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Influence of substituents in the phenyl ring on photophysical properties of 3-[2-(phenyl)benzoxazol-5-yl]alanine derivatives

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Abstract

A series of *N*-(*tert*-butoxycarbonyl)-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives possessing electron-donor and electron-acceptor substitutents in the phenyl ring were synthesized and their photophysical properties in methanol, acetonitrile and methylcyclohexane were studied by means of absorption and fluorescence spectroscopies as well as theoretical calculations. The structures of compounds studied in the ground and excited state were calculated using the MOPAC 2002 package with COSMO model of the solvent whereas the positions of vertical absorption and emission transitions were calculated using a INDO/S or CNDO/Param method within a SCRF approximation for the solvent but they are not in good agreement with the experimental values.

High molar absorption coefficient values and fluorescence quantum yields are characteristic for all compounds studied. Moreover, in the case of derivatives possessing electron-donor substituents in position 2 of the phenyl ring the fluorescence quantum yield increases with the solvent polarity whereas the opposite effect is observed for derivatives substituted by electron-acceptor groups. Also, the position of the absorption and emission band depends on the solvent polarity and is shifted to longer wavelengths in more polar solvents as a result of a charge transfer from a substituent to the benzoxazole moiety. In the case of derivatives substituted by 2-hydroxyphenyl, the intramolecular proton transfer process is observed. However, the character of the group in position 4 of the phenyl ring influences on it. An electron-acceptor substituent facilitates the proton transfer whereas the opposite is true for an electron-donor group.

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

1. Introduction

The 2-phenylbenzoxazoles are known as photostable highly efficient UV dyes [1] and are used as organic brightening agents [2], laser dyes [1], organic plastic scintillators [3] and optical fibre sensors [4]. Moreover, some of their derivatives are biologically active [5–10]. They are also used as fluorescent probes to determine spectrofluorimetrically glutathione [11] and cysteine [12] in biological samples. 2-Phenylbenzoxazole derivatives possess high molar absorption coefficient values and fluorescence quantum yields. 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 [1,13]. Absorption and emission spectra of 2-phenylbenzoxazole and its derivatives with electron-donor substituents in *para* position of the phenyl ring possess well-resolved vibrational structure in nonpolar solvent. This is a result of partially double C_1–$C_{1'}$ bond which restricts rotation of two aromatic subunits of 2-phenylbenzoxazole [13]. The presence of proton-donor substituents such as hydroxyl [13–17] or amino [18] group in position 2 of the phenyl ring

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make the excited state intramolecular proton transfer possible to occur. The resulting tautomers have different absorption and fluorescence spectra.

3-(2-Benzoxazol-5-yl)alanine derivatives are new unnatural amino acids which can be used as fluorescent probes [19–24]. An amino acid moiety allows to incorporate them into a peptide chain [20,23] whereas a benzoxazole moiety is responsible for their fluorescent properties which can be modified by changing a substituent in position 2 of the benzoxazole ring. The presence of acid moiety does not influence on the spectroscopic properties of the benzoxazole moiety [21].

As a response for a need of new, simple and small fluorophores to study structures of biologically active compounds, we synthesized a series of *N*-(*tert*-butoxycarbonyl)-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives substituted in the phenyl ring by different groups (hydroxyl, methoxyl, allyloxyl, phenoxyl, benzoyloxyl, dimethylamino, diethylamino, fluoro, trifluoromethyl and cyano groups) (Fig. 1). Their photophysical properties in methanol, acetonitrile and methylcyclohexane were studied in order to find out how substituent properties as well as its position influence on the 3-[2-(phenyl)benzoxazol-5-yl]alanine derivative spectroscopic properties and if there is a correlation between a compound structure and its properties which may enable to design more rationally probes possessing required properties. To complete the data, the ground and excited state properties were calculated theoretically using semi-empirical methods (PM3 and INDO/S or CNDO/Param).

2. Materials and methods

2.1. Synthesis

2,4-Dihydroxybenzaldehyde, 2,4-bis(benzoyloxy)benzaldehyde and 2,4,5-trimethoxybenzaldehyde were a gift from Vasyl G. Pivovarenko (Kiev Taras Shevchenko National University, Kiev, Ukraine). All the other aldehydes were purchased from Avocado (4-allyloxybenzaldehyde, 2-methoxy-4-dimethylaminobenzaldehyde, 4-cyanobenzaldehyde), Aldrich (2,5-dimethoxybenzaldehyde, 3,4,5-trimethoxybenzaldehyde) and Lancaster (3-phenoxybenzaldehyde, 4-fluorobenzaldehyde, 4-diethylaminosalicylaldehyde, 3,5-bis(trifluoromethyl)benzaldehyde) 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 [25,26], 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 [22,24] (Fig. 1). The products were isolated by means of column chromatography (Merck, Silica gel 60, 0.040–0.063 mm) and then recrystallized. The purity of the obtained compounds was checked by means of TLC (Merck plates, Kieselgel 60 F_{254}) and analytical RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, i.d. = 4.5 mm). 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, infrared spectra recorded on a Bruker IFS-66 instrument, mass spectra recorded on a MASSLAB TRIO-3 or Bruker Biflex III instrument and elemental analysis taken on a Carlo Erba CNSO Eager 200 instrument. Melting points were determined in capillary tubes using Gallenkamp Griffin MPA-350.MB2.5 apparatus and are uncorrected (Supplementary data).

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 [27]. 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 Laboratory of Ultrafast Laser Spectroscopy, Adam Mickiewicz University, Poznań, Poland [28]. 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})

