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New 2,4,5-triarylimidazoles based on a phenylalanine core: synthesis, photophysical characterization and evaluation as fluorimetric chemosensors for ion recognition

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ABSTRACT

Novel fluorescent 4,5-diarylimidazolyl-phenylalanines **3a**-**d** were prepared by reaction of *N-tert*-butyloxycarbonyl-4-formylphenylalanine methyl ester and appropriate (hetero)aromatic diones. The photophysical properties of these new unnatural amino acids were evaluated by UV–Vis absorption and fluorescence spectroscopy in solvents of different character and aqueous mixtures with acetonitrile. They were evaluated as novel amino acid based fluorimetric chemosensors for ions through spectrophotometric and spectrofluorimetric titrations with biologically and analytically important anions and cations such as F^-, OH^-, Cu^{2+} and Fe^{3+}. The results indicate that there was a strong interaction through the donor N, O and S atoms at the side chain, especially for 4,5-di(furan-2′-yl)imidazolyl-phenylalanine **3a** and 4,5-di(thiophen-2′-yl)imidazolyl-phenylalanine **3b** with Cu^{2+} and Fe^{3+}, in a 1:1 complex stoichiometry. The photophysical and metal ion sensing properties of these amino acids suggest that they can be suitable for incorporation into chemosensory peptidic frameworks.

1. Introduction

Optical sensing based on colorimetric and fluorimetric probes is a very appealing topic of research, considering the large number of chromo/fluorophores available and the simple instrumentation required (when using colorimetric probes), and the low detection limits (when using fluorescence probes) [1]. Hence, the design of novel organic fluorophores that can be used in biomolecule labelling and chemosensing of organic and inorganic molecules involved in biological pathways is worth exploring [2]. The development of probes containing binding sites capable of sensing anions and cations, preferably in aqueous media, is a very active area within the chemical sensing field [3]. Imidazole derivatives are known as neutral binding groups for anions [4], and the coordination ability of the imidazole group depends on the acidity of the NH proton that can be modulated by suitable substituents such as heteroaromatic rings like thiophene, pyrrole or furan. On the other hand, the two nitrogen atoms in imidazole enable the coordination of metal cations by this five-membered heterocycle [5]. 2,4,5-Triarylimidazoles have found application in materials sciences for their interesting optoelectronic properties that can be tuned by careful selection of substituents at positions 2, 4 and 5: replacement of the aryl group by an heterocyclic group results in larger π-conjugated systems with improved optical properties for application in two-photon absorption applications, two-photon fluorescence microscopy, high density storage and 3D microfabrication, nonlinear optics, OLEDs, and chemosensors [6]. 2,4,5-Triarylimidazoles have also been applied in medicinal chemistry, 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 [7].

Synthetically modified amino acids are useful for the preparation of intrinsically labelled peptides with tailored properties such as increased fluorescence, conformational rigidity, and metal complexation ability, among others. Recent examples in the literature refer the use of fluorescent unnatural 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 [8]. 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,

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can arise from amino acid side and main chain OH and NH groups, or from NH groups in heterocycles [9]. Therefore, the insertion of suitable heterocyclic systems at the side chain of natural amino acids can add extra functionality to the resulting amino acid, bearing a metal ion chelating site for stable complex formation and subsequent incorporation into a peptide.

Given these facts, our current research interests include the synthesis and characterization of unnatural amino acids bearing fluorescent oxygen, nitrogen and sulphur heterocycles [10], imidazole and benz-X-azole derivatives with interesting optical properties [11] and innovative heterocyclic colorimetric/fluorimetric chemosensors for anions and cations based on amino acid moieties [12]. To the best of our knowledge, this is the first report on the synthesis of 2,4,5-triaryl-imidazole derivatives combined with an amino acid core, the evaluation of the photophysical properties in different solvents and the chemosensing ability in the presence of anions and cations.

