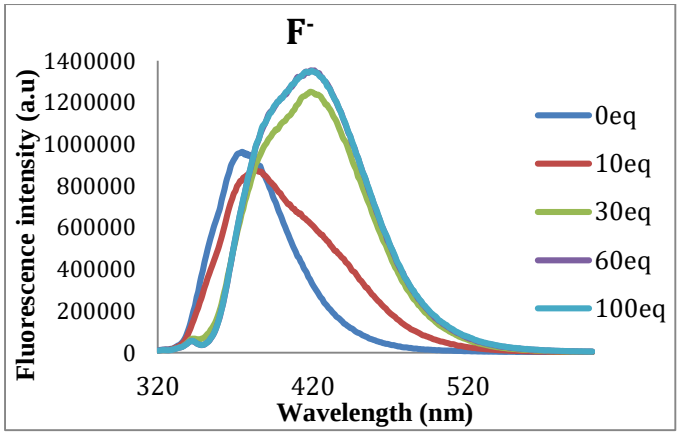
3. Spectrofluorimetric titrations of compound **2h** with all the tested ions (in acetonitrile)

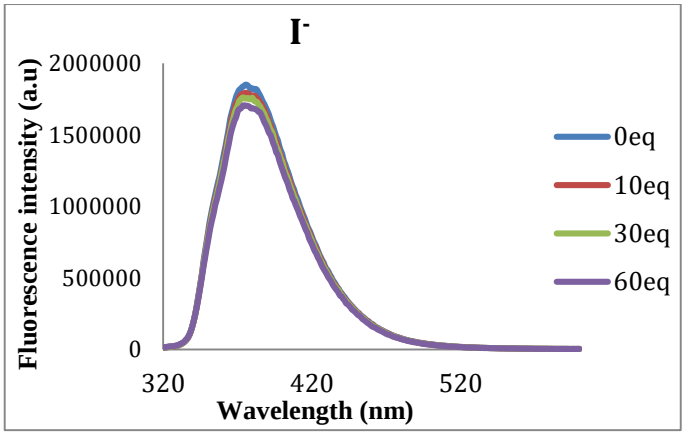


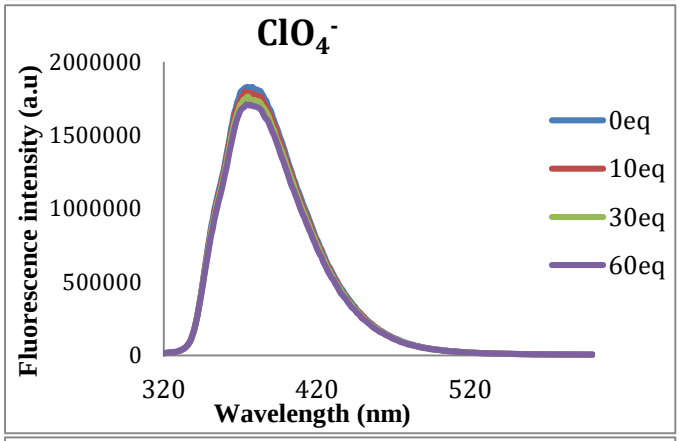


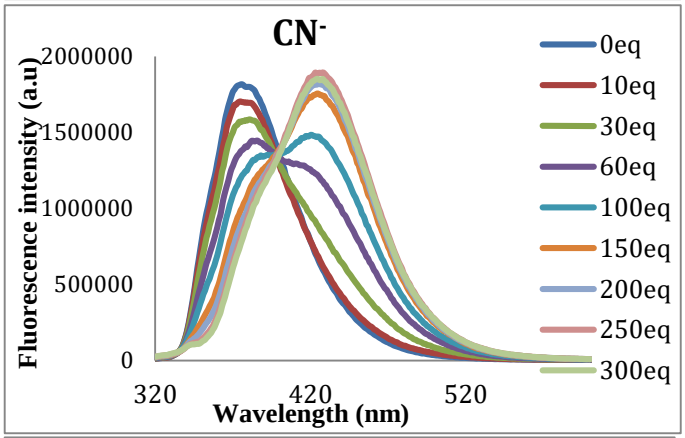


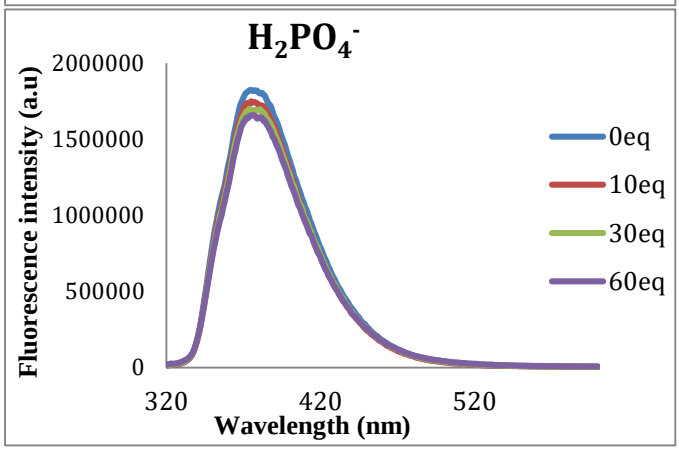


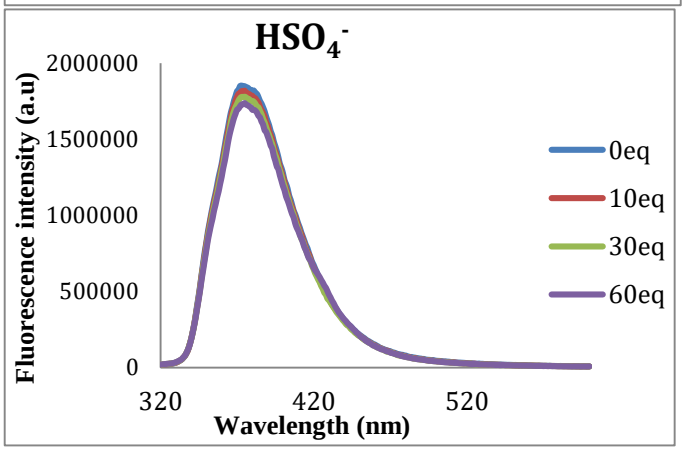

F-
Fluorescence intensity (a.u)
1400000
1200000
