Synthesis and ion recognition studies of novel benzimidazol-5-yl-L-alanines

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Abstract: *N*-fluorenylmethoxycarbonyl-4-amino-phneylalanine and appropriate heterocyclic aldehydes were used in the synthesis of novel fluorescent unnatural amino acids, namely benzimidazol-5-yl-L-alanines. These new compounds were characterised by the usual spectroscopic techniques. The photophysical properties of the new amino acids were evaluated by UV-Vis absorption and fluorescence spectroscopy in acetonitrile. Interaction studies with biologically and analytically important ions such as Cu^{2+}, Fe^{3+}, Hg^{2+} and Pd^{2+}, through spectrophotometric and spectrofluorimetric titrations were carried out to assess their potential as chemosensors.

Keywords*:* Benzimidazole; Thiophene; Furan; Amino acids; Chemosensors; Fluorescence.

1. Introduction

Some natural amino acids can be the precursors for unnatural amino acids by suitable synthetic transformations. Modification at the side chain is the basis for the synthesis of new amino acids, allowing functional interaction between the new modified amino acids and other compounds, by altering their chemical and photophysical properties, to have a wide variety of applications.[1] Heteroaromatic systems containing in its structure potential chelating groups have the ability to act both in the recognition of ions and in the signaling of the recognition event because, when complexed, variation of their fluorescent properties may occur. Benzimidazole and its derivatives have been studied in ion recognition systems that display color changes or fluorescence quenching or enhancement upon binding.[2] Furan and thiophene are also known for their very interesting photophysical properties, which enable their application as sensors and/or fluorescent markers. Therefore, new amino acids containing these associated units could be potential fluorimetric sensors for ions with improved photophysical properties.[3,4] Metallic cations can be complexed by N, O and S donor atoms at the main and side chain of amino acids, while anions can be coordinated based on hydrogen bonds and electrostatic interactions, and it is very important to insert suitable heterocyclic systems at the side chain of natural amino acids to add additional binding sites.[5]

The synthesis and photophysical characterization of novel benzimidazol-5-yl-L-alanines is now reported, by formation of an imidazole ring fused to the phenyl ring at the side chain of phenylalanine, to give a benzimidazole, with different five membered heterocycles as substituents. An interaction study with analytically important ions (F^-, CN^-, OH^-, Co^{2+}, Pd^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+} and Hg^{2+}) was carried out through spectrofluorimetric titrations.

2. Experimental

2.1. General procedure for the synthesis of benzimidazol-5-yl-L-alanines 3a-b

A solution of *N*-fluorenylmethoxycarbonyl-3-nitro-4-amino-L-phenylalanine[6] **1** (1 equiv.), and the appropriate heterocyclic aldehyde **2** (1 equiv.) in absolute ethanol (3 mL) was treated with $Na_2S_2O_4$ (3 equiv.) dissolved in a small volume of water, and heated at reflux for 38 h. The mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 50 mL). The organic layer was dried with magnesium sulphate and evaporated under reduced pressure to give the crude benzimidazolylphenylalanines **3** which were submitted to silica gel column

chromatography using mixtures of dichloromethane and methanol of increasing polarity as eluent.

***N*-fluorenylmethoxycarbonyl-[2-(furan-2'-yl)benzimidazol-5-yl]-L-alanine, (3a).** The product was isolated as a white solid (0.1407 g, 0.285 mmol, 42%). Mp = 229.0-229.9 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.01-3.25 (m, 2H, β-CH_2), 4.12-4.25 (m, 4H, CH_2, CH and α-H), 6.86 (br s, 1H, H4''), 7.20-7.28 (m, 2H, H3 and H10), 7.36 (br s, 2H, H4, H9 and H3''), 7.58-7.61 (m, 5H, H5, H8, H4', H6' and H7'), 7.79 (d, *J* = 8.40 Hz, 1H, NH), 7.83 (d, *J* = 7.20 Hz, 2H, H2 and H11), 8.14 (br s, 1H, H5'') ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 36.41, 46.42, 55.70, 65.56, 113.12, 113.93, 114.00, 114.50, 120.00, 125.11, 126.06, 126.95, 127.51, 140.53, 140.56, 141.02, 143.58, 143.62, 147.14, 155.91, 173.13 ppm. UV/Vis (ACN, nm): λ_{max} (log ε) = 316 (4.38).

