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Synthesis and spectral characterization of novel 1,5-benzodiazepine oxime derivatives

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Abstract: Three new 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes were synthesized and characterized by the methods of ^{1}H- and ^{13}C-NMR, IR and elemental analysis. Along with previously described compounds bearing one additional methyl group on the 5th nitrogen atom, the new compounds were characterized in bulk by UV–Vis and fluorescence spectroscopy in various solvents. The influence of the nature of the organic solvent on the spectra of the title compounds was investigated and is discussed.

Keywords: *N*-hydroxy; UV–Vis; extinction coefficient; fluorescence; quantum yield; bathochromic shift.

INTRODUCTION

Nitrogen-containing heterocyclic compounds are of exceptional importance in living organisms.[1] A variety of contemporary drugs include heterocyclic ring moieties. *In vivo* small molecular weight exogenic compounds, xenobiotics, are often oxidized at nitrogen centers by various enzymatic systems (cytochrome P450, P448) with the production of reactive or toxic metabolites. An example of such active primary metabolites are *N*-hydroxy-substituted compounds that are prone to subsequent oxidation to give nitroso and nitro derivatives as secondary metabolites.[2] Thus, exocyclic (electron-poor) *N*-hydroxy compounds and heterocycles containing endocyclic *N*-hydroxy-portions have been used in the production of various types of bioactive molecules with enhanced metabolic stability, since they are less likely to generate reactive intermediates, such as nitrenium ions and nitrones.[3]

Oximes (C=N–OH) belong to a broad class of *N*-hydroxy compounds and are known to be sources of nitric oxide (NO) – the key signaling molecule affecting the biology of living cells.[4] In 1998, R. F. Furchgott, L. J. Ignarro and F.

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Murad were awarded the Nobel Prize in physiology and medicine "for their discoveries concerning nitric oxide as a signaling molecule in the cardiovascular system". Deficient bioavailability of nitric oxide is associated with many cardiovascular diseases, such as hypertension, atherosclerosis and restenosis.[5] A number of simple alkyl and aryl oximes show vasoactive properties due to their NO-donating capabilities.[4,6] New oxime derivatives are interesting as therapeutically relevant nitric oxide releasing agents for the treatment of various diseases, such as cardiovascular, central nervous system diseases, as well as illnesses related to immunity and other physiological disorders.[3]

Moreover, oxime derivatives are used in organic synthesis for the purification and characterization of carbonyl compounds and the preparation of amides *via* Beckmann rearrangements.[7] In addition, oximes may be synthesized from noncarbonyl compounds to provide an alternative for preparing aldehydes and ketones.[8] The 1,4 and 1,5-benzodiazepine oximes are used in organic synthesis as precursors of fused polycyclic benzodiazepine derivatives with a broad spectrum of pharmaceutical properties.[9,10] In a previous investigation, it was shown that determination of the spectral properties of 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes allows the application of these compounds in practice.[10,12]

Research of the spectral properties of novel 1,5-benzodiazepine oxime derivatives is necessary for the further determination of the parameters of their chemical and enzymatic reactivity.

EXPERIMENTAL

Chemistry

Thiolactams **1a**–**6a** and 1,5-benzodiazepine oximes **4**–**6** were previously described.[11,12] Heterocyclic *N*-hydroxy compounds, 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes (**1**–**3**) were synthesized. Acetonitrile, 96 % ethanol, dimethyl sulfoxide (DMSO), 1,4-dioxane were received from Sigma. Sodium acetate, acetic acid, potassium phosphate, potassium hydroxide and other reagents were of analytical grade and received from Sigma. Buffer solutions were prepared using double distilled water.

The absorbance, excitation and emission spectra of the synthesized compounds **1**–**6** in ethanol, acetonitrile, DMSO, dioxane and 50 mM acetate buffer solution (pH 5.5) were registered spectrophotometrically and spectrofluorimetrically. Values of extinction coefficients (ε) were determined as the slope of the plot of absorbance *vs*. concentration in ethanol and acetate buffer solution.

The fluorescence excitation and emission spectra as well as absorption spectra of compounds **1**–**6** were measured in Britton–Robinson buffer solution of different pH value (obtained by titrating mixture of 20 mM H_3BO_3, 20 mM KH_2PO_4, 20 mM CH_3COOH with 0.3 M NaOH until required pH)[13] and pK_a values were determined.