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq
100eq

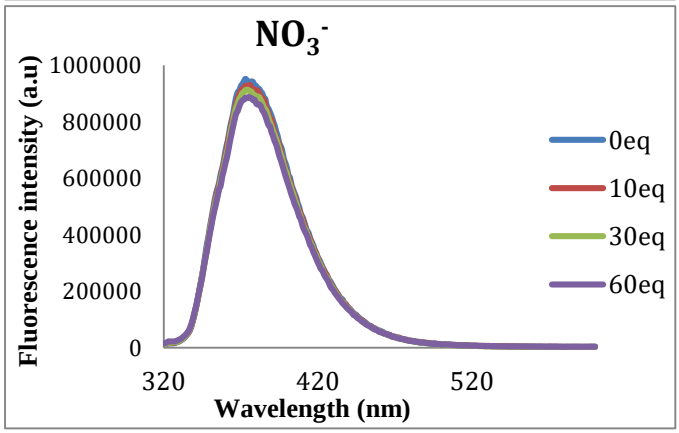

I-
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

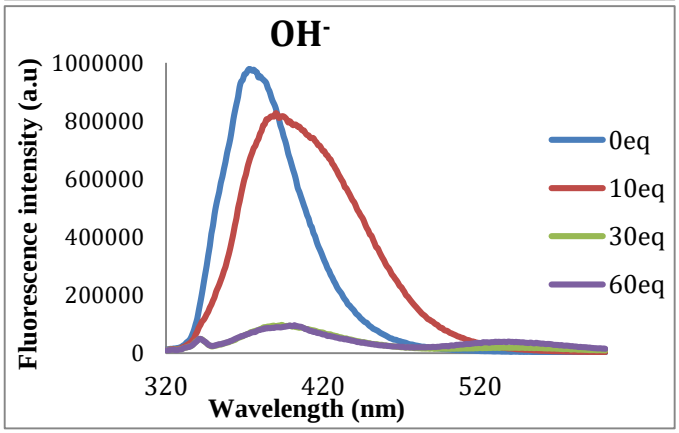

ClO4-
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

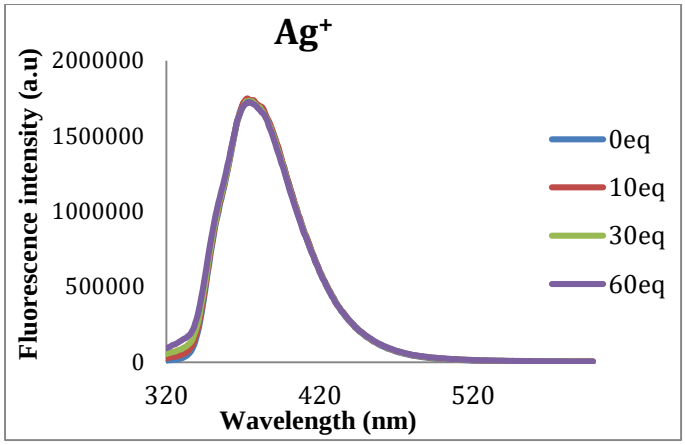

CN-
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq
100eq
150eq
200eq
250eq
300eq