***N*-fluorenylmethoxycarbonyl-[2-(thiophen-2'-yl)benzimidazol-5-yl]-L-alanine (3b).** The product was isolated as a white solid (0.1508 g, 0.296 mmol, 45%). Mp = 239.4-240.0 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.94-3.21 (m, 2H, β-CH_2), 4.13-4.22 (m, 4H, CH_2, CH and α-H), 7.11 (d, *J* = 8.00 Hz, 1H, H4', H6' or H7'), 7.20-7.29 (m, 3H, H3, H10 and H4''), 7.45 (br s, 2H, H4', H6' or H7'), 7.58-7.63 (m, 2H, H5 and H8), 7.70 (dd, *J* = 5.20 and 1.00 Hz, 1H, H5''), 7.76 (d, *J* = 8.40 Hz, 1H, NH), 7.80 (dd, *J* = 3.30 and 0.80 Hz, 1H, H3''), 7.84 (d, *J* = 7.60 Hz, 2H, H2 and H11), 12.88 (br s, 1H, NH) ppm. ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 36.74, 46.52, 56.07, 65.67, 111.34, 117.90, 120.08, 123.68, 125.23, 125.29, 126.57, 127.06, 127.61, 128.26, 128.64, 133.74, 140.64, 143.71, 143.73, 147.02, 155.99, 173.49 ppm. UV/Vis (ACN, nm): λ_{max} (log ε) = 320 (4.50).

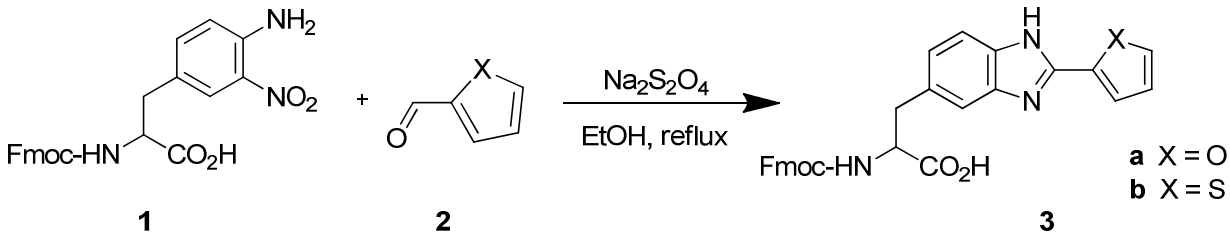
2.2 Spectrophotometric and spectrofluorimetric titrations of benzimidazol-5-yl-L-alanines 3a-b

Solutions of benzimidazol-5-yl-L-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^-, CN^- and OH^-, tetrafluoroborate salt for Co^{2+} and Pd^{2+} and hydrated perchlorate salt for Cu^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+} and Hg^{2+}). Titration of the compounds with the several ions was performed by the sequential addition of equivalents of ion to the amino acid 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 benzimidazol-5-yl-L-alanines **3a-b** were synthesised by reaction of *N*-fluorenylmethoxycarbonyl-3-nitro-4-amino-L-phenylalanine[6] **1** with 2-furancarbaldehyde **2a** and 2-thiophenecarbaldehyde **2b**, by a one-pot reaction in which a reduction is followed by an intramolecular cyclization reaction. The reaction was carried out in absolute ethanol in the presence of $Na_2S_2O_4$ as reducing agent and heated at reflux for 38 h. The pure compounds were isolated in 42% (**3a**) and 45% (**3b**) yield, and were characterized by the usual spectroscopic techniques (Scheme).