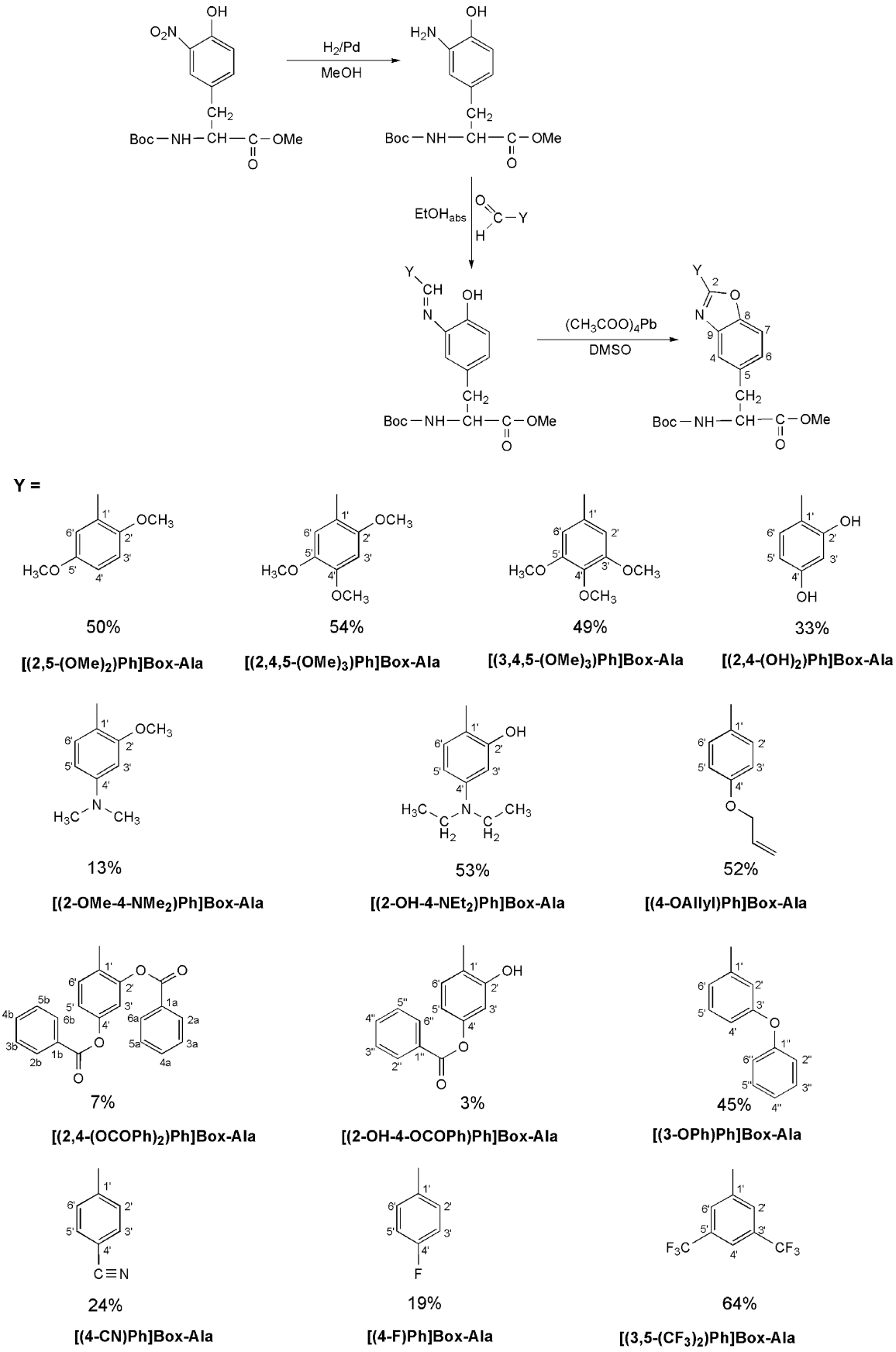

Fig. 1. The synthesis scheme (number below the structure denotes the reaction yield).

rate constants were calculated from the equations:

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

2.3. Theoretical calculations

Vertical absorption and emission transitions were calculated using INDO/S CI or CNDO/Param (in the case of substituents possessing fluorine atoms) semi-empirical methods [29,30]. The semi-empirical molecular-orbital method PM3 [31] was used to optimize the geometry in the ground and first excited state. First, unconstrained geometry optimization has been carried out for the ground state of all compounds considered by FE procedure [32]. These structures were subsequently used as starting geometries in unconstrained optimization of their first excited state (S^1). Due to the relatively large size of the relevant systems, we carried out CI [33] optimization in the minimal active space, restricted to the HOMO and LUMO orbitals with conductor-like screening model for solvation (COSMO) [34]. All calculations were carried out with the MOPAC 2002 code [35]. However, final estimation of the energy of the absorption and fluorescence vertical transitions have been obtained by calculations in the space that consisted of all singly excited configuration interactions. The influence of the solvent on the predicted spectra was described within the self-consisted reaction field (SCRF) methods [34]. The absorption and emission transitions were calculated with the spherical Onsager cavity radius calculated as a half of the longest molecule axis enlarged by 1 Å to

