Amino acids based on 2,4,5-triarylimidazoles: synthesis and evaluation as new chemosensors for ion recognition

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Abstract: *N-tert*-butyloxycarbonyl-4-formylphenylalanine methyl ester and appropriate heterocyclic diones were used in the synthesis of novel fluorescent unnatural amino acids, namely 2,4,5-triarylimidazolyl-alanines. These new compounds were fully characterised by the usual spectroscopic techniques, such as IR and NMR. The photophysical properties of the amino acids were evaluated by UV-Vis absorption and fluorescence spectroscopy in solvents of different character. Interaction studies with biologically and analytically important ions such as F^-, OH^-, Cu^{2+} and Fe^{3+}, through spectrophotometric and spectrofluorimetric titrations were carried out to assess their potential as chemosensors.

Keywords*:* 2,4,5-triarylimidazoles; Unnatural amino acids; Chemosensors; Fluorescence.

1. Introduction

2,4,5-Triaryl-imidazoles have found application in medicinal chemistry due to their properties, for example as ligands for Ru(II) and Pt(II) complexes, as probes of DNA structure or new therapeutic agents due to their capacity to bind or interact with DNA.[1] Recently we reported the synthesis of several 2,4,5-tri(heteroaryl)imidazoles with interesting photophysical properties (high fluorescence quantum yields and large Stokes' shifts) which could be used in diverse optical applications (*e.g.* nonlinear optics, fluorimetric chemosensors, OLEDs and DNA intercalators).[2]

Metallic cations can be complexed through N, O and S donor atoms in amino acids, at the main and side chains, and in aromatic heterocycles, whereas anion coordination, based on hydrogen bonding and electrostatic interactions, can arise from amino acid side and main chain OH and NH groups, or from NH groups in heterocycles.[3,4] Therefore, the insertion of suitable heterocyclic systems at the side chain of natural amino acids can add extra functionality to the amino acid.

Fluorescent sensors are preferred because they are well suited to meet the need for in vivo probes, such as mapping the spatial and temporal distribution of the biological analytes, and they have other advantages including multiple modes of detection (such as fluorescence quenching, enhancing, life time), extremely high sensitivity, relatively low cost and easy availability.[5] Our current research interests include the synthesis and characterization of unnatural amino acids,[6] imidazole and benz-X-azole derivatives with interesting optical properties[7] and innovative heterocyclic colorimetric/fluorimetric chemosensors for anions and cations containing (oligo)thiophene, benzoxazole and amino acid moieties.[8] We now report the synthesis and photophysical characterization of novel 2,4,5-triarylimidazolyl-alanines and the interaction study with biologically important ions such as F^-, OH^-, Cu^{2+} and Fe^{3+}, through spectrophotometric and spectrofluorimetric titrations.

2. Experimental

2.1. General procedure for the synthesis of 2,4,5-triarylimidazolyl-alanines 3a-b

N-tert-butyloxycarbonyl-4-formylphenylalanine methyl ester **1** (1 equiv.) and the appropriate heterocyclic dione **2** (1 equiv.) were dissolved in acetic acid (5 mL/equiv), in the presence of ammonium acetate (20 equiv.) and heated at reflux for 2 hours. After cooling, the mixture was poured over crushed ice and extracted with ethyl acetate (3 x 5 mL). After drying the organic layer over anhydrous $MgSO_4$, the solvent was removed in a rotary evaporator and a solid was

obtained. The crude solid was purified by column chromatography, using mixtures of dichloromethane and methanol of increasing polarity as eluent.

Methyl 2-amino-3-(4-(4,5-di(furan-2-yl)-1*H*-imidazol-2-yl)phenyl)propanoate (3a). The product was isolated as an orange solid (0.035 g, 0.093 mmol, 57%). Mp = 209.8-210.7 ºC. IR (KBr 1%): ν = 3340, 3078, 2928, 1737, 1691, 1633, 1596, 1526, 1518, 1437, 1389, 1368, 1322, 1291, 1256, 1220, 1166, 1109, 1086, 1059, 1029, 994, 971, 920, 854, 813, 796, 772, 684 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 3.06-3.20 (2H), 3.72 (3H,), 6.11 (2H), 6.53 (2H), 6.99 (2H), 7.14 (2H), 7.50 (2H), 7.84 (2H) ppm. UV/Vis (ethanol, nm): λ_{max} (log ε) = 317 (4.21).

