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New fluorescent probes based on 3-(2-benzoxazol-5-yl)alanine skeleton—Synthesis and photophysical properties

Katarzyna Guzow [a,*], Dagmara Szmigiel [a,b], Dominik Wróblewski [a], Magda Milewska [a], Jerzy Karolczak [c,d], Wiesław Wiczk [a]

[a] *Faculty of Chemistry, University of Gdańsk, Sobieskiego 18, 80-952 Gdańsk, Poland*
[b] *Intercollegiate Faculty of Biotechnology, University of Gdańsk & Medical University of Gdańsk, Kładki 24, 80-822 Gdańsk, Poland*
[c] *Quantum Electronics Laboratory, Faculty of Physics, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland*
[d] *Centre of Ultrafast Laser Spectroscopy, Adam Mickiewicz University, Umultowska 85, 61-614 Poznań, Poland*

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Abstract

N-(*tert*-butoxycarbonyl)-3-(benzoxazol-5-yl)alanine methyl ester derivatives substituted in position 2 by heterocyclic group were synthesized and their photophysical properties in methanol, acetonitrile and methylcyclohexane were studied by means of absorption and fluorescence spectroscopies. The positions of their emission bands depend on the solvent polarity and are shifted to longer wavelengths in more polar solvents as a result of a charge transfer from a substituent to the benzoxazole moiety. High molar absorption coefficient values and fluorescence quantum yields are characteristic for most of the compounds studied making them efficient fluorescent probes. Moreover, the presence of additional heteroatom in the substituent enables those compounds to act as chemosensors for metal ions or protons.

Keywords: Benzoxazol-5-yl-alanine; Unnatural amino acid; Fluorescence; Absorption; Chemosensor

1. Introduction

Benzoxazoles are a group of heterocyclic compounds which are widely used in chemistry, industry and medicine. Among them 2-phenylbenzoxazoles are known as photostable highly efficient UV dyes [1] used as organic brightening agents [2], laser dyes [1], organic plastic scintillators [3] and optical fibre sensors [4]. Some benzoxazole derivatives are also used as dopants in organic light-emitting diodes [5], chromophores in nonlinear optical polymers [6–10], chemosensors for metal ions [11,12] or pH sensors [13]. Moreover, some benzoxazole derivatives are biologically active [14–28]. They are also used as fluorescent probes to determine spectrofluorimetrically glutathione [29] and cysteine [30] in biological samples. Such many applications of those compounds are a result of their photophysical properties among which high molar absorption coefficient values and fluorescence quantum yields are most important. Furthermore, substituent in position 2 of the benzoxazole ring may be quite easily changed modifying the photophysical properties of the compound. For example, an electron-donor substituent in a phenyl ring of 2-phenylbenzoxazole shifts the absorption and emission spectra to the longer wavelengths because of a charge transfer in the excited state from a phenyl ring to the benzoxazole moiety [1,31].

The amino acid derivatives of benzoxazoles, 3-(2-benzoxazol-5-yl)alanines, may be incorporated into a peptide chain [32]. Moreover, they have photophysical properties similar to the parent compounds because a benzoxazole moiety is mainly responsible for their fluorescent properties [33–38]. Some of those compounds are potential micropolarity probes [38,39] or chemosensors for H_3O^+ [40] and metal ions [40,41]. Because of that we synthesized a series of new 3-(2-benzoxazol-5-yl)alanine derivatives with a heteroaromatic substituent in position 2 which enable those compounds to form a complex with a metal ion or be protonated (Fig. 1). Their photophysical

* Corresponding author. Tel.: +48 58 523 54 13; fax: +48 58 523 54 72.
E-mail address: kasiag@chem.univ.gda.pl (K. Guzow).

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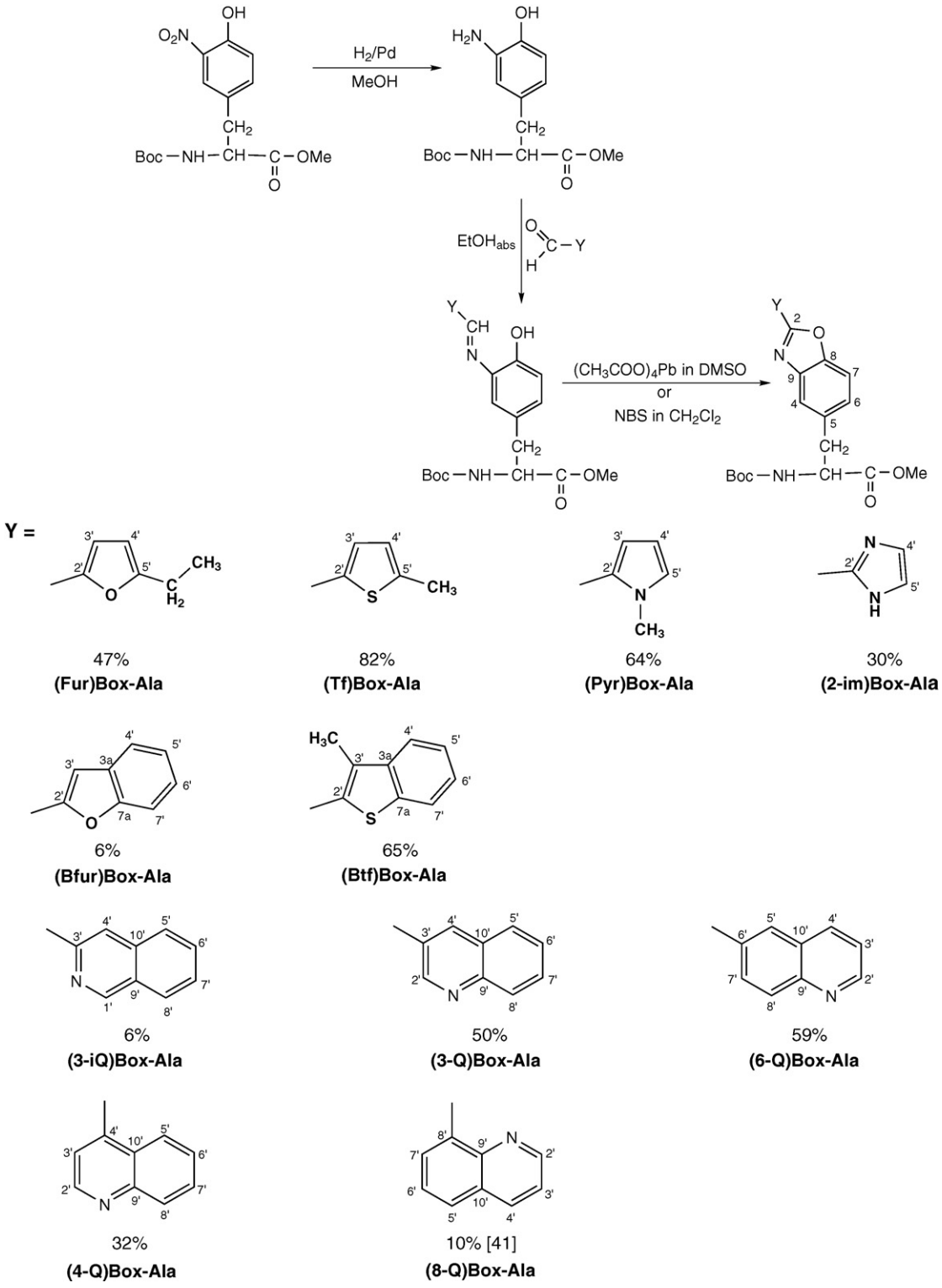

Fig. 1. The synthesis scheme (number below the structure denotes the reaction yield).

properties in methanol, acetonitrile and methylcyclohexane were studied in order to find out how substituent properties influence on the compound spectroscopic properties and if there is a structure–properties relationship which may enable to design more rationally probes possessing required properties.

2. Materials and methods

2.1. Synthesis

Quinoline carboxaldehydes, except for 3-quinolinecarboxaldehyde, were synthesized from corresponding methyl derivative

using selenium dioxide according to the literature procedure [42,43] or its modified version. All other aldehydes were commercially available. 3-Methyl[*b*]thiophene-2-carboxaldehyde and 1-methylpyrrole-2-carboxaldehyde were purchased from Avocado, 5-ethyl-2-furaldehyde, 2-benzofurancarboxaldehyde, 5-methyltiophene-2-carboxaldehyde, imidazole-2-carboxaldehyde, quinoline-3-carboxaldehyde from Lancaster, lepidine from Aldrich, 3-methylisoquinoline from Fluka, 6-methylquinoline from Koch-Light Laboratories Ltd., whereas lead tetraacetate and 3-nitro-L-tyrosine from Lancaster and Fluka, respectively. 3-Nitro-L-tyrosine methyl ester and *N*-Boc-3-nitro-L-tyrosine methyl ester were prepared according to literature procedures published in [44,45], respectively.