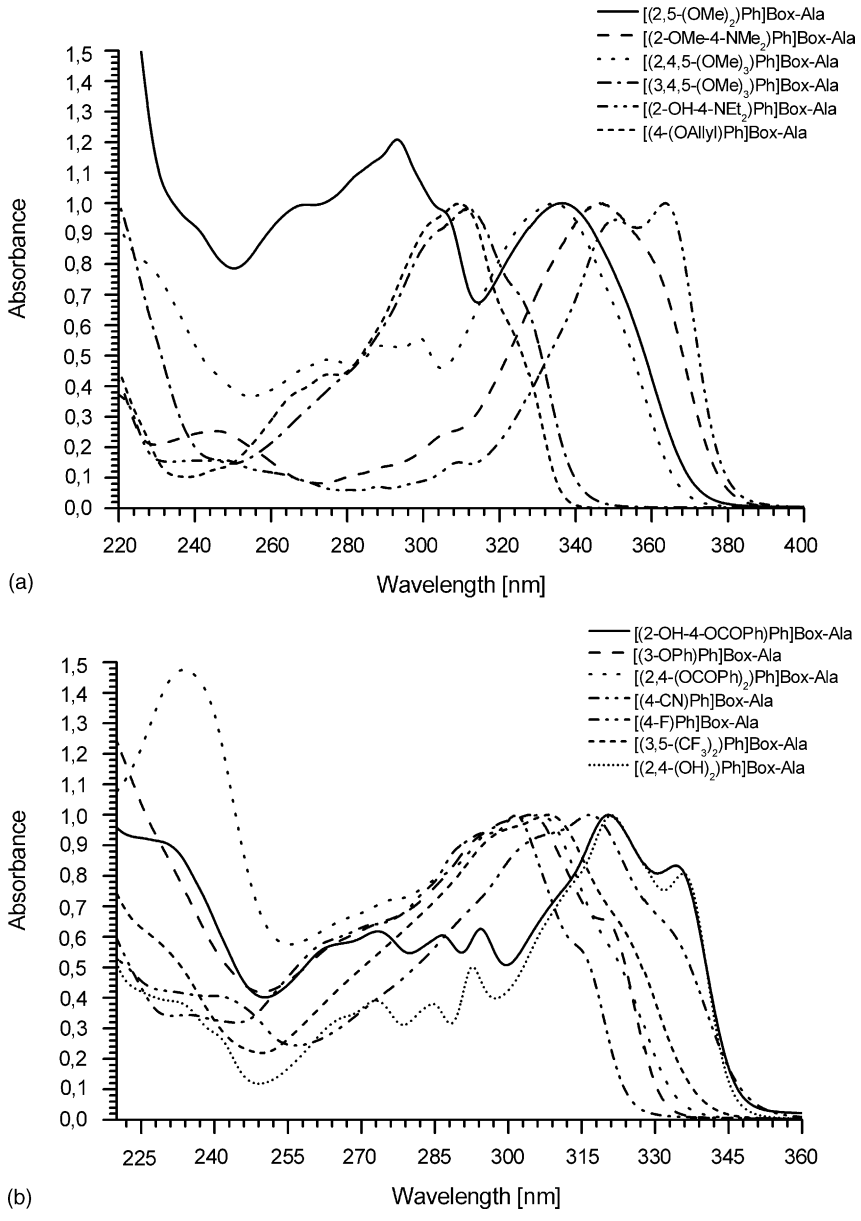

Fig. 2. The absorption spectra of *N*-Boc-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives in methanol.

prevent overlapping solvent molecules with a solute. All calculations were carried out with the MOS-F v 4.2D code [36].

3. Results and discussion

Absorption and emission spectra of all compounds studied were measured in MeOH, MeCN and methylcyclohexane and those obtained in MeOH are presented in Figs. 2 and 3. The fluorescence spectra of all compounds studied look like mirror reflections of their absorption spectra (Fig. 3a and b). The positions of the absorption and emission bands (Table 1) depend on the solvent polarity and its capacity to form hydrogen bonds. They shift bathochromically with the increase of those properties (data not shown) indicating that the excited state is more polar than the ground state because of a charge transfer from a substituent to the benzoxazole moiety [1,24]. It is additionally confirmed by the vibrational structure of the spectra which is well-resolved only in the case of methylcyclohexane, the least polar among the solvents used, and becomes more diffuse in MeCN and MeOH. Moreover, the fluorescence band shift depends on the electron donor–acceptor properties of the substituent in the phenyl ring as in the case of *para* substituted [2-(phenyl)benzoxazol-5-yl]alanine derivatives [21]. A distinct vibrational structure in methylcyclohexane indicates

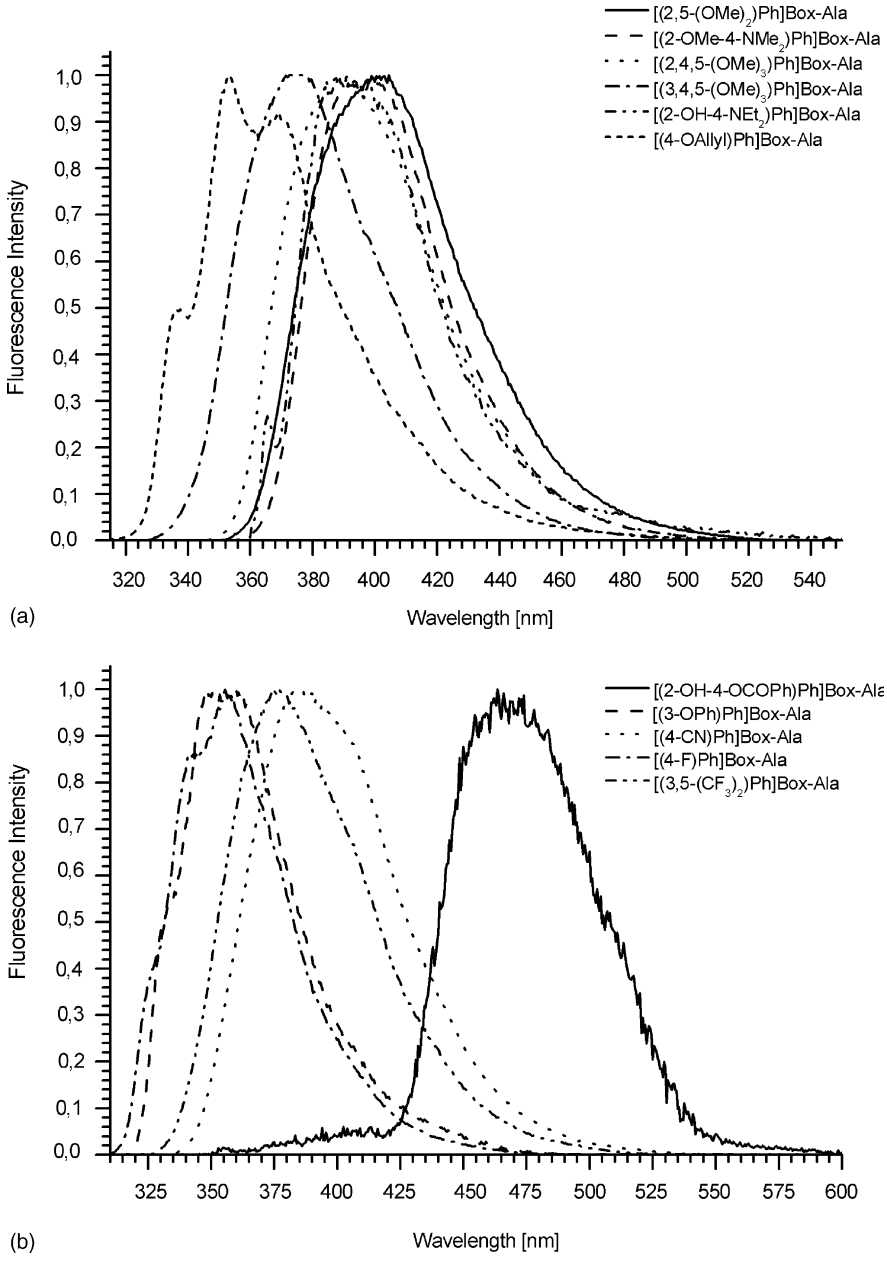

Fig. 3. The emission spectra of *N*-Boc-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives in methanol.