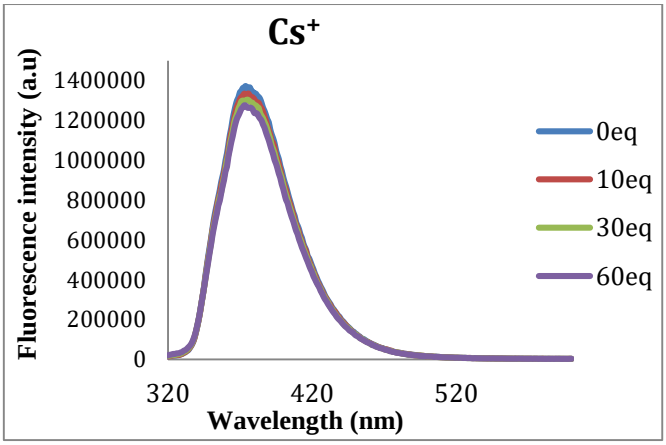

H2PO4-
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

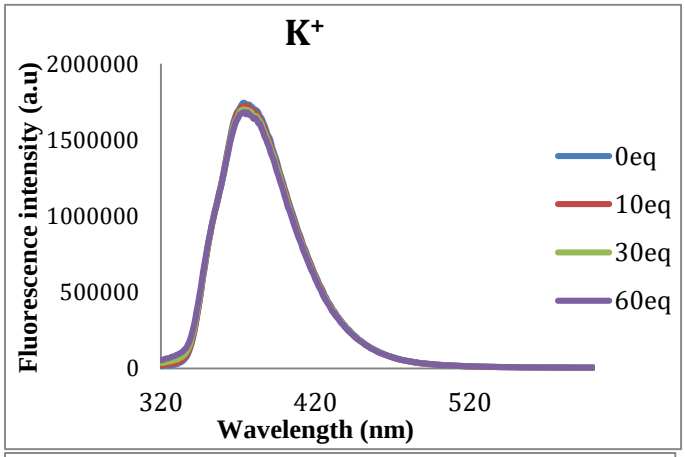

HSO4-
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

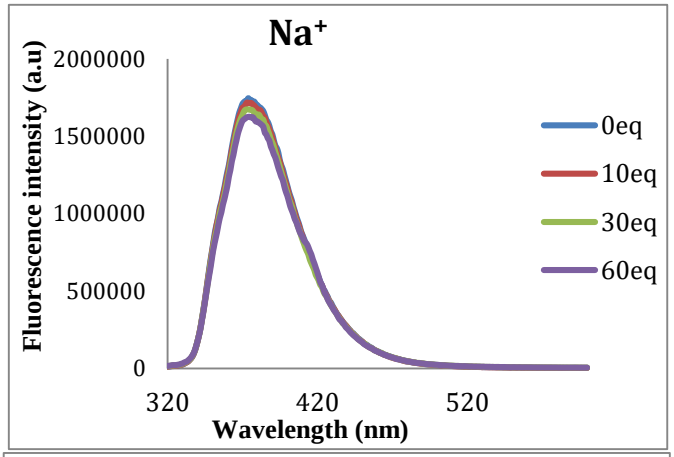

NO3-
Fluorescence intensity (a.u)
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

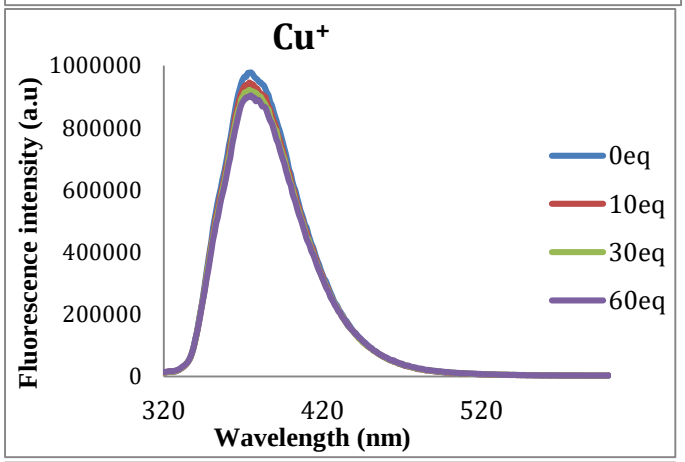

OH-
Fluorescence intensity (a.u)
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

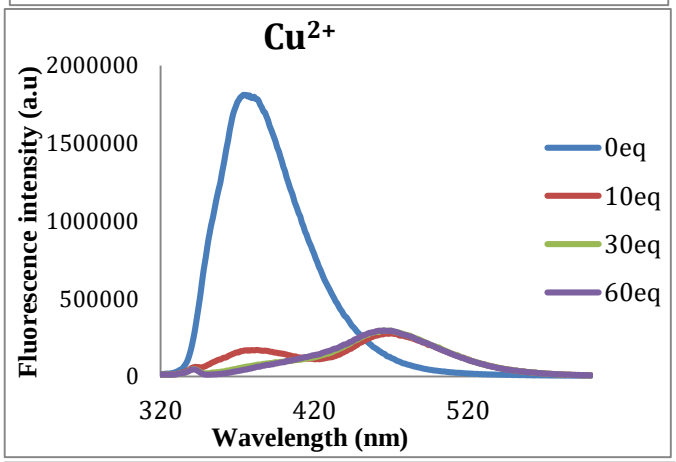

Ag+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

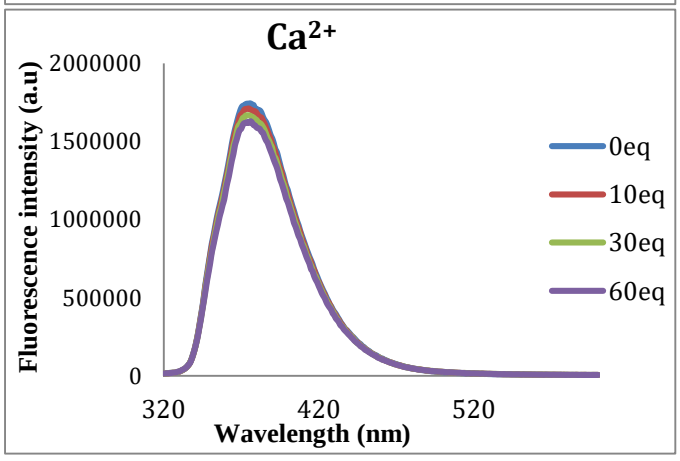

Cs+
Fluorescence intensity (a.u)
1400000
1200000
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