2.1.1. General procedure

All compounds were synthesized from *N*-Boc-protected 3-amino-L-tyrosine methyl ester as a substrate, via the intermediate Schiff base, which underwent oxidative cyclization to the heterocyclic compound in the presence of lead tetraacetate in DMSO according to the procedure published previously [35,37] (Fig. 1). Only in the case of *N*-Boc-3-[2-(2-benzofuryl)benzoxazol-5-yl]alanine methyl ester ((Bfur)Box-Ala), NBS was used as an oxidizing agent [33] (the detailed procedure in supplementary data). The products were isolated by means of column chromatography (Merck, Silica gel 60, 0.040–0.063 mm) and then recrystallized. In the case of (Bfur)Box-Ala, additional purification by means of semi-preparative RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, i.d. = 20 mm) was necessary. The purity of the obtained compounds was checked by means of TLC (Merck plates, Kieselgel 60 F_{254}, spots revealed using a UV lamp (254 nm, 366 nm), solvent ratios in volume parts) and analytical RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, i.d. = 4.5 mm, detection at 223 or 320 nm (quinolinecarboxaldehydes)). The mobile phase was a gradient running from 0% to 100% of B (A = water with addition of 0.01% trifluoroacetic acid, B = 80% of an aqueous solution of acetonitrile with addition of 0.08% trifluoroacetic acid) over 60 min plus 100% of B over 10 min. The identification of the product was based on the ^{1}H and ^{13}C NMR spectra recorded on Varian, Mercury-400 BB spectrometer (400 and 100 MHz, respectively) in $CDCl_3$ or DMSO-d_6 (δ was referenced internally to the residual proton resonance of the solvent or $SiMe_4$), infrared spectra recorded on a Bruker IFS-66 instrument, mass spectra recorded on a MASSLAB TRIO-3 (FAB) or Bruker Biflex III (MALDI-TOF) instrument and elemental analysis taken on a Carlo Erba CNSO Eager 200 instrument. Melting points (mp) were determined in capillary tubes using Gallenkamp Griffin MPA-350.MB2.5 apparatus and are uncorrected.

2.1.2. Identification data for all compounds studied

2.1.2.1. N-Boc-3-[2-[2-(5′-ethyl)furyl]benzoxazol-5-yl]alanine methyl ester ((Fur)Box-Ala). The Schiff base: brown oil, $R_f = 0.77$ (AcOEt/petroleum ether 1:1).

Purification: eluent—AcOEt/petroleum ether 1:2 (v/v), yellow solid (47% yield).

Identification: $R_f = 0.50$ (AcOEt/petroleum ether 1:2); $t_R = 53.4$ min; mp 115–117 °C; IR (KBr): ν_{max} (cm^{-1}) 3352.0, 3140.0, 2977.4, 2939.7, 1758.8, 1716.0, 1632.9, 1563.7, 1502.3, 1477.6, 1436.7, 1392.1, 1365.7, 1285.8, 1214.9, 1174.3, 1144.6, 1063.6, 1019.9, 981.5, 856.4, 815.9, 786.3, 726.1; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.33 (t, 3H, CH_3, $J = 7.61$ Hz), 1.42 (s, 9H, $(CH_3)_3$), 2.81 (q, 2H, CH_2, $J = 7.21$ Hz, $J = 15.42$ Hz), 3.16–3.27 (m, 2H, $C^{\beta}H_2$), 3.73 (s, 3H, OCH_3), 4.64 (q, 1H, $C^{\alpha}H$, $J = 5.21$ Hz, $J = 13.42$ Hz), 5.02 (d, 1H, NH, $J = 8.61$ Hz), 6.23 (dt, 1H, $C^{4'}H$, $J = 0.80$ Hz, $J = 3.40$ Hz), 7.09 (dd, 1H, C^6H, J = 2.00 Hz, $J = 8.21$ Hz), 7.18 (d, 1H, $C^{3'}H$, $J = 3.60$ Hz), 7.46 (d, 2H, C^4H, C^7H, $J = 8.41$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 12.17 CH_3, 21.89 CH_2, 28.51 $(CH_3)_3$, 38.49 C^{β}, 52.52 OCH_3, 54.80 C^{α}, 80.13 $C^{t\text{-Bu}}$, 107.37 $C^{4'}$, 110.51 C^7, 115.85 $C^{3'}$, 120.68 C^4, 126.42 C^6, 133.01 C^5, 141.20 C^9, 148.33 C^8, 149.78 $C^{2'}$, 153.33 C^2, 157.33 $C^{5'}$, 162.57 NHCO, 171.33 CO; MS *m/z* (FAB): 415 (MH^+); anal. calcd. for $C_{22}H_{26}N_2O_6$ (%): C, 63.76; H, 6.32; N, 6.76; found: C, 64.07; H, 6.46; N, 6.71.

2.1.2.2. N-Boc-3-[2-(2-benzofuryl)benzoxazol-5-yl]alanine methyl ester ((Bfur)Box-Ala). The Schiff base: yellow solid (65% yield), $R_f = 0.59$ (AcOEt/petroleum ether 2:5).

Purification: column chromatography (eluent—AcOEt/petroleum ether 1:5 (v/v)), RP-HPLC (gradient: 30–100% B in 120 min, λ = 223 nm), white solid (6% yield).

Identification: $R_f = 0.49$ (AcOEt/petroleum ether 2:5); $t_R = 42.9$ min (gradient: 30–100% B in 60 min, λ = 223 nm); mp 85–87 °C; IR (KBr): ν_{max} (cm^{-1}) 3344.5, 3125.3, 2977.1, 2932.4, 1740.8, 1690.9, 1632.3, 1596.5, 1529.7, 1478.4, 1436.4, 1391.9, 1367.5, 1345.5, 1321.8, 1289.6, 1255.5, 1167.9, 1056.1, 955.3, 881.9, 855.8, 813.0, 798.4, 776.0, 740.6, 719.4; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.44 (s, 9H, $(CH_3)_3$), 3.20-3.32 (m, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, OCH_3), 4.67 (q, 1H, $C^{\alpha}H$, $J = 6.01$ Hz, $J = 14.02$ Hz), 5.06 (d, 1H, NH, $J = 7.61$ Hz), 7.19 (dd, 1H, C^6H, $J = 1.60$ Hz, $J = 8.61$ Hz), 7.33–7.37 (m, 1H, $C^{6'}H$), 7.44–7.49 (m, 1H, $C^{5'}H$), 7.55 (d, 2H, C^4H, C^7H, $J = 8.81$ Hz), 7.63 (d, 1H, $C^{3'}H$, $J = 0.80$ Hz), 7.67 (dd, 1H, $C^{4'}H$, $J = 0.80$ Hz, $J = 8.41$ Hz), 7.73 (d, 1H, $C^{7'}H$, $J = 7.61$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 28.51 $(CH_3)_3$, 38.53 C^{β}, 52.56 OCH_3, 54.83 C^{α}, 80.10 $C^{t\text{-Bu}}$, 110.62 C^7, 110.83 $C^{3'}$, 112.33 $C^{7'}$, 121.23 C^4, 122.52 $C^{4'}$, 124.19 $C^{5'}$, 127.26 $C^{6'}$, 127.41 C^{3a}, 127.78 C^6, 133.56 C^5, 141.30 $C^{2'}$, 143.81 C^9, 149.91 C^8, 153.80 NHCO, 156.12 C^2, 157.92 C^{7a}, 172.26 CO; MS *m/z* (MALDI): 437 (MH^+); anal. calcd. for $C_{24}H_{24}N_2O_6$ (%): C, 66.04; H, 5.54; N, 6.42; found: C, 66.10; H, 5.46; N, 6.61.

2.1.2.3. N-Boc-3-[2-[2-(5′-methyl)thienyl]benzoxazol-5-yl]alanine methyl ester ((Tf)Box-Ala). The Schiff base: yellow solid (91% yield), $R_f = 0.61$ (AcOEt/petroleum ether 2:5).

Purification: eluent—AcOEt/petroleum ether 1:2 (v/v), white solid (82% yield).