Table 1
Absorption and emission maxima (experimental and calculated), oscillator strength and molar absorption coefficient values of *N*-Boc-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives in methanol, acetonitrile and methylcyclohexane

Compound	Absorption		Oscillator strength	log ε	Emission	
	λ (nm)	λ_{calc} (nm)		λ (nm)	λ (nm)	λ_{calc} (nm)
MeOH						
[(2,5-$(OMe)_2$)Ph]Box-Ala	293, *337*	304	0.297	4.10 (337)	405	399
[(2,4,5-$(OMe)_3$)Ph]Box-Ala	299, *335*	–	–	4.39 (335)	389	–
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	313	323	0.583	4.57 (313)	373	393
[(2,4-$(OH)_2$)Ph]Box-Ala	273, 284, 293, *321*, 335	319	0.568	4.52 (321)	*365*, 457	394
[(2-OMe-4-NMe_2)Ph]Box-Ala	346	320	0.659	4.66 (346)	398	400
[(2-OH-4-NEt_2)Ph]Box-Ala	350, *364*	327	0.742	4.81 (364)	391	380
[(4-OAllyl)Ph]Box-Ala	274, *309*	334	0.711	4.56 (309)	337, *353*, 369	395
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	234, *306*	323	0.002	4.47 (306)	362	384
[(2-OH-4-OCOPh)Ph]Box-Ala	274, 287, 294, 321, *334*	328	0.003	4.53 (334)	405, *466*	390
[(3-OPh)Ph]Box-Ala	*305*, 320	318	0.522	4.41 (305)	348, *362*	387
[(4-CN)Ph]Box-Ala	*317*, 334	328	0.637	4.51 (317)	385	409
[(4-F)Ph]Box-Ala	295, *302*	342	0.47	4.47 (302)	342, *355*	404
[(3,5-$(CF_3)_2$)Ph]Box-Ala	309	345	0.424	4.37 (309)	377	415
MeCN						
[(2,5-$(OMe)_2$)Ph]Box-Ala	296, 306, *333*	312	0.243	4.05 (333)	390	399
[(2,4,5-$(OMe)_3$)Ph]Box-Ala	299, *333*	–	–	4.36 (333)	383	–
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	*312*, 326	324	0.592	4.52 (312)	371	393
[(2,4-$(OH)_2$)Ph]Box-Ala	272, 284, 292, *320*, 334	320	0.574	4.39 (320)	*380*, 464	394
[(2-OMe-4-NMe_2)Ph]Box-Ala	343	321	0.66	4.63 (343)	393	400
[(2-OH-4-NEt_2)Ph]Box-Ala	352, *364*	327	0.741	4.80 (364)	394	380
[(4-OAllyl)Ph]Box-Ala	274, 302, *309*, 324	332	0.697	4.59 (309)	333, *349*, 364	395
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	233, *306*	328	0.003	4.46 (306)	362	384
[(2-OH-4-OCOPh)Ph]Box-Ala	294, 320, *334*	326	0.009	4.51 (334)	471	390
[(3-OPh)Ph]Box-Ala	*305*, 320	323	0.569	4.40 (305)	360	387
[(4-CN)Ph]Box-Ala	*316*, 333	328	0.637	4.50 (316)	384	409
[(4-F)Ph]Box-Ala	294, *301*, 314	343	0.47	4.47 (301)	341, *355*	404
[(3,5-$(CF_3)_2$)Ph]Box-Ala	308	345	0.422	4.35 (308)	378	415
Methylcyclohexane						
[(2,5-$(OMe)_2$)Ph]Box-Ala	293, 306, *332*	302	0.231	4.06 (332)	*364*, 378	399
[(2,4,5-$(OMe)_3$)Ph]Box-Ala	299, *327*	–	–	4.32 (327)	357, *375*, 396	–
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	*312*, 327	335	0.671	4.43 (312)	345, *360*, 374	393
[(2,4-$(OH)_2$)Ph]Box-Ala	272, 284, 292, *320*, 336	306	0.333	–[a]	470	394
[(2-OMe-4-NMe_2)Ph]Box-Ala	336	304	0.342	4.58 (336)	363, *382*, 404	400
[(2-OH-4-NEt_2)Ph]Box-Ala	347, 354, *364*	303	0.334	4.89 (364)	*389*, 411	414
[(4-OAllyl)Ph]Box-Ala	275, 297, 302, *310*, 316, 324	336	0.718	4.61 (310)	329, *346*, 362	395
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	231, 301, *309*, 323	328	0.003	4.50 (309)	351	384
[(2-OH-4-OCOPh)Ph]Box-Ala	274, 287, 296, 324, *338*	327	0.002	4.50 (338)	480	390
[(3-OPh)Ph]Box-Ala	293, 299, *306*, 313, 321	327	0.598	4.42 (306)	325, *341*, 357	387
[(4-CN)Ph]Box-Ala	306, *313*, 320, 337	341	0.732	4.51 (313)	342, *359*, 375	409
[(4-F)Ph]Box-Ala	284, 290, 296, *303*, 310, 317	358	0.546	4.50 (303)	320, 336, *352*	404
[(3,5-$(CF_3)_2$)Ph]Box-Ala	299, *306*, 312, 328	359	0.494	4.37 (306)	334, *350*, 365	415

[a] Low solubility.

also small geometry changes in the excited state. Theoretical calculations demonstrated that all 3-[2-(phenyl)benzoxazol-5-yl]alanine derivatives have permanent non-zero dipole moment in the ground state which increases in the excited state in all cases except [(2-OH-4-NEt_2)Ph]Box-Ala and [(2-OH-4-OCOPh)Ph]Box-Ala in MeOH, MeCN and methylcyclohexane, [(2,4-$(OCOPh)_2$)Ph]Box-Ala in MeCN and [(2-OMe-4-NMe_2)Ph]Box-Ala in MeOH and MeCN (Table 3). The greatest change in the dipole moment is observed for derivatives with electron-acceptor substituents in the phenyl ring such as fluoro, trifluoromethyl or cyano group.

The most bathochromically shifted absorption bands possess compounds with electron-donor substituents in positions 2 and 4 in the phenyl ring, especially dialkyl amino groups. There are two exceptions: [2,4-$(OCOPh)_2$Ph]Box-Ala, which has a large substituent in position 2 causing an increase of torsional angle between two subunits and decreasing electronic coupling, and [(4-CN)Ph]Box-Ala in which a cyano group increases electronic coupling.