Fluorescence quantum yields (Φ_f) for all synthesized compounds in 10 mM potassium phosphate buffer solution pH 7.2 were calculated. The fluorescence quantum yields (Φ_f) of compounds **1**–**6** were determined *via* the comparison method, using tryptophan as a standard sample in 0.1 M potassium phosphate buffer solution, pH 7.2.[14]

The melting points were measured using a Barnstead International MEL-TEMP capillary melting point apparatus and are not corrected. Elemental analyses (C, H, N) were performed on a CE-440 elemental analyzer. Absorption spectra were measured on a computer-controlled Nicolet evolution 300 spectrophotometer (Thermo Electron Corporation, USA) and fluorescence spectra on an MPF-4 Hitachi spectrofluorimeter (Japan). The optical path of a quartz cuvette was 1 cm and the spectra were collected at a resolution of one data point per nm. The IR spectra (4000–650 cm^{-1}) were recorded on a Perkin Elmer Frontier FT-IR ATR spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity Inova 300 and Bruker Ascend^{TM} 400 at 302 K. The chemical shifts (δ) are reported relative to TMS (δ = 0 ppm) for ^{1}H-NMR with the solvent reference DMSO-d_6 (δ = 2.5 ppm) for ^{1}H-NMR and DMSO-d_6 (δ = = 39.5 ppm) for ^{13}C-NMR. The values of chemical shifts are expressed in ppm and coupling constants (J) in Hz. The reactions were monitored by TLC using silica gel 60 F_{254} (Merck) plates in the system: benzene–methanol (volume ratio 6:1). Visualization was made with UV light (254 nm) and with iodine vapor. The physical, analytical and spectral data for the compounds are given in the Supplementary material to this paper.

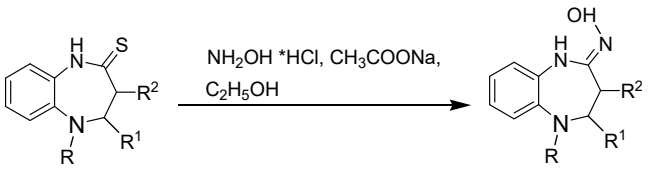
*General procedure for the synthesis of tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes (**1**–**3**)*

The mixture of thiolactam (**1a**–**3a**, 2.0 mmol), hydroxylamine hydrochloride (2.08 g, 3.0 mmol) and sodium acetate (2.52 g, 3.0 mmol) in anhydrous ethanol (70 mL) was heated at 60 °C temperature for 1–3 h. After cooling, the precipitated NaCl was filtered off and the solvent was removed under vacuum to give a solid. The settled precipitate was recrystallized from ethanol to give the pure crystalline compound **1**–**3**.

RESULTS AND DISCUSSION

Chemistry

The synthesis pathway of 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes is represented in Scheme 1. The structures of compounds **1**–**3** were proved by ^{1}H- and ^{13}C-NMR, and IR spectroscopy and elemental analysis (Supplementary material). The synthesis and characterization of the starting thiolactams **1a**–**6a** was published earlier.[11] The 1,5-benzodiazepine oximes **4**–**6** were obtained and characterized as previously described by Kosychova and Strumbreiciute.[12]



1a–**6a** **1**–**6**

1a, 1 R = H; R^1 = H; R^2 = H
2a, 2 R = H; R^1 = CH_3; R^2 = H
3a, 3 R = H; R^1 = H; R^2 = CH_3
4a, 4 R = CH_3; R^1 = H; R^2 = H
5a, 5 R = CH_3; R^1 = CH_3; R^2 = H
6a, 6 R = CH_3; R^1 = H; R^2 = CH_3

Scheme 1. Synthesis pathway for 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes **1**–**6**.

*Absorption spectra of compounds **1**–**6** in organic solvents*

The structures of the investigated oximes **1**–**6** are very similar, they differ only in the position and number of methyl groups in the diazepine ring. Compounds **4**–**6** contain methyl group in the diazepine ring at the 5th nitrogen atom, whereas compounds **1**–**3** do not. This determined the physicochemical properties of these compounds.

The influence of the nature of the organic solvent on the absorbance and fluorescence spectra of compounds **1**–**6** was investigated in different solvents, *i.e.*, polar protic ethanol, polar aprotic acetonitrile and dimethyl sulfoxide (DMSO), nonpolar aprotic 1,4-dioxane and 50 mM acetate buffer solution (pH 5.5), by spectrophotometry.

The UV–Vis spectra of compounds **1**–**6** in various solvents were recorded in the region 210–350 nm and are presented in Fig. 1.