Identification: $R_f = 0.44$ (AcOEt/petroleum ether 1:2); $t_R = 53.6$ min; mp 108–110 °C; IR (KBr): ν_{max} (cm^{-1}) 3333.3, 3074.2, 2968.6, 2933.2, 1736.1, 1687.5, 1627.3, 1584.0, 1526.5, 1478.8, 1453.8, 1436.8, 1391.3, 1367.5, 1348.0, 1324.9, 1294.1, 1274.6, 1254.7, 1223.5, 1195.8, 1164.0, 1057.4, 1007.7, 993.6, 916.0, 872.8, 851.2, 808.2, 793.9, 757.2, 736.3, 716.0, 678.3, 501.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.42 (s, 9H,

(CH_3)$_3$), 2.58 (s, 3H, CH_3), 3.15–3.26 (m, 2H, $C^{\beta}H_2$), 3.73 (s, 3H, OCH_3), 4.64 (q, 1H, $C^{\alpha}H$, J = 6.01 Hz, J = 7.61 Hz), 5.02 (d, 1H, NH, J = 8.01 Hz), 6.85 (dd, 1H, $C^{4'}H$, J = 0.80 Hz, J = 3.60 Hz), 7.08 (dd, 1H, $C^{6}H$, J = 1.60 Hz, J = 8.21 Hz), 7.44 (d, 2H, $C^{4}H$, $C^{7}H$, J = 8.41 Hz), 7.70 (d, 1H, $C^{3'}H$, J = 3.60 Hz).; ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 15.87 CH_3, 28.50 (CH_3)$_3$, 38.53 C^{β}, 52.52 OCH_3, 54.83 C^{α}, 81.74 $C^{t\text{-Bu}}$, 110.38 C^{7}, 120.37 C^{4}, 126.25 C^{6}, 127.00 $C^{4'}$, 127.16 $C^{3'}$, 130.59 $C^{2'}$, 132.86 C^{5}, 143.70 C^{9}, 144.07 $C^{5'}$, 147.04 C^{8}, 151.85 C^{2}, 159.26 NHCO, 172.37 CO; MS *m/z* (FAB): 417 (MH$^+$); anal. calcd. for $C_{21}H_{24}N_2O_5S$ (%): C, 60.56; H, 5.81; N, 6.73; S, 7.70; found: C, 60.98; H, 5.91; N, 6.58; S, 7.40.

2.1.2.4. N-Boc-3-[2-[2-(3′-methyl)thionaphthenyl]benzoxazol-5-yl]alanine methyl ester ((Btf)Box-Ala). The Schiff base: yellow solid (98% yield), R_f = 0.70 (AcOEt/petroleum ether 2:5).

Purification: eluent—AcOEt/petroleum ether 1:3 (v/v), white solid (65% yield).

Identification: R_f = 0.45 (AcOEt/petroleum ether 1:3); t_R = 39.8 min; mp 143-145 °C; IR (KBr): ν_{max} (cm^{-1}) 3365.1, 3055.4, 2969.9, 2936.7, 1743.1, 1692.8, 1612.0, 1571.2, 1505.0, 1479.2, 1433.8, 1388.8, 1361.6, 1337.2, 1319.3, 1260.2, 1210.8, 1171.3, 1058.9, 1020.3, 1003.0, 941.1, 904.6, 875.1, 822.9, 785.5, 753.9, 723.1, 711.1, 657.8; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.43 (s, 9H, (CH_3)$_3$), 2.94 (s, 3H, CH_3), 3.21–3.25 (m, 2H, $C^{\beta}H_2$), 3.74 (s, 3H, OCH_3), 4.65 (d, 1H, $C^{\alpha}H$, J = 7.61 Hz), 5.03 (d, 1H, NH, J = 8.01 Hz), 7.14 (dd, 1H, $C^{6}H$, J = 1.20 Hz, J = 8.41 Hz), 7.45–7.47 (m, 2H, $C^{5'}H$, $C^{6'}H$), 7.53 (d, 1H, $C^{7}H$, J = 8.41 Hz), 7.56 (s, 1H, $C^{4}H$), 7.85–7.89 (m, 2H, $C^{4'}H$, $C^{7'}H$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 13.73 CH_3, 28.52 (CH_3)$_3$, 38.62 C^{β}, 52.56 OCH_3, 54.87 C^{α}, 80.32 $C^{t\text{-Bu}}$, 110.64 C^{7}, 120.82 C^{4}, 122.79 $C^{4'}$, 123.45 $C^{7'}$, 124.89 C^{6}, 126.72 $C^{6'}$, 126.81 $C^{5'}$, 128.06 $C^{3'}$, 133.14 C^{5}, 136.77 $C^{2'}$, 140.41 C^{7a}, 140.80 C^{9}, 142.58 C^{3a}, 147.10 C^{8}, 149.68 C^{2}, 162.06 NHCO, 172.04 CO; MS *m/z* (MALDI): 467 (MH$^+$); anal. calcd. for $C_{25}H_{26}N_2O_5S$ (%): C, 64.38; H, 5.58; N, 6.01; S, 6.87; found: C, 64.68; H, 5.53; N, 6.08; S, 6.88.

2.1.2.5. N-Boc-3-[2-[2-(1′-methyl)pyrrolo]benzoxazol-5-yl]alanine methyl ester ((Pyr)Box-Ala). The Schiff base: brown oil, R_f = 0.11 (AcOEt/petroleum ether 2:5).

Purification: eluent—AcOEt/petroleum ether 2:5 (v/v), white solid (64% yield).

Identification: R_f = 0.40 (AcOEt/petroleum ether 2:5); t_R = 39.2 min; mp 110-112 °C; IR (KBr): ν_{max} (cm^{-1}) 3365.0, 3122.4, 3103.2, 2988.5, 2961.6, 2948.0, 2922.2, 1743.7, 1691.8, 1627.6, 1588.9, 1510.7, 1475.5, 1448.1, 1436.8, 1411.6, 1392.6, 1362.2, 1338.3, 1317.4, 1260.7, 1212.6, 1169.1, 1074.1, 1062.3, 1021.9, 1006.9, 960.1, 922.9, 875.7, 862.3, 820.5, 790.3, 765.7, 742.0, 723.8, 663.5, 614.6, 576.1, 560.4; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.42 (s, 9H, (CH_3)$_3$), 3.14–3.25 (m, 2H, $C^{\beta}H_2$), 3.73 (s, 3H, OCH_3), 4.14 (s, 3H, N–CH_3), 4.63 (q, 1H, $C^{\alpha}H$, J = 6.01 Hz, J = 13.62 Hz), 5.01 (d, 1H, NH, J = 8.01 Hz), 6.25 (dd, 1H, $C^{4'}H$, J = 2.40 Hz, J = 4.21 Hz), 6.87 (t, 1H, $C^{5'}H$, J = 2.00 Hz), 7.03–7.08 (m, 2H, $C^{6}H$, $C^{3'}H$), 7.42 (d, 1H, $C^{7}H$, J = 8.41 Hz), 7.44 (s, 1H, $C^{4}H$); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 28.45 (CH_3)$_3$, 37.17 *N*-CH_3, 38.53 C^{β}, 52.46 OCH_3, 54.83 C^{α}, 81.07 $C^{t\text{-Bu}}$, 109.01 $C^{4'}$, 110.06 C^{7}, 115.34 $C^{3'}$, 120.02 C^{4}, 120.76 $C^{5'}$, 125.57 C^{6}, 128.76 $C^{2'}$, 132.35 C^{5}, 137.14 C^{9}, 142.86 C^{8}, 150.71 C^{2}, 162.86 NHCO, 172.86 CO; MS *m/z* (MALDI): 400 (MH$^+$); anal. calcd. for $C_{21}H_{25}N_3O_5$ (%): C, 63.14; H, 6.31; N, 10.52; found: C, 63.33; H, 6.37; N, 10.46.

2.1.2.6. N-Boc-3-[2-(2-imidazolyl)benzoxazol-5-yl]alanine methyl ester ((2-im)Box-Ala). The Schiff base: brown oil, R_f = 0.12 (AcOEt/petroleum ether 2:5).

Purification: eluent—AcOEt, recrystallization from the mixture of AcOEt/EtOH, grey solid (30% yield).

Identification: R_f = 0.51 (AcOEt); t_R = 31.2 min; mp 221-223 °C; IR (KBr): ν_{max} (cm^{-1}) 3344.7, 3163.7, 3126.4, 2976.8, 2925.0, 2875.4, 2792.1, 2726.8, 2628.3, 1737.4, 1690.0, 1636.4, 1596.6, 1526.2, 1478.4, 1436.8, 1388.7, 1368.6, 1346.5, 1322.7, 1293.4, 1271.4, 1257.2, 1220.4, 1165.0, 1106.4, 1085.3, 1059.9, 993.7, 971.5, 919.6, 868.0, 853.2, 813.5, 796.6, 769.0, 735.0, 685.0; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H (ppm) 1.30 (s, 9H, (CH_3)$_3$), 2.96–3.18 (m, 2H, $C^{\beta}H_2$), 3.63 (s, 3H, OCH_3), 4.23–4.28 (m, 1H, $C^{\alpha}H$), 7.31–7.35 (m, 3H, $C^{4'}H$, $C^{5'}H$, $C^{6}H$), 7.67–7.71 (m, 2H, $C^{4}H$, $C^{7}H$), 13.59 (s, 1H, $N^{1'}H$); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C (ppm) 28.02 (CH_3)$_3$, 36.26 C^{β}, 51.73 OCH_3, 55.37 C^{α}, 78.22 $C^{t\text{-Bu}}$, 110.39 C^{7}, 120.02 C^{4}, 124.05 $C^{4'}$, $C^{5'}$, 126.75 C^{6}, 131.95 C^{5}, 134.66 C^{2}, 134.80 $C^{2'}$, 140.99 C^{9}, 148.57 C^{8}, 155.34 NHCO, 172.41 CO; MS *m/z* (FAB): 388 ((M + 2H)$^+$); anal. calcd. for $C_{19}H_{22}N_4O_5$ (%): C, 59.06; H, 5.74; N, 14.50; found: C, 58.83; H, 5.70; N, 14.20.