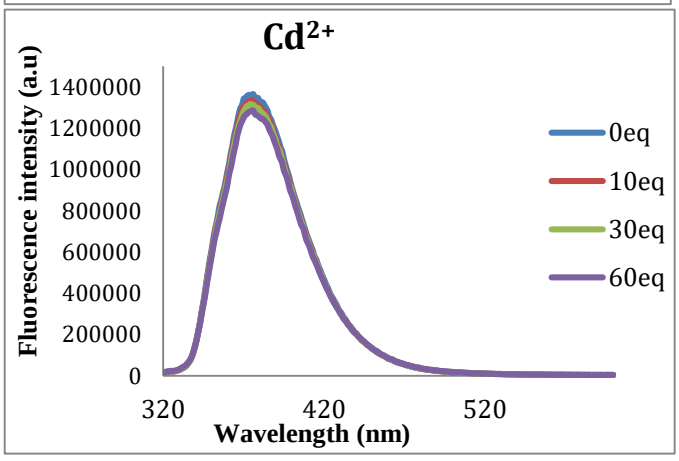

K+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

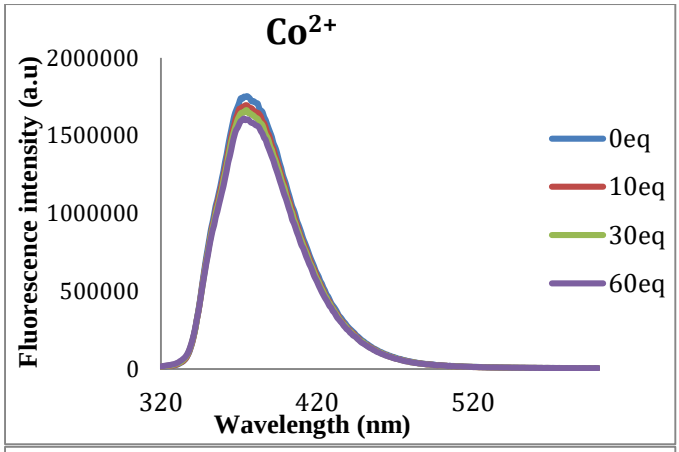

Na+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

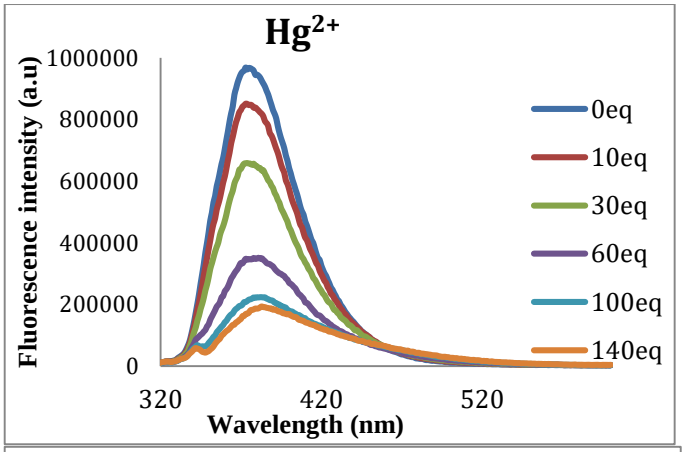

Cu+
Fluorescence intensity (a.u)
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