Scheme. Synthesis of benzimidazol-5-yl-L-alanines **3a**-**b**.

3.2. Photophysical study of benzimidazol-5-yl-L-alanines 3a-b

The photophysical properties of alanines **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).[7] Both benzimidazol-5-yl-L-alanines **3** showed high relative fluorescence quantum yields, and large Stokes' shifts.

Table 1. UV-visible absorption and emission data for benzimidazol-5-yl-L-alanines **3a-b** in acetonitrile.

Compound	UV/Vis		Fluorescence		
	λ_{max} (nm)	log ε	λ_{emi} (nm)	Stokes' shift (cm^{-1})	Φ_F
3a	316	4.38	344	2576	0.66
3b	320	4.50	383	5140	0.57

Considering the photophysical results obtained in acetonitrile, alanine **3a** with a furyl substituent was the most fluorescent (with Φ_F 0.66) whereas alanine **3b** bearing a thienyl moiety displayed the largest Stokes' shifts (5140 cm^{-1}).

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 selected ions such as F^-, CN^-, OH^-, Co^{2+}, Pd^{2+}, Cu^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+} and Hg^{2+}, the interaction of benzimidazol-5-yl-L-alanines **3a-b** with these ions was evaluated through UV-Vis and fluorescence spectroscopies in spectrophotometric and spectrofluorimetric titrations in acetonitrile.

Studies were performed for various ions, but Figures 1 and 2 show the best results for compounds **3a**-**b**. In the spectrophotometric titrations, no changes were seen in the absorption spectra bands of benzimidazol-5-yl-L-alanines **3a-b** after addition of up to 300 equiv. of each ion.

In the spectrofluorimetric titrations with Cu^{2+}, Fe^{3+}, Hg^{2+} and Pd^{2+} a decrease of the fluorescence intensity (a chelation enhancement of quenching, CHEQ effect) was observed for both alanines, with an almost complete fluorescence quenching. In Figure 1 it can be seen that alanine **3a** was more sensitive to Pd^{2+}, when compared to Fe^{3+}, Hg^{2+} and Cu^{2+}: total quenching occurred after addition of 0.8 equivalents of Pd^{2+}, whereas 5 equiv. for Fe^{3+} and 2 equiv. for Hg^{2+} were necessary. Addition of 10 equiv. of Cu^{2+} caused a 90% quenching of fluorescence.