2.1.2.7. N-Boc-3-[2-(3-isoquinolinyl)benzoxazol-5-yl]alanine methyl ester ((3-iQ)Box-Ala). 3-isoquinolinecarboxaldehyde was synthesized according to the literature procedure [42] (28% yield).

The Schiff base: lightbrown solid (86% yield), R_f = 0.31 (CH_2Cl_2/MeOH/AcOH 100:10:1).

Purification: eluent—CH_2Cl_2/MeOH 50:1 (v/v), recrystallization from the mixture of CH_2Cl_2/petroleum ether, pinkish solid (6% yield).

Identification: R_f = 0.26 (CH_2Cl_2/MeOH 50:1); t_R = 48.1 min; mp 182-183 °C; IR (KBr): ν_{max} (cm^{-1}) 3335.8, 3005.2, 2976.1, 2932.5, 1752.7, 1703.3, 1550.5, 1435.2, 1364.5, 1148.8, 1060.3, 979.8, 763.1; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H (ppm) 1.31 (s, 9H, (CH_3)$_3$), 3.00–3.21 (m, 2H, $C^{\beta}H_2$), 3.65 (s, 3H, OCH_3), 4.28 (s, 1H, $C^{\alpha}H$), 7.29 (d, 1H, NH, J = 7.61 Hz), 7.36 (dd, 1H, $C^{6}H$, J = 1.60 Hz, J = 8.41 Hz), 7.75 (d, 2H, $C^{4}H$, $C^{7}H$, J = 8.41 Hz), 7.82–7.93 (m, 2H, $C^{6'}H$, $C^{7'}H$), 8.25 (q, 2H, $C^{5'}H$, $C^{8'}H$, J = 8.01 Hz, J = 12.22 Hz), 8.87 (s, 1H, $C^{4'}H$), 9.50 (s, 1H, $C^{1'}H$); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C (ppm) 27.96 (CH_3)$_3$, 36.30 C^{β}, 51.64 OCH_3, 55.33 C^{α}, 78.17 $C^{t\text{-Bu}}$, 110.47 C^{7}, 120.39 C^{4}, 121.13 $C^{4'}$, 126.99 C^{6}, 127.57 $C^{5'}$, 127.72 $C^{7'}$, 128.80 $C^{8'}$, 129.30 $C^{6'}$, 131.44 C^{5}, 134.57 $C^{10'}$, 135.08 $C^{9'}$, 138.92 $C^{3'}$, 141.48 C^{9}, 149.27 C^{8}, 153.04 $C^{1'}$, 153.12 NHCO, 162.04 C^{2}, 172.30 CO; MS *m/z* (MALDI): 448 (MH$^+$); anal. calcd. for $C_{25}H_{25}N_3O_5$ (%): C, 67.10; H, 5.63; N, 9.39; found: C, 67.20; H, 5.35; N, 9.20.

2.1.2.8. N-Boc-3-[2-(3-quinolinyl)benzoxazol-5-yl]alanine methyl ester ((3-Q)Box-Ala). The Schiff base: yellow solid (96% yield), $R_f = 0.84$ (AcOEt).

Purification: eluent—AcOEt/petroleum ether 1:1 (v/v), white solid (50% yield).

Identification: $R_f = 0.54$ (AcOEt/petroleum ether 1:1); $t_R = 47.1$ min; mp 154-155 °C; IR (KBr): ν_{max} (cm^{-1}) 3209.6, 3025.6, 2975.7, 1745.1, 1711.7, 1627.4, 1477.5, 1449.2, 1435.0, 1281.8, 1133.6, 1061.9, 1049.9, 974.4, 714.6; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.43 (s, 9H, $(CH_3)_3$), 3.20–3.32 (m, 2H, $C^{\beta}H_2$), 3.75 (s, 3H, OCH_3), 4.67 (q, 1H, $C^{\alpha}H$, $J = 6.41$ Hz, $J = 13.62$ Hz), 5.05 (d, 1H, NH, $J = 8.01$ Hz), 7.19 (dd, 1H, C^6H, $J = 2.00$ Hz, $J = 8.01$ Hz), 7.58 (d, 2H, C^4H, C^7H, $J = 8.41$ Hz), 7.64–7.68 (m, 1H, $C^{6'}H$), 7.82–7.86 (m, 1H, $C^{7'}H$), 7.98 (d, 1H, $C^{5'}H$, $J = 7.61$ Hz), 8.20 (d, 1H, $C^{8'}H$, $J = 8.41$ Hz), 9.00 (d, 1H, $C^{4'}H$, $J = 2.00$ Hz), 9.73 (d, 1H, $C^{2'}H$, $J = 2.40$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 28.50 $(CH_3)_3$, 38.60 C^{β}, 52.58 OCH_3, 54.85 C^{α}, 73.50 $C^{t\text{-Bu}}$, 110.86 C^7, 121.06 C^4, 127.27 C^6, 127.95 $C^{4'}$, 128.95 $C^{6'}$, 129.88 $C^{3'}$, 131.60 $C^{10'}$, 133.82 C^5, 135.56 $C^{8'}$, 142.00 $C^{7'}$, 143.00 $C^{5'}$, 144.67 $C^{2'}$, 146.25 C^9, 148.77 C^8, 149.55 $C^{9'}$, 151.67 NHCO, 165.27 C^2, 171.65 CO; MS *m/z* (MALDI): 448 (MH^+); anal. calcd. for $C_{25}H_{25}N_3O_5$ (%): C, 67.10; H, 5.63; N, 9.39; found: C, 67.26; H, 5.81; N, 9.35.

2.1.2.9. N-Boc-3-[2-(4-quinolinyl)benzoxazol-5-yl]alanine methyl ester ((4-Q)Box-Ala). 4-Quinolinecarboxaldehyde: It was synthesized according to modified literature procedure [43]. To the solution of 4-methylquinoline (0.66 ml, 5 mmol) in 1,4-dioxane (6 ml) selenium dioxide (0.56 g, 5.06 mmol) was added and the reation mixture was refluxed for 8 h. Then the solvent was evaporated in vacuo and the product was isolated by means of column chromatography using as an eluent AcOEt. Recrystallization from the mixture of AcOEt/petroleum ether gave the product as a yellow solid (0.39 g, 2.51 mmol, 52% yield).

Identification: $R_f = 0.23$ (AcOEt/petroleum ether 1:1); $t_R = 24.6$ min; mp 135-136 °C; IR (KBr): ν_{max} (cm^{-1}) 3064.2, 2853.7, 2742.0, 1702.1, 1614.5, 1587.1, 1508.6, 1464.6, 1352.1, 1300.0, 1265.8, 1236.8, 1213.3, 1161.2, 1138.6, 1077.3, 1046.4, 884.4, 845.6, 813.9, 760.9, 709.7, 649.9; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.73-7.89 (m, 3H, $C^{6'}H$, $C^{7'}H$, $C^{8'}H$), 8.23 (dd, 1H, $C^{4'}H$, $J = 0.80$ Hz, $J = 8.41$ Hz), 9.03 (m, 1H, $C^{2'}H$), 9.21 (d, 1H, $C^{5'}H$, $J = 4.41$ Hz), 10.53 (s, 1H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 124.14 $C^{3'}$, 124.64 $C^{10'}$, 126.01 $C^{5'}$, 129.64 $C^{7'}$, 130.16 $C^{8'}$, 130.45 $C^{6'}$, 137.07 $C^{4'}$, 149.41 $C^{9'}$, 150.61 $C^{2'}$, 199.04 CHO; MS *m/z* (FAB): 159 ($(M + 2H)^+$); anal. calcd. for $C_{10}H_7NO$ (%): C, 76.42; H, 4.49; N, 8.91; found: C, 76.41; H, 4.41; N, 8.77.

The Schiff base: brown oil, $R_f = 0.39$ (AcOEt).

Purification: eluent—(1) AcOEt, (2) AcOEt/petroleum ether 1:1 (v/v), white solid (32% yield).