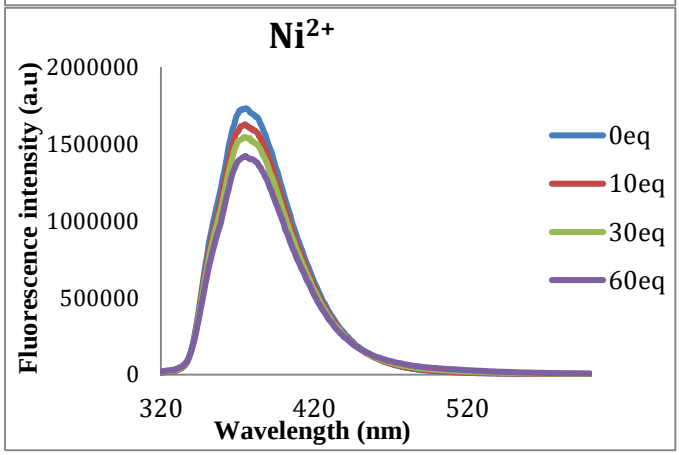

Cu2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

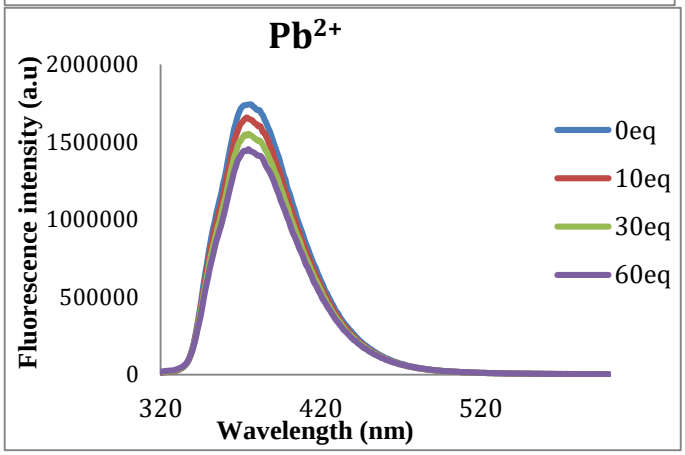

Ca2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

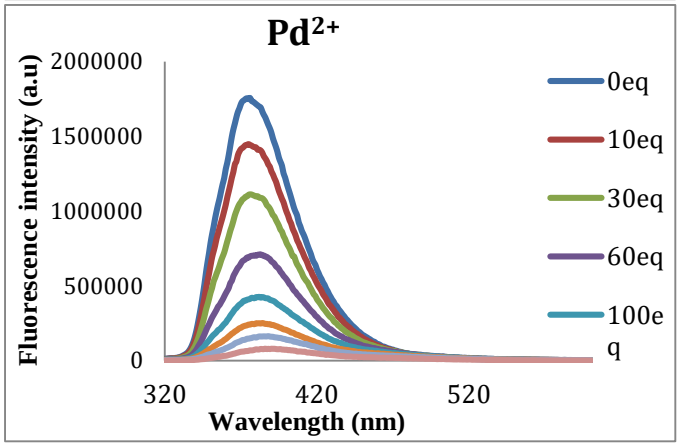

Cd2+
Fluorescence intensity (a.u)
1400000
1200000
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

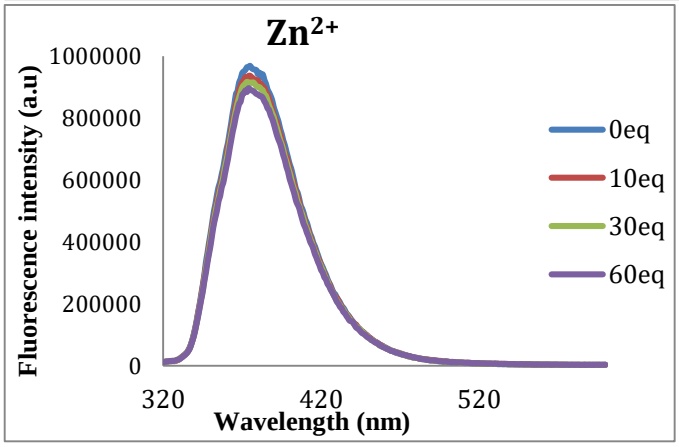

Co2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

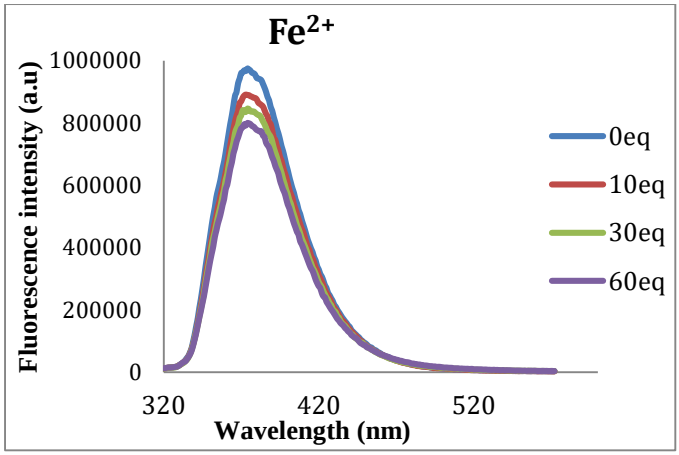

Hg2+
Fluorescence intensity (a.u)
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq
100eq
140eq