2. Experimental

2.1. Synthesis 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 using KBr discs. NMR spectra were obtained on 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 °C. All chemical shifts are given in ppm using δ_H $Me_4Si = 0$ ppm as reference. Assignments were supported by spin decoupling-double resonance and bidimensional heteronuclear correlation techniques. Low and high resolution mass spectra were obtained at "C.A.C.T.I. Unidad de Espectrometria de Masas" at the University of Vigo, Spain. Commercially available reagents **2a-d** were purchased from Sigma–Aldrich, ACROS, or TCI and used as received. Compound **1** was synthesised as reported elsewhere [13].

2.2. General procedure for the synthesis of imidazolyl-phenylalanines *3a-d*

N-tert-Butyloxycarbonyl-4-formylphenylalanine methyl ester **1** (1 equiv) and the appropriate dione **2** (1 equiv) were dissolved in acetic acid (5 mL/mmol) in the presence of ammonium acetate (20 equiv) and heated at reflux for 2 h. After cooling, the mixture was poured over crushed ice and extracted with ethyl acetate (3 × 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.

2.2.1. N-Acetyl-4-(4′,5′-di(furan-2″-yl)imidazol-2′-yl) phenylalanine methyl ester (*3a*)

Starting from aldehyde **1** (0.050 g, 0.163×10^{-3} mol) and 1,2-di(furan-2-yl)ethane-1,2-dione **2a** (0.031 g, 0.163×10^{-3} mol), compound **3a** was isolated as an orange solid (0.035 g, 0.084×10^{-3} mol, 42%). mp = 209.8–210.7 °C. IR (KBr 1%, cm^{-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. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.99 (s, 3H, CH_3 Ac), 3.06–3.20 (m, 2H, β-CH_2), 3.72 (s, 3H, OCH_3), 4.85–4.88 (m, 1H, α-H), 6.11 (d, *J* 7.6 Hz, 1H, NH Ac), 6.53 (dd, *J* 3.6 and 1.8 Hz, 2H, 2 × H4″), 6.99 (d, *J* 3.6 Hz, 2H, 2 × H3″), 7.14 (d, *J* 8.0 Hz, 2H, H2 and H6), 7.50 (d, *J* 1.8 Hz, 2H, 2 × H5″), 7.84 (d, *J* 8.0 Hz, 2H, H3 and H5). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 23.07 (CH_3 Ac), 37.72 (β-CH_2), 52.43 (OCH_3), 53.16 (α-C), 107.80 (C3″), 111.76 (C4″), 125.81 (C3 and C5), 127.24 (C4′ or C5′), 127.87 (C4′or C5′), 129.69 (C2 and C6), 130.28 (C4), 137.20 (C1), 141.54 (C5″), 145.83 (C2′) 155.33 (C2″), 169.89 (C=O Ac), 171.90 (C=O ester). UV/Vis (ethanol, nm): λ_{max} (log ε) = 317 (4.21). MS *m/z* (ESI, %): 420 ($[M+H]^+$, 100). HRMS: *m/z* (ESI) calcd for $C_{23}H_{22}N_3O_5$ 420.15606; found 420.15508.

2.2.2. N-Acetyl-4-(4′,5′-di(thiophen-2″-yl)imidazol-2′-yl) phenylalanine methyl ester (*3b*)

Starting from aldehyde **1** (0.050 g, 0.162×10^{-3} mol) and 1,2-di(thiophen-2-yl)ethane-1,2-dione **2b** (0.036 g, 0.162×10^{-3} mol), compound **3b** was isolated as a yellow solid (0.038 g, 0.084×10^{-3} mol, 45%). mp = 215.9–217.0 °C. IR (KBr 1%, cm^{-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. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.79 (s, 3H, CH_3 Ac), 2.88–3.07 (m, 2H, β-CH_2), 3.59 (s, 3H, OCH_3), 4.47–4.52 (m, 1H, α-H), 7.00 (dd, *J* 5.2 and 3.6 Hz, 1H, H4″), 7.15 (dd, *J* 3.6 and 0.8 Hz, 1H, H3″), 7.20 (dd, *J* 4.2 and 3.6 Hz, 2H, H4‴), 7.31 (d, *J* 8.4 Hz, 2H, H2 and H6), 7.40–7.42 (m, 2H, H5″ and H3‴), 7.69 (d, *J* 5.2 and 1.2 Hz, 1H, H5‴), 7.93 (d, *J* 8.4 Hz, 2H, H3 and H5), 8.36 (d, *J* 8.0 Hz, 1H, NH Ac), 12.80 (br s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 22.23 (CH_3 Ac), 36.57 (β-CH_2), 51.84 (OCH_3), 53.43 (α-C), 120.60 (C4′ or C5′), 123.39 (C3″), 124.84 (C3‴), 125.23 (C3 and C5), 127.31 (C4″), 127.40 (C5‴), 127.59 (C4‴), 128.09 (C4), 128.41 (C5″), 129.43 (C2 and C6), 130.79 (C2‴), 133.55 (C4′ or C5′), 137.45 (C2″), 137.83 (C1), 145.66 (C2′), 169.35 (C=O Ac), 172.13 (C=O ester). UV/Vis (ethanol, nm): λ_{max} (log ε) = 313 (4.21). MS *m/z* (ESI, %): 452 ($[M+H]^+$, 100). HRMS: *m/z* (ESI) calcd for $C_{23}H_{22}N_3O_3S_2$ 452.10911; found 452.10971.