Methyl 2-amino-3-(4-(4,5-di(thiophen-2-yl)-1H-imidazol-2-yl)phenyl)propanoate (3b). The product was isolated as a yellow solid (0.038 g, 0.094 mmol, 58%). Mp = 215.9-217.0 ºC. IR (KBr 1%): ν = 3431, 3146, 2977, 1711, 1655, 1596, 1534, 1513, 1446, 1419, 1391, 1366, 1332, 1259, 1195, 1168, 1120, 1060, 1020, 994, 959, 939, 912, 892, 871, 817, 735, 695, 646, 613 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 2.88-3.07 (2H), 3.59 (3H), 4.47-4.52 (1H), 7.00 (1H), 7.15 (1H), 7.20 (1H), 7.31 (2H), 7.40-7.42 (2H), 7.69 (1H), 7.93 (2H), 8.36 (1H), 12.80 (1H) ppm. UV/Vis (ethanol, nm): λ_{max} (log ε) = 313 (4.21).

2.2 Spectrophotometric and spectrofluorimetric titrations of 2,4,5-triarylimidazolyl-alanines 3a-b

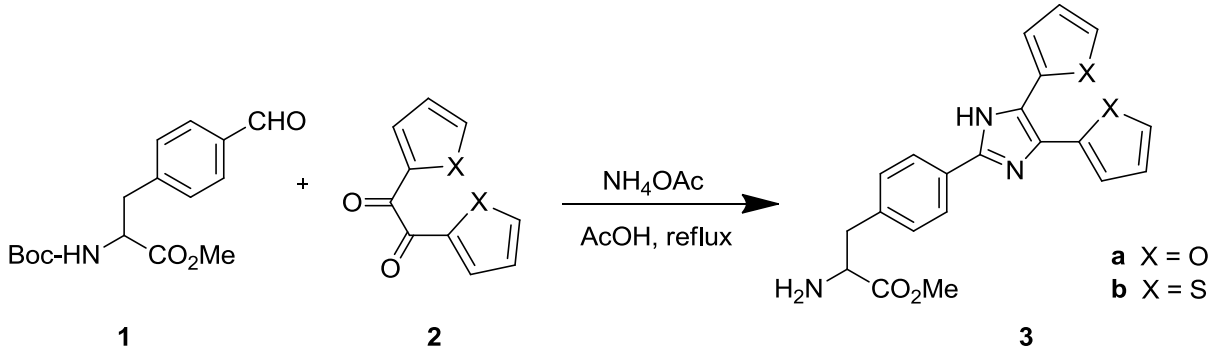
Solutions of 2,4,5-triarylimidazolyl-alanines **3** (ca. 1.0×10^{-5} to 1.0×10^{-6} M) and of the ions under study (ca. 1.0×10^{-1} to 1.0×10^{-3} M) were prepared in UV-grade acetonitrile (in the form of hydrated tetrabutylammonium salt for F^- and OH^- and hydrated perchlorate salt for Cu^{2+} and Fe^{3+}). Titration of the compounds with the several ions was performed by the sequential addition of equivalents of ion to the alanine 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.

3. Results and discussion

3.1. Synthesis

Novel 2,4,5-triarylimidazolyl-alanines **3a-b** were synthesised by reaction of *N-tert*-butyloxycarbonyl-4-formylphenylalanine methyl ester **1** with the appropriate heterocyclic

dione **2**, in the presence of ammonium acetate in acetic acid at reflux for 2 hours. The pure compounds were isolated in 57% (**3a**) and 58% (**3b**) yield, and were characterized by the usual spectroscopic techniques. The acidic reaction media yielded the *N*-deprotected form of the amino acids (Scheme).



Scheme. Synthesis of 2,4,5-triarylimidazolyl-alanines **3a**-**b**.

3.2. Photophysical study of 2,4,5-triarylimidazolyl-alanines 3a-b

The photophysical properties of compounds **3** were evaluated and the UV-vis absorption and emission spectra of degassed 10^{-6}-10^{-5} M solutions in absolute ethanol of both compounds were measured (Table 1). Relative fluorescence quantum yields were calculated using 9,10-diphenylanthracene as standard (Φ_F = 0.95 in ethanol).[9] Both 2,4,5-triarylimidazolyl-alanines **3** showed modest to high fluorescence quantum yields, and displayed large Stokes' shifts.

Table 1. UV-visible absorption and emission data for 2,4,5-triarylimidazolyl-alanines **3a-b** in absolute ethanol.