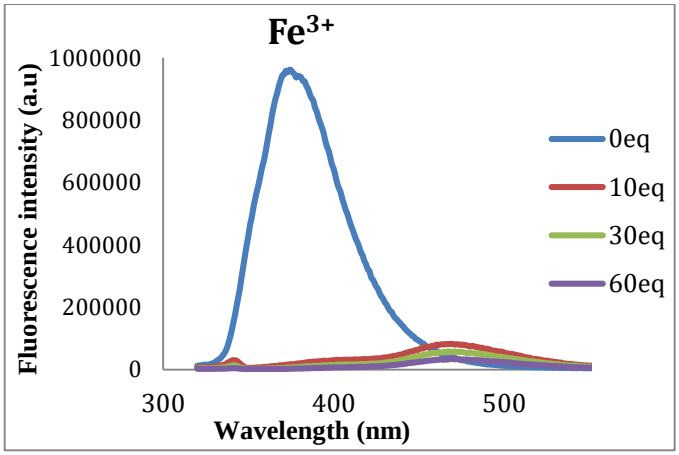

Ni2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

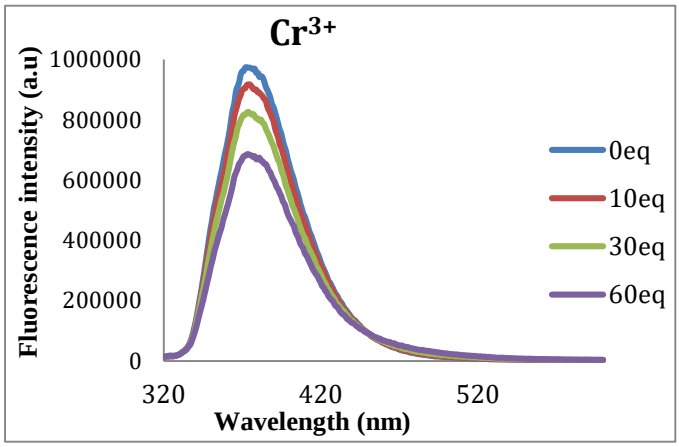

Pb2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq


Pd2+
Fluorescence intensity (a.u)
2000000
1500000
1000000
500000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq
100eq

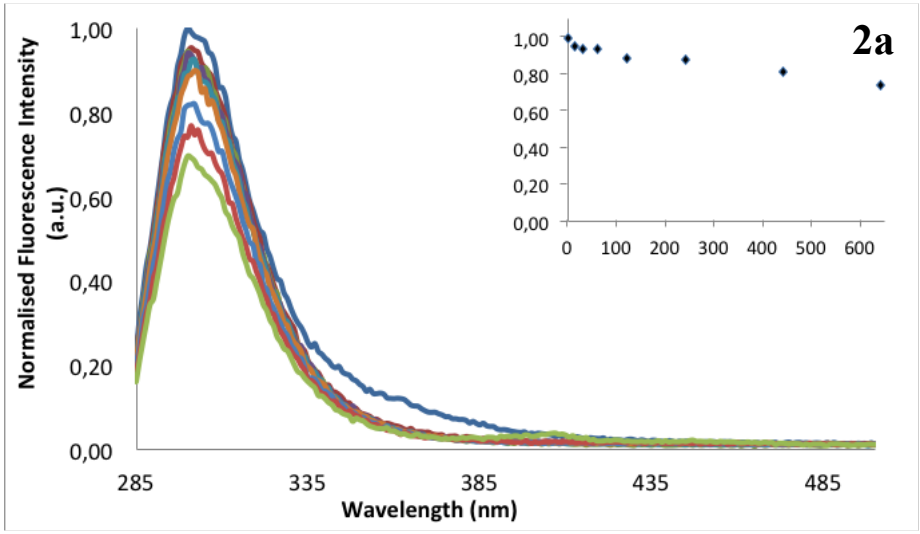

Zn2+
Fluorescence intensity (a.u)
1000000
800000
600000
400000
200000
0
320
420
520
Wavelength (nm)
0eq
10eq
30eq
60eq