Identification: $R_f = 0.73$ (AcOEt); $t_R = 53.9$ min; mp 133-134 °C; IR (KBr): ν_{max} (cm^{-1}) 3363.6, 2968.9, 1758.2, 1696.1, 1536.6, 1505.9, 1479.6, 1440.7, 1390.6, 1368.0, 1350.2, 1325.8, 1271.6, 1251.8, 1215.0, 1173.7, 1124.6, 1114.3, 1054.8, 1000.9, 977.7, 931.9, 923.6, 908.4, 851.1, 798.5, 764.4, 687.6, 663.5, 634.7, 607.0; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.43 (s, 9H, $(CH_3)_3$), 3.21–3.34 (m, 2H, $C^{\beta}H_2$), 3.77 (s, 3H, OCH_3), 4.68 (q, 1H, $C^{\alpha}H$, $J = 6.01$ Hz, $J = 13.62$ Hz), 5.08 (d, 1H, NH, $J = 8.41$ Hz), 7.24 (d, 1H, C^6H, $J = 1.60$ Hz), 7.61 (d, 1H, C^7H, $J = 8.41$ Hz), 7.69 (s, 1H, C^4H), 7.75–7.86 (m, 2H, $C^{6'}H$, $C^{7'}H$), 8.22–8.24 (m, 2H, $C^{3'}H$, $C^{8'}H$), 9.10 (d, 1H, $C^{2'}H$, $J = 4.41$ Hz), 9.45 (dd, 1H, $C^{5'}H$, $J = 1.20$ Hz, $J = 8.61$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 28.51 $(CH_3)_3$, 38.66 C^{β}, 52.62 OCH_3, 54.88 C^{α}, 80.74 $C^{t\text{-Bu}}$, 110.96 C^7, 121.60 $C^{3'}$, 124.82 C^4, 126.53 C^6, 127.97 $C^{10'}$, 128.58 $C^{6'}$, 130.17 $C^{5'}$, 130.45 $C^{7'}$, 131.25 $C^{8'}$, 133.53 C^5, 142.67 $C^{4'}$, 149.45 C^9, 149.66 $C^{9'}$, 150.04 C^8, 154.22 $C^{2'}$, 155.34 CONH, 161.22 C^2, 172.59 CO; MS *m/z* (MALDI): 448 (MH^+); anal. calcd. for $C_{25}H_{25}N_3O_5$ (%): C, 67.10; H, 5.63; N, 9.39; found: C, 67.15, 5.68; N, 9.30.

2.1.2.10. N-Boc-3-[2-(6-quinolinyl)benzoxazol-5-yl]alanine methyl ester ((6-Q)Box-Ala). 6-Quinolinecarboxaldehyde: 6-methylquinoline (1 g, 7 mmol) was heated to 180 °C. Selenium dioxide (0.78 g, 7 mmol) was added in small portions with stirring during 1 h. The reaction mixture was refluxed at about 200 °C for additional 1 h and then cooled to the room temperature and extracted with boiling diethyl ether. After solvent evaporation, the product was isolated by means of column chromatography using as an eluent AcOEt. Recrystallization from the mixture of AcOEt/petroleum ether gave the product as an orange solid (0.29 g, 1.82 mmol, 26% yield).

Identification: $t_R = 15.2$ min; mp 75–77 °C; IR (KBr): ν_{max} (cm^{-1}) 3039.9, 1697.3, 1621.1, 1460.6, 894.3, 773.4; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 7.52–7.56 (m, 1H, $C^{3'}H$), 8.20–8.24 (m, 2H, $C^{4'}H$, $C^{8'}H$), 8.33–8.37 (m, 2H, $C^{5'}H$, $C^{7'}H$), 9.06–9.07 (m, 1H, $C^{2'}H$), 10.22 (s, 1H, CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 122.76 $C^{3'}$, 127.29 $C^{5'}$, 128.26 $C^{10'}$, 131.40 $C^{7'}$, 134.16 $C^{8'}$, 134.87 $C^{6'}$, 137.99 $C^{4'}$, 151.46 $C^{9'}$, 153.68 $C^{2'}$, 192.01 CHO; MS *m/z* (FAB): 159 ($(M + 2H)^+$); anal. calcd. for $C_{10}H_7NO$ (%): C, 76.42; H, 4.49; N, 8.91; found: C, 76.27; H, 4.44; N, 8.82.

The Schiff base: yellow solid (99% yield), $R_f = 0.17$ (AcOEt/petroleum ether 1:1).

Purification: eluent—AcOEt, white solid (59% yield).

Identification: $R_f = 0.53$ (AcOEt); $t_R = 42.7$ min; mp 127-128 °C; IR (KBr): ν_{max} (cm^{-1}) 3362.7, 3009.5, 2978.0, 2928.5, 1739.8, 1691.5, 1554.0, 1435.0, 1274.0, 1119.7, 1054.9, 952.6, 720.3; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H (ppm) 1.43 (s, 9H, $(CH_3)_3$), 3.20–3.31 (m, 2H, $C^{\beta}H_2$), 3.76 (s, 3H, OCH_3), 4.67 (q, 1H, $C^{\alpha}H$, $J = 5.61$ Hz, $J = 13.42$ Hz), 5.07 (d, 1H, NH, $J = 8.41$ Hz), 7.18 (dd, 1H, C^6H, $J = 2.00$ Hz, $J = 8.41$ Hz), 7.49–7.52 (m, 1H, $C^{3'}H$), 7.56 (d, 2H, C^4H, $C^{5'}H$, $J = 8.81$ Hz), 8.25 (d, 1H, $C^{4'}H$, $J = 8.81$ Hz), 8.31 (d, 1H, C^7H, $J = 8.41$ Hz), 8.55 (dd, 1H, $C^{7'}H$, $J = 2.00$ Hz, $J = 9.01$ Hz), 8.75 (d, 1H, $C^{8'}H$, $J = 2.00$ Hz), 9.01 (dd, 1H, $C^{2'}H$, $J = 1.60$ Hz, $J = 4.21$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C (ppm) 28.51 $(CH_3)_3$, 38.58 C^{β}, 52.59 OCH_3, 54.84 C^{α}, 80.57 $C^{t\text{-Bu}}$, 110.81 C^7, 120.98 C^4, 122.33 $C^{3'}$, 125.35 C^6, 127.03 $C^{5'}$, 127.86 $C^{6'}$, 128.13 $C^{7'}$, 128.25 $C^{10'}$, 130.70 $C^{8'}$, 133.18 C^5, 137.10 $C^{4'}$, 142.86 C^9, 148.49 C^8, 149.69 $C^{2'}$, 150.36 $C^{9'}$, 152.28 CONH, 163.20

C^2, 172.37 CO; MS *m/z* (MALDI): 448 (MH^+); anal. calcd. for $C_{25}H_{25}N_3O_5$ (%): C, 67.10; H, 5.63; N, 9.39; found: C, 67.02; H, 5.65; N, 9.23. N-Boc-3-[2-(8-quinolinyl)benzoxazol-5-yl]alanine methyl ester ((8-Q)Box-Ala) [41].

2.2. Spectroscopic measurements

Absorption spectra of all compounds studied in methanol, acetonitrile and methylcyclohexane were measured using a Perkin-Elmer Lambda 40P spectrophotometer whereas emission spectra were measured using a Perkin-Elmer LS 50B spectrofluorimeter. The solvents used were either spectroscopic or HPLC grade. Quantum yields (QY) were calculated using as a reference quinine sulphate in 0.5 M H_2SO_4 (QY = 0.53 ± 0.02) or 2-aminopyridine in 0.05 M H_2SO_4 (QY = 0.605) and were corrected for different refractive indexes of solvents [46]. In all fluorometric measurements the optical density of the solution does not exceed 0.1.

Fluorescence intensity decays were collected using a time-correlated single-photon counting apparatus (the pico/femtosecond laser system, Ti:sapphire 'Tsunami' laser pumped with an argon ion laser 'BeamLok' and thermoelectrically cooled MCP-PTM R3809U-05) at the Centre of Ultrafast Laser Spectroscopy, Adam Mickiewicz University, Poznań, Poland [47]. The excitation wavelength was 270 nm, whereas the fluorescence intensity decay was measured at the maximum of the emission band which wavelength was selected by means of monochromator (about 7.5 nm bandwidth). The fluorescence intensity decays were recorded at 20 °C with a polarizer set up at a magic-angle. The Ludox solution was used as a reference. The fluorescence lifetimes were calculated using software delivered by Edinburgh Analytical Instruments.