Compound	UV/Vis		Fluorescence		
	λ_{max} (nm)	log ε	λ_{max} (nm)	Stokes' shift (nm)	Φ_F
3a	317	4.21	405	88	0.72
3b	313	4.21	420	107	0.26

Considering the results obtained in ethanol, the photophysical properties of 2,4,5-triarylimidazolyl-alanines **3** were evaluated in other solvents of different character. The solvents tested were acetonitrile, dimethylsulphoxide, dichloromethane and diethyl ether, as examples of solvents with different polarity and proticity. The collected data revealed similar wavelengths of maximum absorption and emission and the fluorescence quantum yield did not vary significantly (Table 2).

The overall trend revealed that alanine **3a** with furyl pendants was the most fluorescent (with Φ_F in the range 0.64-0.77) whereas alanine **3b** bearing thienyl pendants displayed the largest Stokes' shifts (between 105-109 nm).

Table 2. UV-visible absorption and emission data for 2,4,5-triarylimidazolyl-alanines **3a-b** in various organic solvents of different character.

Cpd.	Solvent	UV/Vis		Fluorescence		
		λ_{max}	log ε	λ_{em}	Stokes' shift (nm)	Φ_F
3a	ACN	317	4.24	413	96	0.77
	EtOH	317	4.21	405	88	0.72
	DMSO	317	4.26	409	92	0.64
	DCM	324	4.21	410	86	0.64
	Diethyl ether	313	4.23	401	88	0.76
3b	ACN	313	4.28	422	109	0.30
	EtOH	313	4.21	420	107	0.26
	DMSO	320	4.29	428	108	0.14
	DCM	318	4.25	423	105	0.26
	Diethyl ether	312	4.23	419	107	0.29

3.3. Spectrophotometric and spectrofluorimetric titrations of 3a-b with ions

The modification of phenylalanine through the introduction of an extra UV-active and highly fluorescent heterocycle at its side chain was expected to provide additional binding sites for a variety of ions through the heterocycle donor atoms, as well as improved photophysical

properties for the chemosensing studies. With heterocyclic alanines **3** it was intended to assess the influence of the structure in the chemosensing ability of anions and cations. Considering the biological, environmental and analytical relevance of ions such as F^-, OH^-, Cu^{2+} and Fe^{3+}, the interaction of 2,4,5-triarylimidazolyl-alanines **3** with these ions was evaluated through UV-vis and fluorescence spectroscopies in spectrophotometric and spectrofluorimetric titrations in acetonitrile. In the spectrophotometric titrations, no changes were seen in the absorption spectra bands of 2,4,5-triarylimidazolyl-alanines **3a-b** after addition of up to 400 equiv of each ion.

In the spectrofluorimetric titrations with Cu^{2+} and Fe^{3+}, a decrease of the fluorescence intensity (a chelation-enhanced quenching, CHEQ effect) was observed for both the alanines, with an almost complete fluorescence quenching. In Figure 1A is shown the spectrofluorimetric titration of alanine **3a** with Cu^{2+}, where the drastic effect of ion complexation is evident in the band centred at the wavelength of maximum emission at 317 nm.