Compounds with a hydroxyl group in position 2 of the phenyl ring, such as 2-(2-hydroxyphenyl)benzoxazole

Table 2
Fluorescence quantum yields, fluorescence lifetimes, pre-exponential factors, fluorescence rate constant and radiationless rate constant of *N*-Boc-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives in methanol, acetonitrile and methylcyclohexane

Compounds	QY	τ (ns)	α	X_R^2	τ_{av} (ns)	$k_f = QY/\tau_{av}$ ($\times 10^{-8}$ s^{-1})	$k_{nr} = (1-QY)/\tau_{av}$ ($\times 10^{-8}$ s^{-1})
MeOH							
[(2,5-(OMe)$_2$)Ph]Box-Ala	0.37	2.08	1.000	5.04			
		1.57	0.350				
		2.26	0.650	1.67			
		2.22	0.233				
		1.42	0.086				
		0.02	0.681	1.11	2.03	1.82	3.1
[(2,4,5-(OMe)$_3$)Ph]Box-Ala	0.81	2.03	1	1.19	–	3.99	0.94
[(3,4,5-(OMe)$_3$)Ph]Box-Ala	0.7	1.28	1	1.2	–	5.47	2.34
[(2,4-(OH)$_2$)Ph]Box-Ala	0.02	1.33	1.000	1.67			
		1.27	0.984				
		3.01	0.016	1.11	1.33	0.15	7.37
[(2-OMe-4-NMe$_2$)Ph]Box-Ala	0.97	1.32	1	1.15	–	7.35	0.23
[(2-OH-4-NEt$_2$)Ph]Box-Ala	0.08	0.10	1.000	106			
		1.02	0.020	1.11			
		0.14	0.980		0.25	3.2	36.8
[(4-OAllyl)Ph]Box-Ala	0.79	1.21	1	1.17	–	6.53	1.73
[(2,4-(OCOPh)$_2$)Ph]Box-Ala	0.002	0.02	1.000	4.02			
		0.01	1.000				
		1.17	0.000	1.22	0.01	2	998
[(2-OH-4-OCOPh)Ph]Box-Ala	0.003	0.28	1.000	2.21			
		0.27	1.000				
		1.73	0.000	1.10	0.27	0.11	36.9
[(3-OPh)Ph]Box-Ala	0.13	1.25	1.000	178			
		1.21	0.357	1.08			
		0.05	0.643		1.14	1.14	7.63
[(4-CN)Ph]Box-Ala	0.5	1.92	1	1	–	5	5
[(4-F)Ph]Box-Ala	0.48	1.53	1	1.15	–	4.17	3.47
[(3,5-(CF$_3$)$_2$)Ph]Box-Ala	0.44	2.42	1.000	1.30			
		2.45	0.937				
		0.77	0.063	1.02	2.41	1.85	2.32
MeCN							
[(2,5-(OMe)$_2$)Ph]Box-Ala	0.35	2.04	1.000	1.41			
		2.14	0.810				
		1.13	0.190	1.02	2.03	1.72	1.35
[(2,4,5-(OMe)$_3$)Ph]Box-Ala	0.79	1.78	1	1.1	–	4.43	1.18
[(3,4,5-(OMe)$_3$)Ph]Box-Ala	0.72	1.2	1	1.17	–	6	2.33
[(2,4-(OH)$_2$)Ph]Box-Ala	0.01	1.40	1.000	1.64			
		2.10	0.200				
		1.14	0.800	1.07	1.44	0.07	6.88
[(2-OMe-4-NMe$_2$)Ph]Box-Ala	0.88	1.24	1	1.1	–	7.1	0.97
[(2-OH-4-NEt$_2$)Ph]Box-Ala	0.43	0.67	1	1.19	–	6.41	8.51
[(4-OAllyl)Ph]Box-Ala	0.56	1.14	1	1.1	–	4.9	3.86
[(2,4-(OCOPh)$_2$)Ph]Box-Ala	0.003	0.006	1.000	7.79			
		1.10	0.000				
		0.003	1.000	1.21	0.003	10	331
[(2-OH-4-OCOPh)Ph]Box-Ala	0.01	3.01	1.000	24.97			
		0.26	0.977				
		0.86	0.023	1.15	0.3	0.03	3.3
[(3-OPh)Ph]Box-Ala	0.37	1.45	1	1.13	–	3.27	5.57
[(4-CN)Ph]Box-Ala	0.52	1.87	1	1.05	–	4.95	5.57
[(4-F)Ph]Box-Ala	0.46	1.47	1	1.12	–	3.13	3.67
[(3,5-(CF$_3$)$_2$)Ph]Box-Ala	0.46	2.35	1.000	3.90			
		2.37	0.895				
		0.37	0.105	1.12	2.33	1.96	2.3
Methylcyclohexane							
[(2,5-(OMe)$_2$)Ph]Box-Ala	0.51	1.95	1.000	1.43			
		1.99	0.885				
		0.66	0.115	1.19	1.93	2.46	2.54

Table 2 (*Continued*)

Compounds	QY	τ (ns)	α	X_R^2	τ_{av} (ns)	$k_f = QY/\tau_{av}$ ($\times 10^{-8}$ s^{-1})	$k_{nr} = (1 - QY)/\tau_{av}$ ($\times 10^{-8}$ s^{-1})
[(2,4,5-(OMe)$_3$)Ph]Box-Ala	0.58	1.29	1.000	1.49			
		1.33	0.840				
		0.58	0.160	1.19	1.27	4.57	3.31
[(3,4,5-(OMe)$_3$)Ph]Box-Ala	0.66	1.02	1.000	1.38			
		0.66	0.197				
		1.07	0.803	1.12	1.01	6.53	3.37
[(2,4-(OH)$_2$)Ph]Box-Ala	0.02	2.27	1.000	23.3			
		0.19	0.978				
		1.14	0.022	1.17	0.3	0.67	32.7
[(2-OMe-4-NMe$_2$)Ph]Box-Ala	0.44	1.01	1.000	32.4			
		0.92	0.987				
		3.42	0.022	2.25	1.25	3.52	4.48
		1.06	0.624	1.04			
		0.47	0.368				
		6.38	0.008				
[(2-OH-4-NEt$_2$)Ph]Box-Ala	0.11	0.23	1.000	10.9			
		0.25	0.569				
		0.04	0.431	1.00	0.22	5	40.6
[(4-OAllyl)Ph]Box-Ala	0.84	1.05	1	1.17	–	8	1.52
[(2,4-(OCOPh)$_2$)Ph]Box-Ala	0.003	0.01	1.000	31.3			
		0.96	0.006				
		0.01	0.994	1.05	0.358	3.0 (0.008)[a]	99.7 (2.78)[a]
[(2-OH-4-OCOPh)Ph]Box-Ala	0.03	0.64	1	1.07	–	0.47	15.1
[(3-OPh)Ph]Box-Ala	0.5	1.16	1	1.08	–	4.31	4.31
[(4-CN)Ph]Box-Ala	0.55	1.28	1	1.04	–		
[(4-F)Ph]Box-Ala	0.49	1.23	1	1.04	–	3.98	4.15
[(3,5-(CF$_3$)$_2$)Ph]Box-Ala	0.54	1.50	1.000	1.44			
		1.52	0.944				
		0.70	0.056	1.07	1.5	3.6	3.82

[a] Calculated taking into account also longer lifetime.