Fluorescence intensity decay data were fitted by the iterative convolution to the sum of exponents:

$$I(t) = \sum_i \alpha_i \exp\left(-\frac{t}{\tau_i}\right) \quad (1)$$

where α_i and τ_i are the pre-exponential factor and fluorescence lifetime, respectively. The average fluorescence lifetime as well as the fluorescence (k_f) and nonradiative (k_{nr}) rate constants were calculated from the equations:

$$\tau_{av} = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i}, \quad k_f = \frac{QY}{\tau_{av}}, \quad k_{nr} = \frac{(1 - QY)}{\tau_{av}} \quad (2)$$

3. Results and discussion

Absorption and fluorescence spectra of all compounds studied in acetonitrile are presented in Figs. 2 and 3 whereas calculated molar absorption coefficients, absorption and emission maxima, fluorescence quantum yield, fluorescence lifetime, average fluorescence lifetime for multi-exponential decays and quality of the fit in all studied solvents are presented in Table 1. Absorption maxima of the compounds studied are in the range from 300 to 340 nm whereas emission maxima are in the range from 340 to 420 nm. The increase of the solvent polarity and its

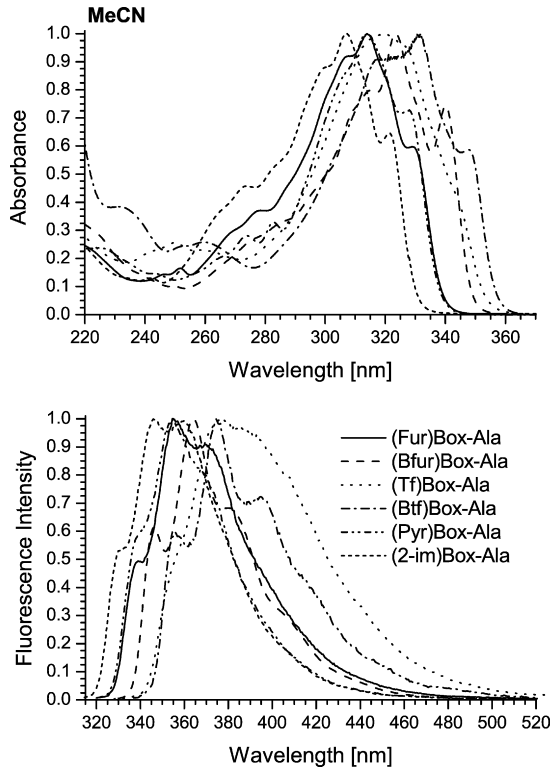

Fig. 2. Normalized to unity absorption (upper panel) and emission (lower panel) spectra of 3-(2-benzoxazol-5-yl)alanine derivatives in acetonitrile.

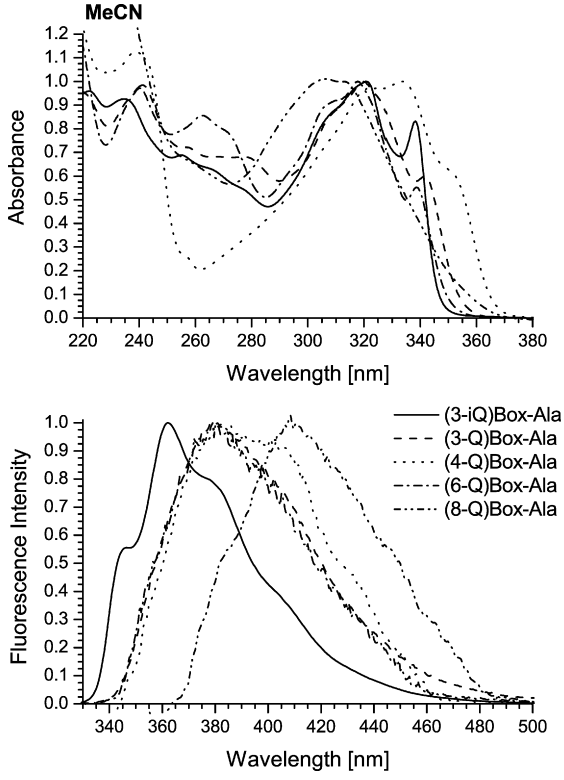

Fig. 3. Normalized to unity absorption (upper panel) and emission (lower panel) spectra of 3-[2-(quinolinyl)benzoxazol-5-yl]alanine derivatives in acetonitrile.

Table 1
Absorption (λ_{abs}) and emission (λ_{em}) maxima, molar absorption coefficient (ε), fluorescence quantum yield (ϕ), fluorescence lifetimes (τ), pre-exponential factors (α), quality of the fit (X^2_R), fluorescence (k_f) and nonradiative (k_{nr}) rate constants of 3-[2-(heteroaromatic)benzoxazol-5-yl]alanine derivatives in MeOH, MeCN and methylcyclohexane

Compound	λ_{abs} (nm) (ε, dm^3 mol^{-1} cm^{-1})	λ_{em} (nm)	ϕ	τ_i (ns)	α_i	$<\tau>^a$ (ns)	X^2_R	k_f ($\times 10^8$/s)	k_{nr} ($\times 10^8$/s)
MeOH									
(Fur)Box-Ala	246, 278, 310, 316 (32090), 333	342, 359, 372	0.863	1.43	1.000	–	1.10	6.03	0.96
(Bfur)Box-Ala	277, 286, 316, 325 (38720), 341	349, 363, 383	0.862	1.12	1.000	–	1.12	7.70	1.23
(Tf)Box-Ala	250, 260, 320 (31060)	354, 377, 395	0.644	1.69	1.000	–	1.18	3.81	2.11
(Btf)Box-Ala	232, 259, 320, 331 (46290), 347	360, 378, 397	0.174	0.30 0.28 0.66	1.000 0.975 0.025	0.30	2.82 1.18	5.80	27.53
(Pyr)Box-Ala	225, 244, 274, 283, 306, 313 (37250), 327	340, 356, 372	0.717	1.37	1.000	–	1.12	5.23	2.07
(2-im)Box-Ala	242, 274, 300, 307 (28860), 322	334, 351, 361	0.906	1.36	1.000	–	1.00	6.66	0.69
(3-iQ)Box-Ala	222, 236, 255, 267, 278, 318, 322 (30610), 339	349, 365, 382	0.631	2.18	1.000	–	1.08	2.89	1.69
(3-Q)Box-Ala	242, 258, 282, 321 (25850), 342	386	0.502	1.74 1.81 0.92	1.000 0.868 0.132	1.75	1.31 1.15	2.87	2.85
(4-Q)Box-Ala	240, 257, 324, 335 (17500), 354	411	0.314	0.54 0.44 0.73	1.000 0.782 0.218	0.53	42.45 1.06	5.92	12.94
(6-Q)Box-Ala	241, 266, 309, 319 (28970), 340	386	0.199	1.04 0.74 1.24	1.000 0.562 0.438	1.02	2.93 1.15	1.95	7.85
(8-Q)Box-Ala	262, 314 (15330)	354, 412	0.076	0.64 0.62 1.98	1.000 0.987 0.013	0.67	1.59 1.14	1.13	13.79
MeCN									
(Fur)Box-Ala	246, 252, 269, 278, 307, 314 (33160), 329	339, 355, 369	0.661	1.32	1.000	–	1.02	5.01	2.57
(Bfur)Box-Ala	276, 286, 308, 314, 323 (43770), 340	346, 368, 380	0.507	1.08	1.000	–	1.07	4.69	4.56
(Tf)Box-Ala	250, 260, 314, 319 (30620), 326	351, 376, 393	0.582 0.87	1.49 1.57 0.186	1.000 0.814	1.49	1.28 1.04	3.91	2.81
(Btf)Box-Ala	232, 259, 318, 331 (48833), 348	356, 375, 395 0.75	0.222 0.000	0.31 0.30	1.000 1.000	0.30	1.40 1.20	7.40	25.93
(Pyr)Box-Ala	225, 244, 274, 283, 306, 314 (38050), 328	338, 354, 369	0.587	1.31	1.000	–	0.96	4.48	3.15
(2-im)Box-Ala	242, 273, 299, 307 (29090), 321	331, 346, 359	0.663	1.30	1.000	–	1.10	5.10	2.59
(3-iQ)Box-Ala	222, 235, 255, 266, 321 (30510), 338	345, 362, 378	0.640	2.03	1.000	–	1.11	3.15	1.77
(3-Q)Box-Ala	241, 257, 278, 308, 320 (27450), 342	381	0.508	1.39 1.49 0.95	1.000 0.739 0.261	1.39	1.22 0.98	3.65	3.54
(4-Q)Box-Ala	239, 257, 322, 334 (18900), 351	383, 402	0.060	0.12 0.11 0.25	1.000 0.964 0.036	0.12	1.82 1.25	5.00	78.33

Table 1 (*Continued*)