2.2.3. N-Acetyl-4-(4′,5′-diphenyl-imidazol-2′-yl) phenylalanine methyl ester (*3c*)

Starting from aldehyde **1** (0.049 g, 0.160×10^{-3} mol) and 1,2-diphenylethane-1,2-dione **2c** (0.033 g, 0.160×10^{-3} mol), compound **3c** was isolated as an orange solid (0.025 g, 0.057×10^{-3} mol, 44%). mp = 203.1–204.2 °C. IR (KBr 1%, cm^{-1}): ν = 3320, 3178, 3028, 1837, 1751, 1633, 1596, 1525, 1418, 1382, 1368, 1300, 1290, 1254, 1222, 1166, 1100, 1085, 1059, 1029, 994, 970, 923, 855, 812, 796, 772, 683. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.80 (s, 3H, CH_3 Ac), 2.88–3.07 (m, 2H, β-CH_2), 3.60 (s, 3H, OCH_3), 4.47–4.52 (m, 1H, α-H), 7.19–7.49 (m, 8H, 2xPh), 7.48 (d, *J* 7.8 Hz, 2H, H2 and H6), 7.98 (d, *J* 7.8 Hz, 2H, H3 and H5), 8.36 (d, *J* 7.6 Hz, 1H, NH Ac), 12.62 (br s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 22.23 (CH_3 Ac), 36.58 (β-CH_2), 51.83 (OCH_3), 53.47 (α-C), 125.11 (C3 and C5), 126.50 (C4″), 127.05 (C3″ and C5″), 127.69 (C4′ or C5′), 127.74 (C4‴), 128.17 (C2″ and C6″), 128.39 (C2 and C6), 128.65 (C3‴ and C5‴), 128.71 (C4), 129.36 (C2‴ and C6‴), 131.07 (C1″), 135.17 (1‴), 137.03 (C4′ or C5′), 137.40 (C1), 145.44 (C2′), 169.35 (C=O Ac), 172.16 (C=O ester). UV/Vis (ethanol, nm): λ_{max} (log ε) = 304 (4.28). MS *m/z* (ESI, %): 440 ($[M+H]^+$, 100). HRMS: *m/z* (ESI) calcd for $C_{27}H_{26}N_3O_3$ 440.19687; found 440.19628.

2.2.4. N-Acetyl-4-(4′,5′-di(pyridin-2″-yl)imidazol-2′-yl) phenylalanine methyl ester (*3d*)

Starting from aldehyde **1** (0.020 g, 0.066×10^{-3} mol) and 1,2-di(pyridin-2-yl)ethane-1,2-dione **2d** (0.014 g, 0.066×10^{-3} mol), compound **3d** was isolated as an orange solid (0.020 g, 0.045×10^{-3} mol, 44%). mp = 123.9–124.7 °C. IR (KBr 1%, cm^{-1}): ν = 3351, 3068, 2918, 1764, 1691, 1632, 1526, 1509, 1437, 1365, 1320, 1293, 1256, 1166, 1109, 1086, 1049, 1019, 991, 920, 853, 812, 796,