(HBO) [15,37,38] or 3-[2-(2-hydroxyphenyl)benzoxazol-5-yl]alanine [21], can exist in the ground state as two rotational conformers (*syn*- or *anti*-enol) as well as the keto form at a low concentration (for structures see [15]). One of the isomers (*syn*-enol) has an internal hydrogen bond whereas the other forms intermolecular hydrogen bond with a solvent. According to Krishnamurthy and Dogra [37] and Woolfe et al. [38], the absorption band of the *syn*-enol is at longer wavelengths (about 330 nm for HBO) than this of *anti*-enol (320 nm) while keto form possesses the absorption band shifted bathochromically in comparison with those of both enol forms (about 370 nm [38]). In our case, the presence of additional substituent in position 4 of the phenyl ring causes that absorption spectra are shifted to the longer wavelengths than those predicted for HBO, however, for [(2,4-(OH)$_2$)Ph]Box-Ala and [(2-OH-4-OCOPh)Ph]Box-Ala a weak absorption in the range 350–380 nm is observed (Fig. 2b). In the excited state the closed *syn*-enol, which has intramolecular hydrogen bond, undergoes efficient proton transfer causing a decrease of the fluorescence quantum yield [13,15,18,21,37,38]. Thus, low quantum yields displayed by [(2-OH-4-OCOPh)Ph]Box-Ala, [(2,4-(OH)$_2$)Ph]Box-Ala and [(2-OH-4-NEt$_2$)Ph]Box-Ala are a result of the excited state intramolecular proton transfer. For 3-[2-(2-hydroxyphenyl)benzoxazol-5-yl]alanine, the emission band of keto form is centred at about 480 nm whereas the emission band of enol form at about 360 nm and the positions of both of them slightly depend on solvent polarity [21]. Also, for [(2-OH-4-OCOPh)Ph]Box-Ala, [(2,4-(OH)$_2$)Ph]Box-Ala and [(2-OH-4-NEt$_2$)Ph]Box-Ala two emission bands are observed (Fig. 3b), however, the fluorescence intensity of each band depends on the kind of substituent in position 4 of the phenyl ring. The electron-acceptor substituent, decreasing the charge density on oxygen atom of the hydroxyl group, facilitates the proton transfer, whereas the opposite is true for electron-donor subsituent. Thus, for [(2-OH-4-OCOPh)Ph]Box-Ala in all solvents studied an intense emission of the keto form and a very weak shoulder of the enol emission are observed, while for [(2-OH-4-NEt$_2$)Ph]Box-Ala a relatively strong emission of the enol form, which coincidences with the emission spectra of derivatives with methoxyl group in position 2, is observed with a very small shoulder at a tail of the emission spectrum (Fig. 3a). The influence of the electron-acceptor substituent on the excited state intramolecular proton transfer of 2-(2-hydroxyphenyl)benzoxazole derivatives was observed by Tanaka et al. [39] and Seo et al. [40]. For [(2,4-(OH)$_2$)Ph]Box-Ala the emission of the keto form is observed only in methylcyclohexane, while in more polar slovents (MeOH, MeCN) the emission of the enol form is

more intense as in parent HBO. Moreover, an increase of the solvent polarity causes the blue shift of both emission bands [15,21,37,38].

The molar absorption coefficient values as well as the fluorescence quantum yields are quite high in most cases (Tables 1 and 2). Those results are in accordance with the theoretical calculations which predict high values of the oscillator strengths in both absorption and emission. The highest molar absorption coefficient has [(2-OH-4-NEt_2)Ph]Box-Ala in methylcyclohexane whereas the lowest—[(2,5-$(OMe)_2$)Ph]Box-Ala in MeCN. Moreover, the molar absorption coefficient values of derivatives possessing methoxyl group in position 2 of the phenyl ring as well as [(2-OH-4-OCOPh)Ph]Box-Ala increase with the solvent polarity.

The highest fluorescence quantum yield is displayed by [(2-OMe-4-NMe_2)Ph]Box-Ala in MeOH (QY = 0.97) while the lowest quantum yield is displayed by [(2,4-$(OCOPh)_2$)Ph]Box-Ala. In the latter case, the fluorescence excitation spectra indicate that this is a real fluorescence of this derivative not impurities fluorescence (data not shown). Such a small fluorescence quantum yield is a result of fast and efficient radiationless energy degradation which is probably caused by a low frequency vibration of a large substituent and lack of the possibility to form a planar structure in the excited state. The fluorescence quantum yield increases with the solvent polarity in the case of derivatives substituted in the phenyl ring by electron-donor groups such as dimethylamino and methoxyl groups whereas for derivatives with electron-acceptor substituents such as

Table 3
Theoretically calculated dipole moment, C_1—C_1, bond length and dihedral angle between benzoxazole and substituent moieties of *N*-Boc-3-[2-(phenyl)benzoxazol-5-yl]alanine methyl ester derivatives in methanol, acetonitrile and methylcyclohexane in the ground and excited states