Compound	λ_{abs} (nm) (ε, dm^3 mol^{-1} cm^{-1})	λ_{em} (nm)	ϕ	τ_i (ns)	α_i	$<\tau>$[a] (ns)	X^2_R	k_f ($\times 10^8$/s)	k_{nr} ($\times 10^8$/s)
(6-Q)Box-Ala	240, 262, 271, 308, 318 (28990), 339	379	0.037	0.35 0.22 0.53 0.12 0.31 0.72	1.000 0.818 0.182 0.364 0.591 0.045	0.33 0.33	382 1.31 1.08	1.12	29.18
(8-Q)Box-Ala	261, 313 (13720)	354, 408	0.024	0.20 0.15 4.10[c]	1.000 0.980 0.020	1.55	36.99 1.01	0.15 (1.60)[b]	6.30 (65.07)[b]
Methylcyclohexane									
(Fur)Box-Ala	245, 269, 278, 306, 314 (30900), 319, 329	333, 351, 366	0.508	1.17	1.000	–	1.12	4.34	4.21
(Bfur)Box-Ala	250, 276, 286, 300, 309, 315, 324 (44960), 341	345, 364, 381	1.000	0.86	1.000	–	1.09	11.63	0
(Tf)Box-Ala	250, 260, 313, 319 (28070), 326, 344	348, 373, 387	0.554	1.20 1.28 0.74	1.000 0.746 0.254	1.19	1.39 1.04	4.66	3.75
(Btf)Box-Ala	234, 261, 317, 323, 332 (49270), 349	353, 373, 394	0.228	0.28 0.27 0.99	1.000 1.000 0.000	0.27	1.43 1.10	8.44	28.59
(Pyr)Box-Ala	227, 244, 274, 283, 306, 314 (40250), 320, 329	332, 349, 364	0.561	1.17	1.000	–	1.13	4.79	3.75
(2-im)Box-Ala	–[d]	327, 343, 357	0.827	1.23 1.17 4.28 1.19 4.69[c] 0.22	1.000 0.985 0.015 0.839 0.000 0.161	1.34 1.15	6.64 1.60 1.17	7.19	1.50
(3-iQ)Box-Ala	222, 233, 257, 307, 321 (32530), 338	343, 361, 380, 404	0.721	1.70	1.000	–	1.09	4.24	1.64
(3-Q)Box-Ala	242, 257, 266, 278, 308, 321 (27160), 328, 344	354, 370, 392	0.079	0.27 0.13 0.35 0.10 0.26 0.50	1.000 0.708 0.292 0.489 0.447 0.064	0.25 0.26	239 1.23 1.06	3.04	35.42
(4-Q)Box-Ala	243, 257, 322, 337 (17680), 355	355, 381, 450	0.002	0.10 0.03 0.18	1.000 0.857 0.143	0.10	36.25 1.09	0.20	99.80
(6-Q)Box-Ala	241, 264, 273, 307, 312, 319 (29410), 325, 342	364	0.006	2.50 2.53 0.04 2.95[c] 0.04 0.35	1.000 0.011 0.989 0.013 0.960 0.027	1.07 1.44 (0.10)[e]	1136 1.43 1.02	0.04 (0.60)[b]	6.90 (99.40)[b]
(8-Q)Box-Ala	234, 260, 316 (13810)	353, 373, 394, 413	0.038	0.19 0.14 0.76	1.000 0.969 0.031	0.23	16.71 1.05	1.65	41.83

[a] The average fluorescence lifetime.
[b] The fluorescence and nonradiative rate constants calculated using the average fluorescence lifetime which do not include the longest fluorescence lifetime component.
[c] The longest fluorescence lifetime component (about 3–4 ns) with very low contribution is probably impurity fluorescence.
[d] Too low solubility.
[e] The average fluorescence lifetime calculated without taking into account the longest fluorescence lifetime component.

ability to form hydrogen bonds influences the emission spectra causing their bathochromic shift whereas the absorption spectra remain almost unchanged. It indicates that electric dipole moment in the excited state is higher than in the ground state because of a charge transfer from the substituent to the benzoxazole moiety [48]. For the quinoline (azaaromatic) derivatives, hipsochromic shift of the absorption spectrum is observed for polar aprotic solvent (MeCN) whereas protic one (MeOH) causes its bathochromic shift. Such behaviour of the absorption spectrum indicates that the long-wavelength transition in the 3-[2-(quinoline)benzoxazol-5-yl]alanine derivatives has $n \rightarrow \pi^*$ character. Moreover, bathochromic shift of the emission spectra as well as the increase of the fluorescence quantum yield with the increase of the solvent polarity and its ability to form hydrogen bond (Table 1) indicate on $\pi^* \rightarrow \pi$ character of the radiative transition [49,50].

Absorption and emission spectra of compounds studied possess the most distinct vibrational structure in methylcyclohexane. In polar solvents it becomes more diffuse. However, the vibrational structure of the absorption spectra of all quinoline derivatives as well as the emission spectra of (3-iQ)Box-Ala and (3-Q)Box-Ala is similar to that of *N*-Boc-3-(2-(2-naphthyl)benzoxazol-5-yl)alanine methyl ester spectra [36] as a result of the isoelectronic character of the substituents in position 2 of the benzoxazole ring (naphthyl and quinoline). The molar absorption coefficients as well as the fluorescence quantum yields of majority of compounds studied are high whereas the fluorescence lifetimes are short indicating on the high probability of the radiative transitions (Table 1). The fluorescence rate constants are in the range from 3 to about 8×10^8/s, except for some quinoline derivatives. In all studied solvents the highest molar absorption coefficients (about $50\,000\,dm^3\,mol^{-1}\,cm^{-1}$) is observed for *N*-Boc-3-[2-[2-(3′-methyl)thionaphthenyl]benzoxazol-5-yl]alanine methyl ester ((Btf)Box-Ala) whereas the lowest (about $14\,000\,dm^3\,mol^{-1}\,cm^{-1}$) for *N*-Boc-3-[2-(8-quinolinyl)benzoxazol-5-yl]alanine methyl ester ((8-Q)Box-Ala). The character of the solvent influences on the values of molar absorption coefficients. For (Bfur)Box-Ala, (Pyr)Box-Ala, (Btf)Box-Ala, (2-im)Box-Ala, (3-Q)Box-Ala and (6-Q)Box-Ala, they are higer in more polar solvents whereas the opposite is true for (Tf)Box-Ala. In all other cases, it is difficult to find any distinct relationship.

Fluorescence quantum yields of compounds studied are relatively high, except for quinoline derivatives. The highest fluorescence quantum yield is observed for (Bfur)Box-Ala in methylcyclohexane (1.000) and the lowest for (4-Q)Box-Ala (0.002) in the same solvent. For the majority of studied compounds, the fluorescence quantum yield is higher in more polar solvents, except for (Btf)Box-Ala and (3-iQ)Box-Ala for which the opposite relationship is observed. Relatively low fluorescence quantum yield of some quinoline derivatives in non-polar solvent suggests that the emissive state (transition) possesses $n \rightarrow \pi^*$ character, for which the radiationless deactivations are more efficient than radiative ones ($k_{nr} \gg k_f$) (Table 1), on the contrary to the $\pi^* \rightarrow \pi$ transition [49]. However, in more polar and/or protic solvents the state inversion takes place and emission is the $\pi \leftarrow \pi^*$ [51] resulting in higher fluorescence quantum yields in those solvents (Table 1). High fluorescence quantum yield of all the other compounds studied in which the ring heteroatom is bounded to carbon atoms by single bonds is probably a result of $\pi^* \rightarrow \pi$ transition because non-bonding orbital of the heteroatom, which is perpendicular to the ring plane, overlays with π orbitals of the neighbouring carbon atoms [49,50].

Most of the compounds studied, especially quinoline derivatives, have multi-exponential fluorescence intensity decays which may be a result of the emission from two states or the presence of different conformers or different complexes with solvent molecule(s) in the excited state. In those cases, the decay is more heterogenous in polar aprotic solvent than in the protic one (Table 1). The fluorescence lifetimes of the compounds studied are rather short (about 1–2 ns) (Table 1). The longest fluorescence lifetime has (3-iQ)Box-Ala in MeOH ($\tau = 2.18$ ns) whereas the shortest—(4-Q)Box-Ala in methylcyclohexane ($<\tau> = 0.10$ ns). In most cases, the fluorescence lifetime lengthens with the increase of the solvent polarity which also confirms a charge transfer from the substituent to the benzoxazole ring in the excited state.

The character of the substituent in position 2 of the benzoxazole ring also influences on the photophysical properties of the compounds studied. The absorption and emission bands shift batochromically with a change of the heteroatom in the substituent in the order nitrogen < oxygen < sulphur ((2-im)Box-Ala < (Pyr)Box-Ala < (Fur)Box-Ala < (Tf)Box-Ala and (Bfur)Box-Ala < (Btf)Box-Ala). It is probably connected with the differences in electronegativity of those heteroatoms. The molar absorption coefficients increase in the order (Pyr)Box-Ala > (Fur)Box-Ala > (Tf)Box-Ala > (2-im)Box-Ala and (Btf)Box-Ala > (Bfur)Box-Ala whereas the fluorescence quantum yields in the order (2-im)Box-Ala > (Fur)Box-Ala > (Pyr)Box-Ala > (Tf)Box-Ala (in MeOH and MeCN) and (Bfur)Box-Ala > (Btf)Box-Ala (in all solvents studied) (Table 1).

The size of the substituent in position 2 of the 3-(benzoxazol-5-yl)alanine derivative is also important as in the case of derivatives with aromatic hydrocarbon groups [36]. The bigger is the aromatic substituent, the more bathochromically shifted the absorption and emission spectra are (Table 1) as a result of greater delocalization of the electrons. Also, the increase of the molar absorption coefficient value, the decrease of the fluorescence quantum yield (except for (Pyr)Box-Ala and (3-iQ)Box-Ala) and in most cases the shortening of the fluorescence lifetime regardless of the heteroatom present in the substituent are observed (Table 1).