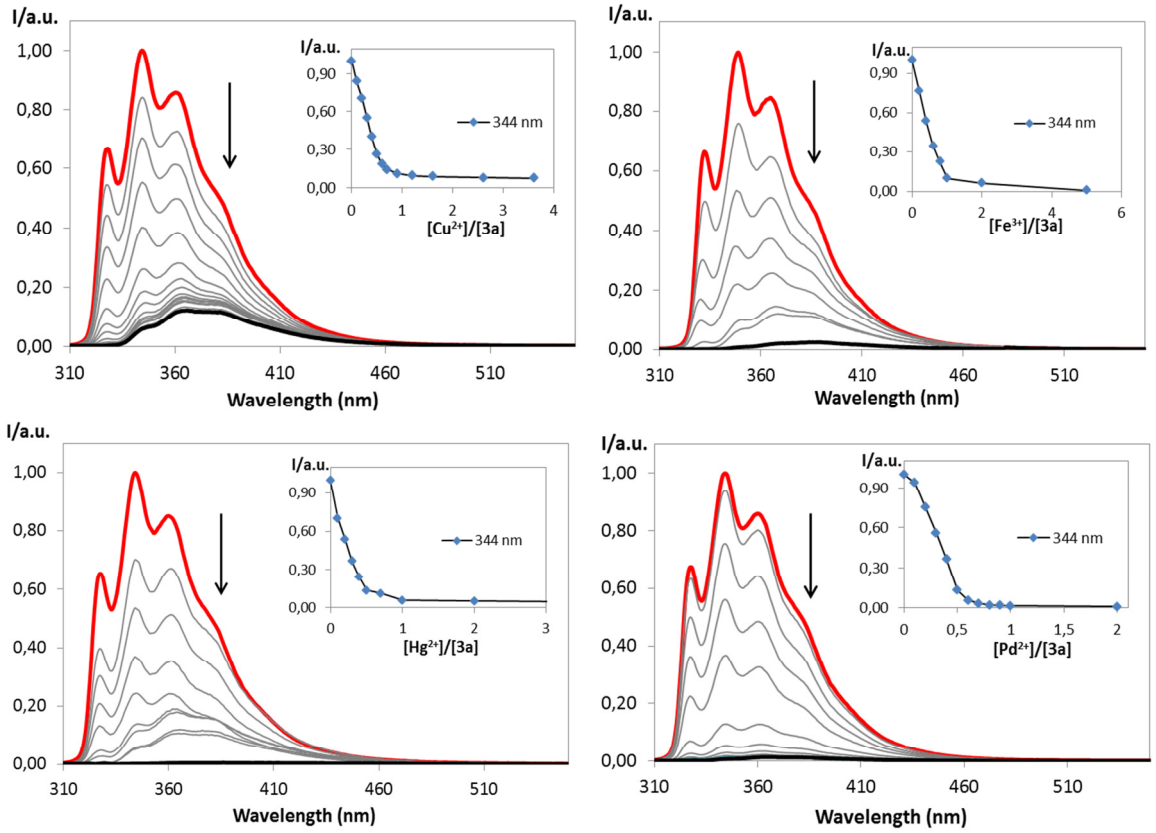


Figure 1. Fluorimetric titrations of benzimidazol-5-yl-L-alanine **3a** with Cu^{2+}, Fe^{3+}, Hg^{2+} and Pd^{2+} in acetonitrile [λ_{exc} **3a** = 316 nm]. Inset: normalised emission at 344 nm as a function of added ion equivalents.

Higher sensitivity towards Pd^{2+} was also seen for compound **3b** with only 1 equiv. of cation causing a total quenching. As for the other cations, the fluorimetric titration revealed that addition of 10 equiv. of Cu^{2+} caused a 70% quenching of fluorescence, 0.8 equiv. of Fe^{3+} caused a 90% quenching (although total quenching was only seen with 30 equiv), and with Hg^{2+} addition of 15 equiv. were necessary for a complete quenching of fluorescence (Figure 2).