Compound	Dipole moment [D]		Bond length [D]		Dihedral angle [deg]	
	Ground state	Excited state	Ground state	Excited state	Ground state	Excited state
MeOH						
[(2,5-$(OMe)_2$)Ph]Box-Ala	4.33	5.07	1.464	1.387	−111	0.5
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	3.24	7.78	1.462	1.392	−35.5	0
[(2,4-$(OH)_2$)Ph]Box-Ala	3.37	3.97	1.455	1.386	44.9	0
[(2-OMe-4-NMe_2)Ph]Box-Ala	3.8	3.62	1.457	1.384	47.9	1.3
[(2-OH-4-NEt_2)Ph]Box-Ala	4.85	3.76	1.454	–	−40.7	–
[(4-OAllyl)Ph]Box-Ala	3.3	5.85	1.456	1.386	−18.1	0.2
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	3.82	5.98	1.464	1.39	−70.7	0
[(2-OH-4-OCOPh)Ph]Box-Ala	4.58	3.17	1.459	1.387	−53.7	−0.8
[(3-OPh)Ph]Box-Ala	2.22	6.83	1.461	1.393	37.7	0.4
[(4-CN)Ph]Box-Ala	4.81	13.32	1.461	1.387	37.8	0.1
[(4-F)Ph]Box-Ala	2.03	9.06	1.46	1.389	35.9	0.6
[(3,5-$(CF_3)_2$)Ph]Box-Ala	4.15	13.12	1.461	1.389	−39.3	0.1
MeCN						
[(2,5-$(OMe)_2$)Ph]Box-Ala	2.98	5.07	1.464	1.387	−110.9	0.5
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	3.28	7.78	1.462	1.392	−34.2	0
[(2,4-$(OH)_2$)Ph]Box-Ala	3.37	3.97	1.455	1.386	44.4	0
[(2-OMe-4-NMe_2)Ph]Box-Ala	3.79	3.62	1.457	1.384	47.9	1.3
[(2-OH-4-NEt_2)Ph]Box-Ala	4.87	3.77	1.464	–	−41	–
[(4-OAllyl)Ph]Box-Ala	3.27	5.85	1.457	1.386	−21.9	0.2
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	6.47	5.98	1.464	1.39	72	0
[(2-OH-4-OCOPh)Ph]Box-Ala	4.67	3.17	1.46	1.387	−54	−0.8
[(3-OPh)Ph]Box-Ala	2.36	6.83	1.46	1.393	30.1	0.4
[(4-CN)Ph]Box-Ala	4.82	13.32	1.461	1.387	37.8	0.1
[(4-F)Ph]Box-Ala	2.03	9.06	1.459	1.389	35.8	0.6
[(3,5-$(CF_3)_2$)Ph]Box-Ala	4.15	13.12	1.461	1.389	−39.9	0.1
Methylcyclohexane						
[(2,5-$(OMe)_2$)Ph]Box-Ala	3.3	5.07	1.466	1.387	−104.2	0.5
[(3,4,5-$(OMe)_3$)Ph]Box-Ala	3.5	7.78	1.461	1.392	−14.1	0
[(2,4-$(OH)_2$)Ph]Box-Ala	2.92	3.97	1.448	1.386	0.5	0
[(2-OMe-4-NMe_2)Ph]Box-Ala	3.37	3.62	1.462	1.384	72.2	1.3
[(2-OH-4-NEt_2)Ph]Box-Ala	4.46	3.63	1.461	–	−76.1	–
[(4-OAllyl)Ph]Box-Ala	3.31	5.85	1.457	1.386	−12.5	0.2
[(2,4-$(OCOPh)_2$)Ph]Box-Ala	4.03	6.03	1.464	1.39	−59.4	0
[(2-OH-4-OCOPh)Ph]Box-Ala	3.49	3.17	1.451	1.387	−0.7	−0.8
[(3-OPh)Ph]Box-Ala	2.47	6.83	1.46	1.393	22	0.4
[(4-CN)Ph]Box-Ala	4.65	13.32	1.46	1.387	16	0.1
[(4-F)Ph]Box-Ala	1.84	9.06	1.459	1.389	5.5	0.6
[(3,5-$(CF_3)_2$)Ph]Box-Ala	3.93	13.12	1.46	1.389	−17.4	0.1