In the case of azaaromatic derivatives, the position of the nitrogen atom in the quinoline ring influences the photophysical properties of Box-Ala derivatives (Table 1). The increase of the distance between the benzoxazole ring and the nitrogen atom in the quinoline ring causes hipsochromic shift of the absorption spectrum whereas the emission spectrum shifts bathochromically and the fluorescence quantum yield as well as the fluorescence lifetime decrease. Thus, the fluorescence quantum yield and the fluorescence lifetime increase in the order

(6-Q)Box-Ala > (3-Q)Box-Ala > (3-iQ)Box-Ala. However, (8-Q)Box-Ala possesses lower fluorescence quantum yield and longer fluorescence lifetime than (4-Q)Box-Ala (Table 1).

4. Conclusion

3-(Benzoxazol-5-yl)alanine derivatives substituted in position 2 by heteroaromatic group are new simple and small fluorophores which may be used as potential chemosensors for metal ions and/or pH sensors. Moreover, absorption and emission spectra of compounds studied display bathochromic shift as compared with tyrosine and tryptophan what make them suitable to use in fluorescence conformational analysis in the presence of natural aromatic amino acids. The positions of their absorption and emission bands as well as the molar absorption coefficient, fluorescence quantum yield and fluorescence lifetime are determined by the character of the substituents as well as the solvent properties.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.photochem.2006.09.019.

References

[1] Y. Kanegae, K. Peariso, S.S. Martinez, Appl. Spectrosc. 50 (1996) 316.
[2] B.M. Krasovitskii, B.M. Bolotin, Organic Luminescent Materials, VCH, Weinheim, 1988.
[3] A. Pla-Dalmau, J. Org. Chem. 60 (1995) 5468.
[4] Y. Wang, W. Liu, K. Wang, G. Shen, R. Yu, Fresen J. Anal. Chem. 360 (1998) 702.
[5] C.-W. Ko, Y.-T. Tao, A. Danel, L. Krzemińska, P. Tomasik, Chem. Mater. 13 (2001) 2441.
[6] K.H. Park, M.G. Kwak, W.S. Jahng, C.J. Lee, N. Kim, React. Funct. Polym. 40 (1999) 41.
[7] K.H. Park, J.T. Lim, S. Song, M.G. Kwak, C.J. Lee, N. Kim, React. Funct. Polym. 40 (1999) 169.
[8] K.H. Park, J.T. Lim, S. Song, J.S. Lee, C.J. Lee, N. Kim, React. Funct. Polym. 40 (1999) 177.
[9] J. Hwang, H. Moon, J. Seo, S.Y. Park, T. Aoyama, T. Wada, H. Sasabe, Polymer 42 (2001) 3023.
[10] T. Beltrani, M. Bösch, R. Centore, S. Concilio, P. Günter, A. Sirigu, Polymer 42 (2001) 4025.
[11] J.-M. Kim, B.-O. Chong, K.H. Park, T.-E. Chang, D.K. Han, D.J. Choo, K.-D. Ahn, Polym. Bull. 44 (2000) 79.
[12] J.L. Bricks, J.L. Slominskii, M.A. Kudinova, A.I. Tolmachev, K. Rurack, U. Resch-Genger, W. Rettig, J. Photochem. Photobiol. A: Chem. 132 (2000) 193.
[13] E.R. Zakhs, A.I. Ponyaev, M.A. Subbotina, A.V. El'tsov, Russ. J. Gen. Chem. 71 (2001) 1076.
[14] M.R. DeLuca, S.M. Kerwin, Tetrahedron Lett. 38 (1997) 199.
[15] M.B. Reynolds, M.R. DeLuca, S.M. Kerwin, Bioorg. Chem. 27 (1999) 326.
[16] D. Kumar, M.R. Jacob, M.B. Reynolds, S.M. Kerwin, Bioorg. Med. Chem. 10 (2002) 3997.
[17] I.H. Hall, N.J. Peaty, J.R. Henry, J. Easmon, G. Heinisch, G. Pürstinger, Arch. Pharm. Pharm. Med. Chem. 332 (1999) 115.
[18] S. Sato, G. Levy, J. Pharm. Sci. 78 (1989) 785.
[19] G. Turan-Zitouni, Ş. Demirayak, A. Özdemir, Z.A. Kaplancikli, M.T. Yildiz, Eur. J. Med. Chem. 39 (2003) 267.
[20] I. Yildiz-Oren, I. Yalçin, E. Aki-Sener, N. Ucarturk, Eur. J. Med. Chem. 39 (2004) 291.
[21] C. Ramalingan, S. Balasubramanian, S. Kabilan, M. Vasudevan, Eur. J. Med. Chem. 39 (2004) 527.
[22] S.M. Rida, F.A. Ashour, S.A.M. El-Hawash, M.M. ElSemary, M.H. Badr, M.A. Shalaby, Eur. J. Med. Chem. 40 (2005) 949.
[23] A. Pinar, P. Yurdakul, I. Yildiz, Ö. Temiz-Arpaci, N. Leyla Acan, E. Aki-Sener, I. Yalçin, Biochem. Biophys. Res. Commun. 317 (2004) 670.
[24] B.B. Wang, N. Maghami, V.L. Goodlin, P.J. Smith, Bioorg. Med. Chem. Lett. 14 (2004) 3221.
[25] D.W. Dunwell, D. Evans, T.A. Hicks, J. Med. Chem. 18 (1975) 53.
[26] D.W. Dunwell, D. Evans, T.A. Hicks, J. Med. Chem. 18 (1975) 1158.
[27] Ö.T. Arpaci, I. Ören, N. Altanlar, Il Farmaco 57 (2002) 175.
[28] Ö.T. Arpaci, E.A. Şener, I. Yalçin, N. Altanlar, Il Farmaco 57 (2002) 771.
[29] S.-C. Liang, H. Wang, Z.-M. Zhang, X. Zhang, H.-S. Zhang, Anal. Chem. Acta 451 (2002) 211.
[30] S.-C. Liang, H. Wang, Z.-M. Zhang, X. Zhang, H.-S. Zhang, Spectochim. Acta A 58 (2002) 2605.
[31] R. Passerini, J. Chem. Soc. (1954) 2256.
[32] M. Szabelski, M. Rogiewicz, W. Wiczk, Anal. Biochem. 342 (2005) 20.
[33] K. Guzow, M. Szabelski, J. Malicka, W. Wiczk, Helv. Chim. Acta 84 (2001) 1086.
[34] A. Rzeska, J. Malicka, K. Guzow, M. Szabelski, W. Wiczk, J. Photochem. Photobiol. A: Chem. 146 (2001) 9.
[35] K. Guzow, M. Szabelski, J. Malicka, J. Karolczak, W. Wiczk, Tetrahedron 58 (2002) 2201.
[36] K. Guzow, K. Mazurkiewicz, M. Szabelski, R. Ganzynkowicz, J. Karolczak, W. Wiczk, Chem. Phys. 295 (2003) 119.
[37] K. Guzow, J. Zielińska, K. Mazurkiewicz, J. Karolczak, W. Wiczk, J. Photochem. Photobiol. A: Chem. 175 (2005) 57.
[38] K. Guzow, M. Milewska, W. Wiczk, Spectrochim. Acta A 61 (2005) 1133.
[39] K. Guzow, M. Szabelski, J. Karolczak, W. Wiczk, J. Photochem. Photobiol. A: Chem. 170 (2005) 215.
[40] M. Milewska, A. Skwierawska, K. Guzow, D. Szmigiel, W. Wiczk, Inorg. Chem. Commun. 8 (2005) 947.
[41] K. Guzow, M. Milewska, D. Wróblewski, A. Giełdoń, W. Wiczk, Tetrahedron 60 (2004) 11889.
[42] C.E. Teague Jr., A. Roe, J. Am. Chem. Soc. 73 (1951) 688.
[43] G. Xue, J.S. Bradshaw, N.K. Dalley, P.B. Savage, R.M. Izatt, L. Prodi, M. Montalti, N. Zaccheroni, Tetrahedron 58 (2002) 4809.
[44] E. Wünsch, Synthese von Peptiden, in: E. Mhller (Ed.), Houben-Weyl, Methoden der organischen Chemie, vol. 15, Georg Thieme Verlag, Stuttgart, 1974, p. 317.
[45] J.M. Stewart, J.D. Young, Solid Phase Peptide Synthesis, 2nd ed., Pierce Chemical Company, Rockford, Illinois, 1984, p. 63.
[46] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd ed., Kluwer Academic/Plenum Publisher, New York, 1999, p. 53.
[47] J. Karolczak, D. Komar, J. Kubicki, T. Wróżowa, K. Dobek, B. Ciesielska, A. Maciejewski, Chem. Phys. Lett. 344 (2001) 154.
[48] T.A. Fayed, J. Photochem. Photobiol. A: Chem. 121 (1999) 17.
[49] B. Valeur, Molecular Fluorescence. Principles and Applications, Wiley-VCH, Weinheim, New York, Chichester, Brisbane, Singapore, Toronto, 2002.
[50] C.A. Parker, Photoluminescence of Solutions, Elsevier Publishing Company, Amsterdam, London, New York, 1968.
[51] K. Sakata, K. Hara, Chem. Phys. Lett. 371 (2003) 164.