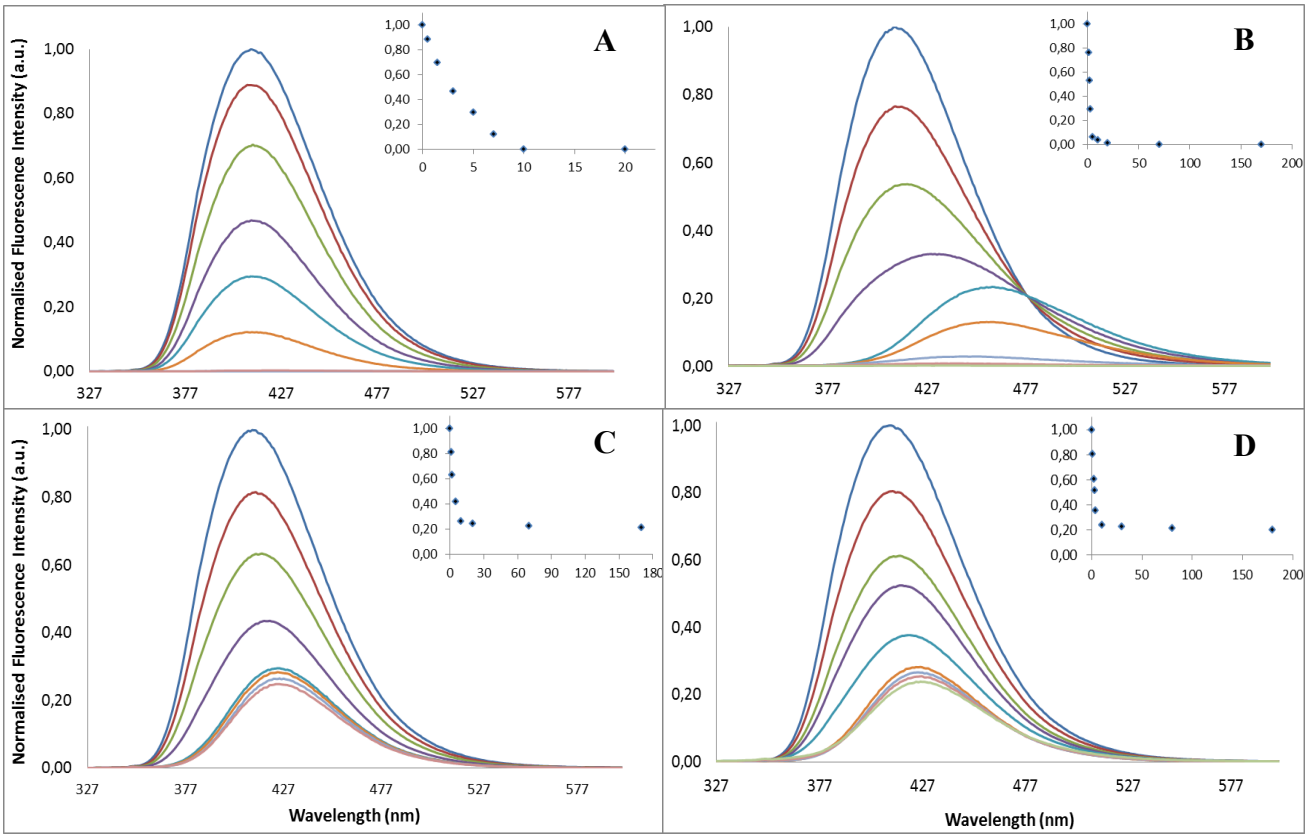


Figure 1. Fluorimetric titrations of 2,4,5-triarylimidazolyl-alanines **3a** with Cu^{2+} (A), Fe^{3+} (B), F^- (C) and OH^- (D) in acetonitrile [λ_{exc} **3c** = 317 nm]. Inset: normalised emission at 413 nm as a function of added ion equivalents.

With regard to the other ions Fe^{3+}, F^- and OH^-, a less pronounced CHEQ effect was also observed after ion addition, without complete quenching of fluorescence, with F^- and OH^-. In Figure 1 B, C and D are shown the spectrofluorimetric titrations of asparagine **3a** with Fe^{3+}, F^- or OH^-. For F^-, there was a maximum decrease of 75% and 45 % in fluorescence of alanines **3a-b** with the addition of 10 equiv of anion; as for OH^-, a fluorescence quenching of 75% and 45% was visible with the addition of 10 equiv to alanines **3a-b**; alanines **3a-b** responded highly to titration with Fe^{3+} with a 100% quenching after 20 and 180 equiv of metal were added.

The spectrofluorimetric titration results indicated that both 2,4,5-triarylimidazolyl-alanines were very sensitive to Cu^{2+}, whereas the sensing of Fe^{3+}, F^- and OH^- had lower sensitivity. Alanine **3a** would be the more interesting candidate as chemosensor due to the higher fluorescence quantum yield, which is important for maximization of response to analyte in the analysis of very dilute samples.

4. Conclusions

The novel 2,4,5-triarylimidazolyl-alanines **3a-b** are highly emissive, with modest to excellent fluorescence quantum yields (Φ_F = 0.64-0.77 for **3a**; Φ_F = 0.14-0.30 for **3b**) and display large Stokes' shifts (between 86 and 109 nm) in organic solvents of different character. Alanine **3a** bearing furyl pendants displayed higher fluorescence than alanine **3b**, bearing thienyl pendants, relating to the heteroatom, and larger Stokes shifts were seen for alanine **3b,** in all the solvents tested. Through spectrophotometric and spectrofluorimetric titration with several ions (F^-, OH^-, Cu^{2+} and Fe^{3+}) it was concluded that alanines **3** show a high sensitivity and ability to interact with Cu^{2+} and Fe^{3+} in ACN.

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