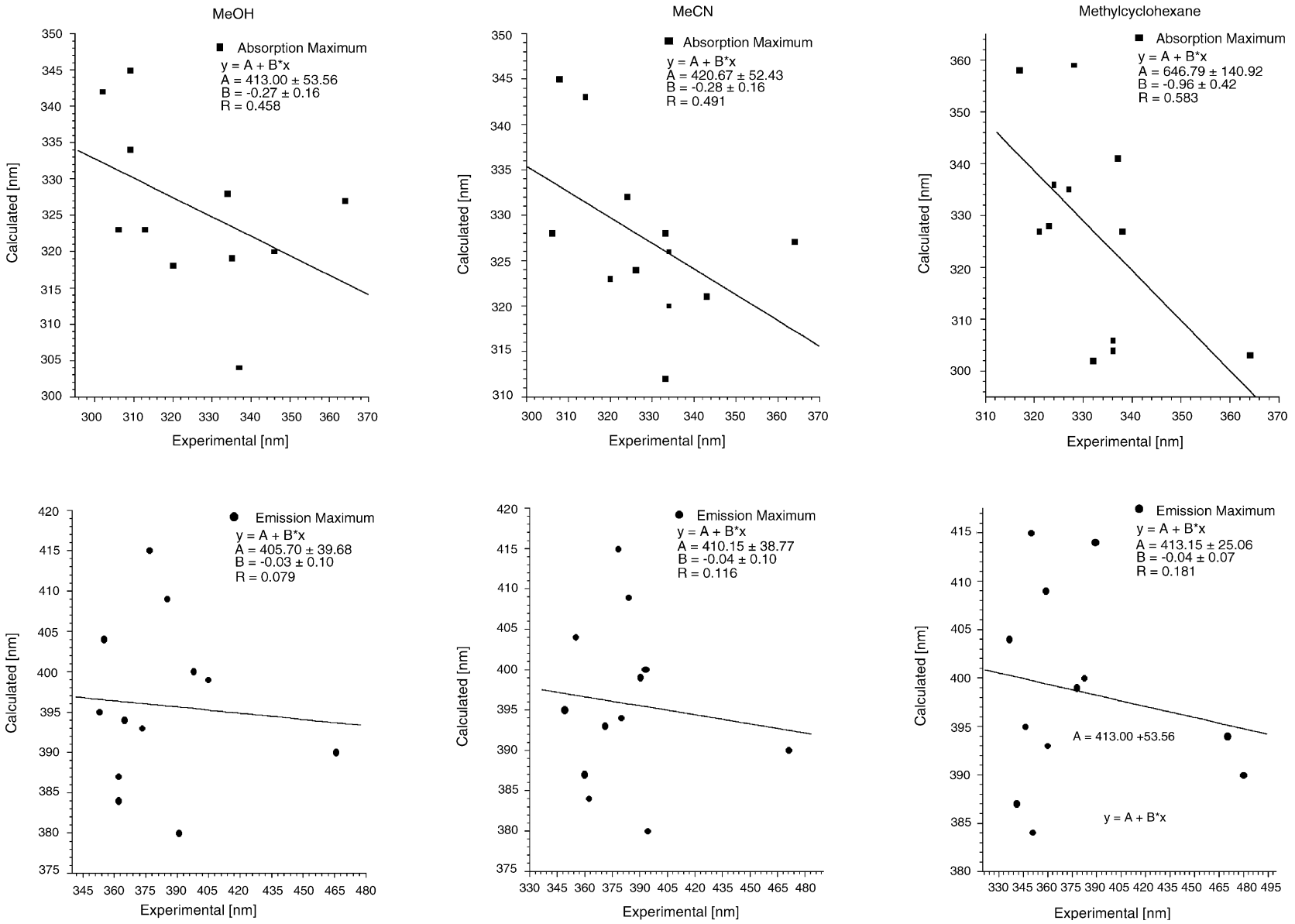

Fig. 4. The correlation between the theoretically calculated absorption (at the top) and emission (at the bottom) vertical transitions and the absorption and emission maxima, respectively, measured in methanol (on the left), acetonitrile (in the middle) and methylcyclohexane (on the right).

cyano, phenoxyl and trifluoromethyl groups the opposite dependence is observed.

Time-resolved fluorescence was applied to measure the fluorescence intensity decays of the studied compounds and calculate fluorescence lifetimes as well as pre-exponential factors and fluorescence and non-radiative rate constants (Table 2). Generally, the fluorescence lifetimes of compounds studied are rather short, in the range from 1 to 3 ns regardless of the solvent used. Derivatives possessing a substituent in position 4 have mono-exponential fluorescence intensity decays, while those with asymmetric situated subsituents—multi-exponential. The fluorescence lifetimes of all derivatives studied, except those substituted by 2-hydroxyphenyl, shorten with the decrease of solvent polarity. Also, the heterogeneity of the fluorescence intensity decay depends on the solvent polarity and its ability to form hydrogen bonds. In methanol and methylcyclohexane the fluorescence intensity decays are more heterogeneous than those in acetonitrile. In most cases in methylcyclohexane as well as in methanol the additional short fluorescence lifetime is present. In methylcyclohexane it is probably a result of the presence of rotational conformers which do not much differ in energy whereas in methanol the presence of conformers forming intramolecular hydrogen bond is responsible for this fact. The smaller heterogeneity of the fluorescence intensity decays in acetonitrile is probably caused by a higher diversification of the energy of the rotational conformers, thus, the emission of the lowest-energy conformer is observed. The presence of such conformers was confirmed, basing on theoretical calculations, in the case of 3-[2-(aryl)benzoxazol-5-yl]alanine derivatives [24] built also from two subunits which may rotate around the bond connecting them as 3-[2-(phenyl)benzoxazol-5-yl]alanine derivatives. The calculated energy barriers between minima were small whereas the energy minima themselves were flat and wide [24]. The heterogeneous fluorescence decays of compounds substituted by 2-hydroxyphenyl derivatives are a result of the presence of different tautomers [15]. Both the fluorescence and non-radiative rate constants are in the range from 2 to $8 \times 10^8\ s^{-1}$, except for [(2,4-(OCOPh)$_2$)Ph]Box-Ala for which the non-radiative rate constant is much higher (the component with a long lifetime has been neglected because of its very low contribution to the fluorescence decay—probably emission of the photoproduct(s)). Short fluorescence lifetimes and high values of the fluorescence rate constants are compatible with high values of the oscillator strengths and molar absorption coefficients. The exceptions are derivatives substituted by 2-hydroxyphenyl for which the non-radiative rate constant is much higher whereas the fluorescence rate constant is generally lower.

Calculated main geometric parameters (bond length between C_1–$C_{1'}$ carbon atoms, dihedral angle between two subunits of the molecule (benzoxazole and substituent moieties) and dipole moment) of all compounds studied in methanol, acetonitrile and methylcyclohexane in the ground and excited states are collected in Table 3. Bond length between C_1–$C_{1'}$ carbon atoms in all compounds studied decreases in the excited state and the dihedral angles between two subunits are close to 0°. A consequence of those facts is a planar structure in the excited state of all 3-[2-(phenyl)benzoxazol-5-yl]alanine derivatives studied.

The vertical absorption and emission transitions were calculated using the SCRF approximation with Onsager solvation model and configuration interaction (CI) with CI = all, i.e. all singly excited configuration were taken into account. The obtained results are presented in Table 1. Generally, the consistence between theoretical and experimental data is poor but in all solvents studied the absorption transitions are in better agreement with the experimental results than the emission transitions which almost do not correlate with the experimental values (Fig. 4). The solvent polarity does not influence on the theoretically calculated emission transition in opposition to the absorption transition. For most derivatives studied the calculated absorption transitions are at longer wavelengths in methylcyclohexane compared to those in acetonitrile and methanol but only for [(2-OH-4-NEt$_2$)Ph]Box-Ala the calculated emission transition displays this kind of dependence. For most derivatives the calculated wavelengths of the emission transitions are higher than experimentally determined. These results indicate that planar structure obtained in the excited state is not correct. Though, the application of semi-empirical methods to calculate spectral properties of the compounds built from two subunits which may rotate around bond connecting these subunits requires caution [24]. However, for all compounds studied in all solvents high oscillator strengths ($f > 0.8$) calculated for vertical transitions between ground and excited states for the excited state geometry (which corresponds to the emission transition) are in agreement with high fluorescence quantum yields.

4. Conclusion

3-[2-(Phenyl)benzoxazol-5-yl]alanine derivatives are new simple and small fluorophores with interesting photophysical properties. 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 and fluorescence quantum yield and fluorescence lifetime depend on the character of the substituents in the phenyl ring as well as the solvent properties. Moreover, 3-[2-(2-hydroxyphenyl)benzoxazol-5-yl]alanine derivatives undergo the excited state proton transfer. However, the substituent in position 4 of the phenyl ring is responsible for the fact which tautomer predominates in the solution. Theoretical calculations performed revealed that semi-empirical methods cannot be used to foresee the spectroscopic parameters of this group of compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.jphotochem.2005.04.034.

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