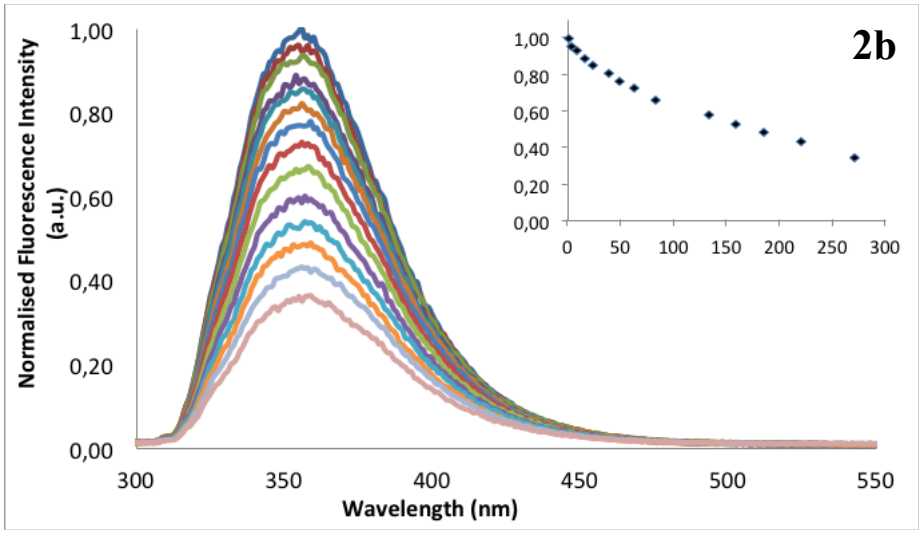


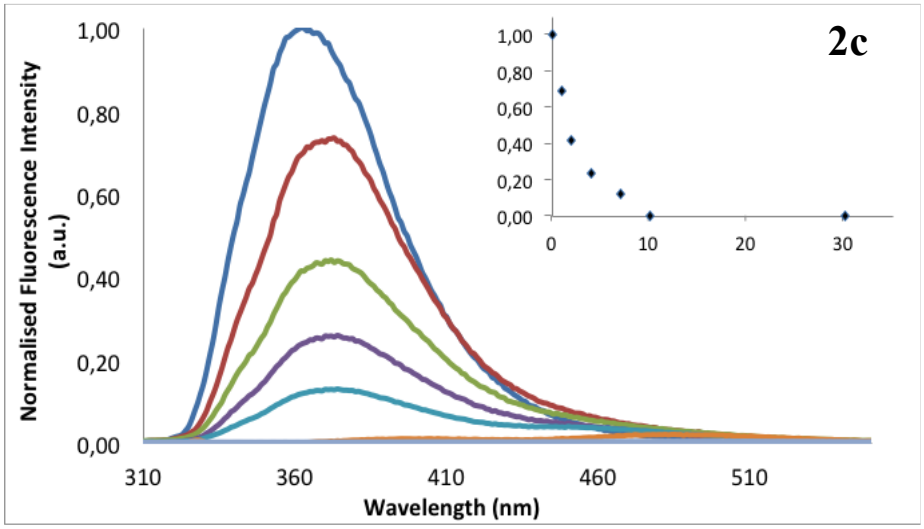


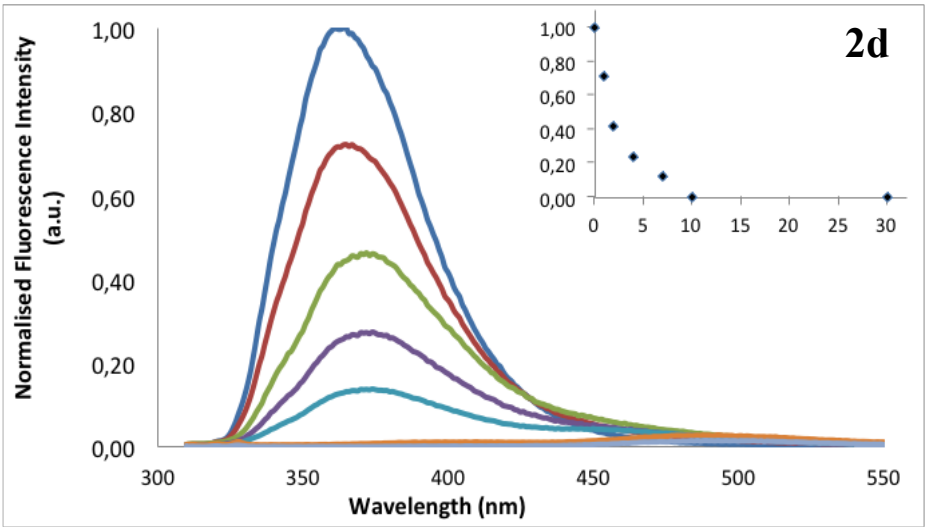


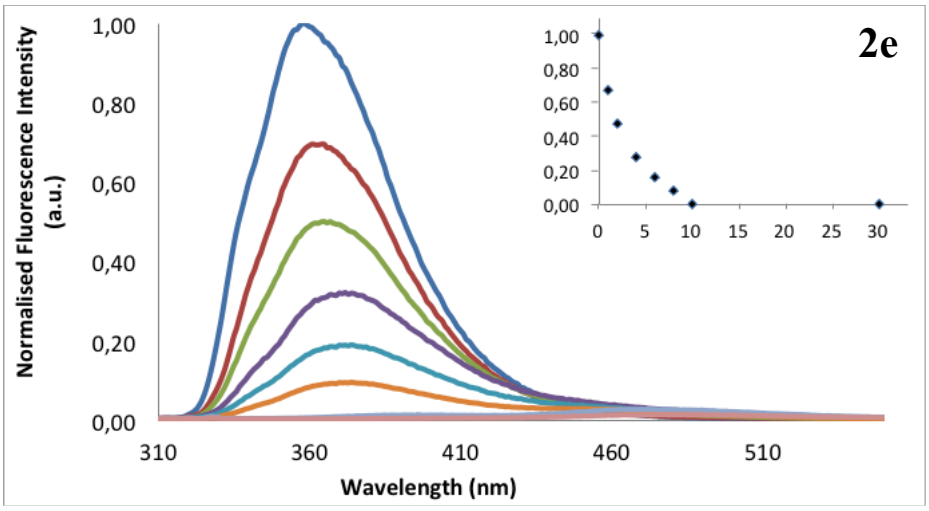
4. Spectrofluorimetric titrations of compounds **2a-j** with Fe^{3+} (in acetonitrile)