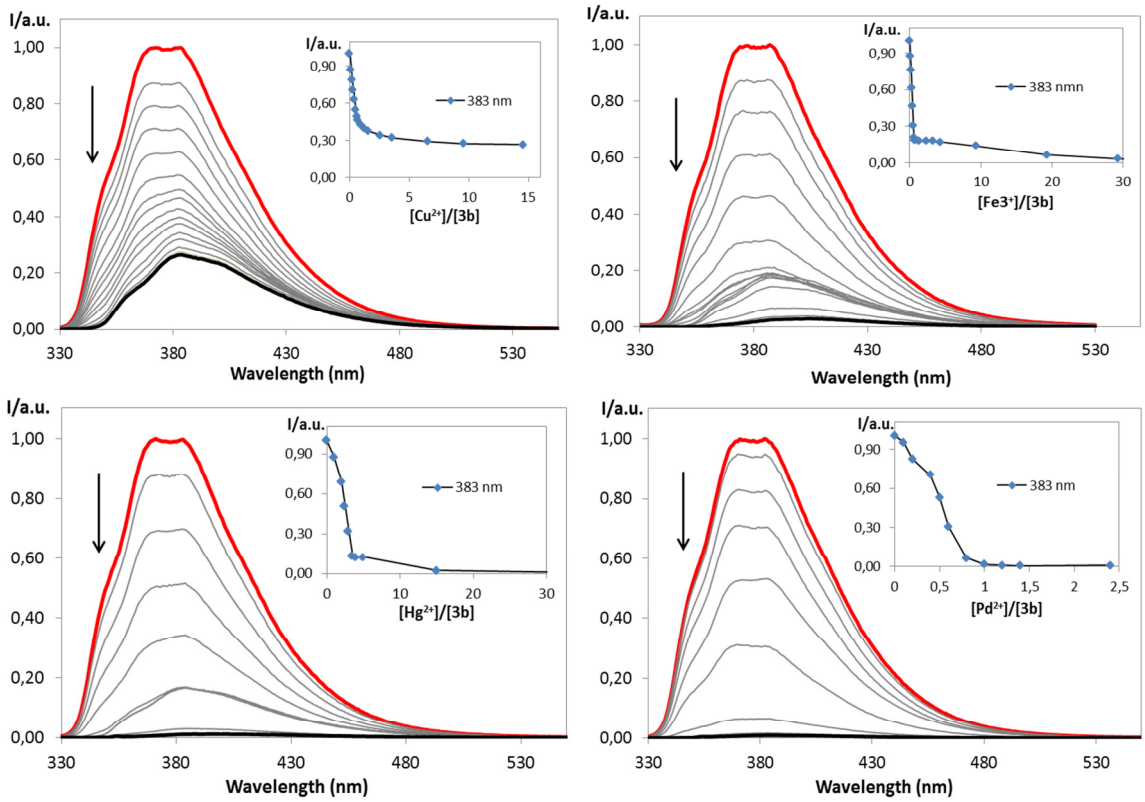


Figure 2. Fluorimetric titrations of benzimidazol-5-yl-L-alanine **3b** with Cu^{2+}, Fe^{3+}, Hg^{2+} and Pd^{2+} in acetonitrile [λ_{exc} **3b** = 320 nm]. Inset: normalised emission at 383 nm as a function of added ion equivalents.

By comparison of the obtained spectrofluorimetric titration results for compounds **3a** and **3b**, it can be concluded that benzimidazolylalanine **3a** is more sensitive to the studied cations as it requires less amount of cation for a higher percentage of quenching. Nevertheless, both alanines **3a-b** can be considered interesting candidates as fluorimetric chemosensors due to the high fluorescence quantum yield, which is important for maximization of response to analyte in the analysis of very dilute samples.

The sensitivity of compounds **3a-b** can be compared qualitatively using as criteria for sensitivity the number of equivalents of cation necessary to achieve the highest fluorescence quenching (Table 2).

Table 2. Comparison of the number of equivalents necessary to quench fluorescence in spectrofluorimetric titrations of compounds **3a** and **3b** with various anions and cations.

Compound	**3a**		**3b**	
Ions	Nº equiv.	% Quenching	Nº equiv.	% Quenching
Cu^{2+}	10	90	10	70
Fe^{3+}	5	100	0.8	90
Hg^{2+}	2	100	15	100
Pd^{2+}	0.8	100	1	100
Zn^{2+}	3	80	250	70
Cd^{2+}	2	70	220	50
Cr^{3+}	1	90	1	70
Co^{2+}	100	90	4	60
Fe^{2+}	17	80	15	60
Pb^{2+}	3	90	32	70
F^-	4	20	3	15
CN^-	3	80	3	50
OH^-	4	90	3	25

4. Conclusions

The novel benzimidazol-5-yl-L-alanines **3a-b** are highly emissive, with good fluorescence quantum yields (Φ_F = 0.66 for **3a**; Φ_F = 0.57 for **3b**) and large Stokes' shifts (2576 and 5140 cm^{-1}, respectively) in acetonitrile. Alanine **3a** bearing a furyl moiety displayed higher fluorescence than alanine **3b**, bearing a thienyl moiety. Through spectrophotometric and spectrofluorimetric titration with several ions it was concluded that alanines **3** show a high sensitivity and ability to interact with Pd^{2+} in ACN.

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