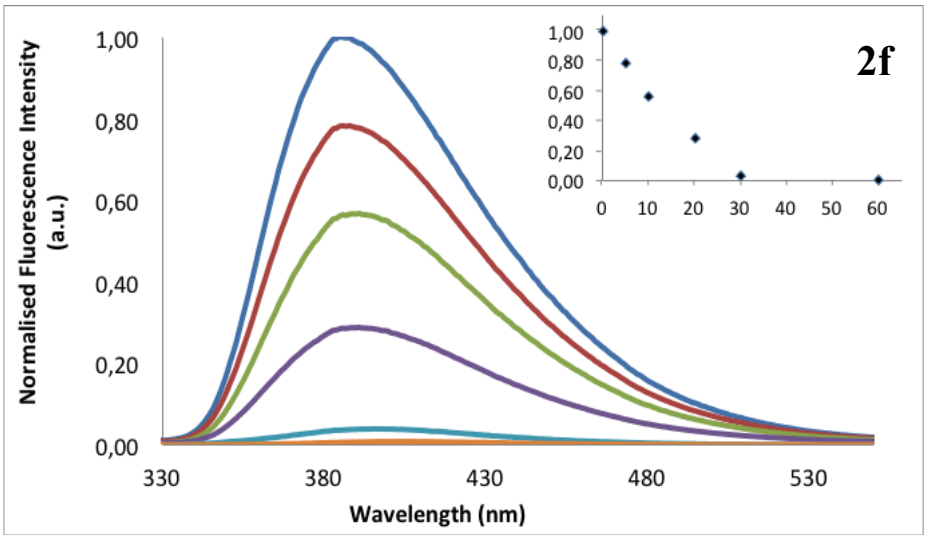


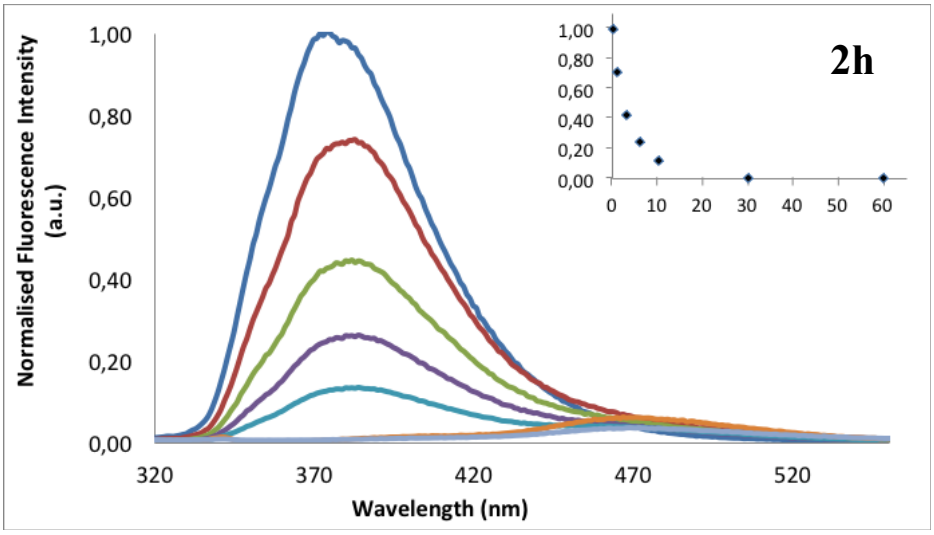

2b
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
300
350
400
450
500
550
Wavelength (nm)
1,00
0,80
0,60
0,40
0,20
0,00
0
50
100
150
200
250
300

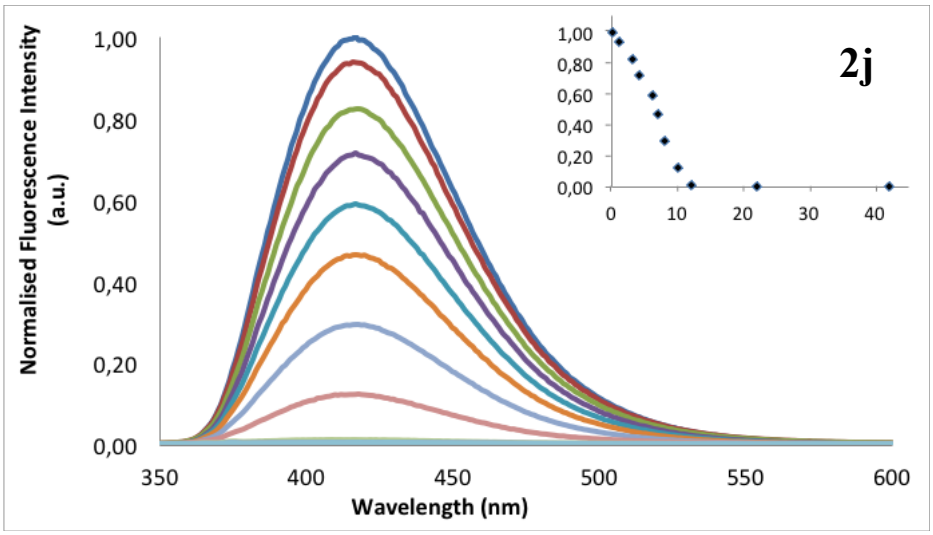

2c
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
310
360
410
460
510
Wavelength (nm)
1,00
0,80
0,60
0,40
0,20
0,00
0
10
20
30


2d
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
300
350
400
450
500
550
Wavelength (nm)
1,00
0,80
0,60
0,40
0,20
0,00
0
5
10
15
20
25
30


2e
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
310
360
410
460
510
Wavelength (nm)
0
5
10
15
20
25
30


2f
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
330
380
430
480
530
Wavelength (nm)
0
10
20
30
40
50
60


2h
Normalised Fluorescence Intensity (a.u.)
1,00
0,80
0,60
0,40
0,20
0,00
320
370
420
470
520
Wavelength (nm)
0
10
20
30
40
50
60



5. Job's plot for compound **2i** with Cu^{2+} (in acetonitrile)