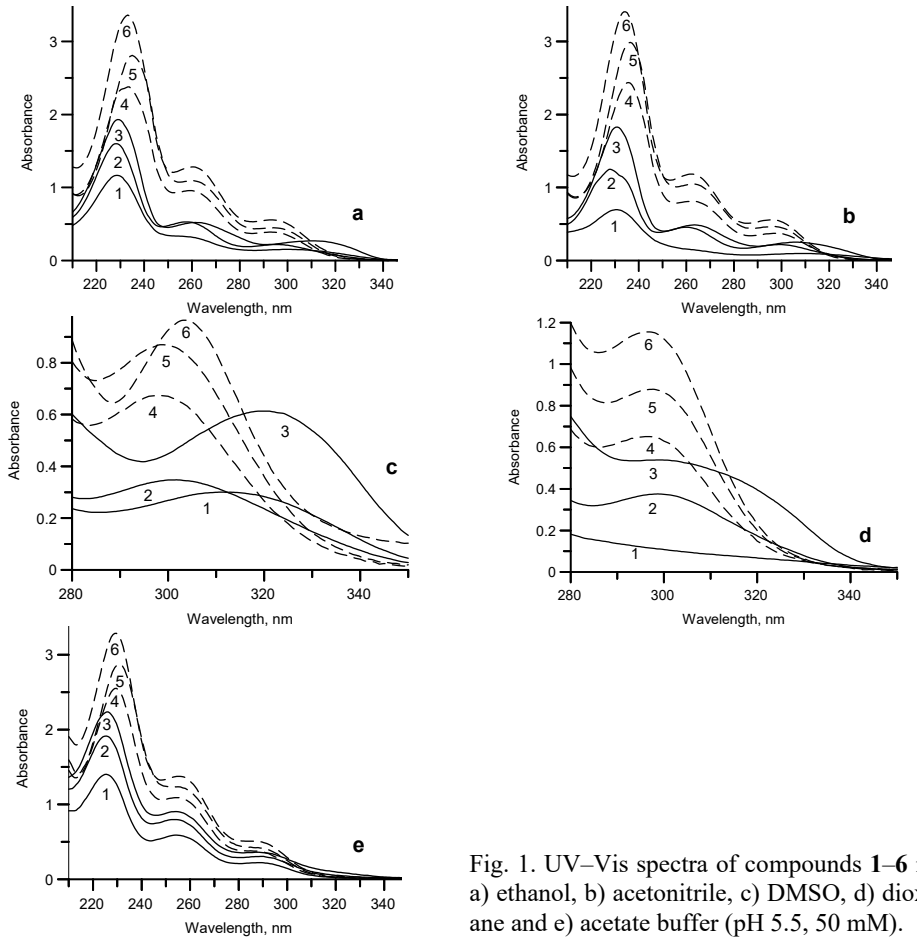


Fig. 1. UV–Vis spectra of compounds **1**–**6** in a) ethanol, b) acetonitrile, c) DMSO, d) dioxane and e) acetate buffer (pH 5.5, 50 mM).

The absorption spectra of compounds **1**–**6** in ethanol, acetonitrile and acetate buffer were compatible and had three bands in the 225–237, 255–265 and 290––322 nm regions (Table I). However, the spectra of compound **1** were different from those of the other compounds with only the first maximum being well defined, whereas the other two were almost flat. In DMSO and dioxane, due to the optical properties of these solvents, only one broad absorption maximum of the investigated compounds in the 280–350 nm region was observed (Fig. 1c and d). In addition, it was noted that the first absorption maxima of compounds **4**–**6** were shifted by about 5 nm to longer wavelengths in comparison to the spectra of compounds **1**–**3** in all the analyzed solvents (Table I).

TABLE I. The absorption spectra maxima of compounds **1**–**6** in organic solvents and acetate buffer solution

Solvent	Maxima	Compound					
		1	**2**	**3**	**4**	**5**	**6**
		λ / nm					
Acetate buffer	λ1	225	225	225	229	231	229
	λ2	255	255	255	255	260	255
	λ3	290	290	290	290	290	290
Ethanol	λ1	230	230	233	235	237	235
	λ2	260	260	264	262	262	262
	λ3	305	295–300	315	295	296	295
Acetonitrile	λ1	229	229	229	232	236	234
	λ2	–	260	262	265	265	265
	λ3	–	300	308	300	300	300
DMSO	λ1	314	302	322	300	300	304
Dioxane	λ1	–	300	310	298	298–300	298

The second absorption spectra maxima were registered in the 255–265 nm region for all investigated compounds. However, noticeable variations appeared for the position of the third absorption maxima in organic solvents, *e.g.*, in ethanol it was shifted to the longer wavelength region for compounds **1**–**3** in comparison to **4**–**6** (Fig. 1a).

Moreover, the most obvious difference in the spectra of all compounds appeared in the strongly polar, aprotic solvent DMSO. This allowed the analyzed compounds to be arranged in the following row **3**>**1**>**2**, **4**–**6** based on their absorption spectra wavelengths (322, 314 and 300–304 nm).

As was already mentioned, the absorption spectra were different for the compound without a methyl group in diazepine ring (**1**). It had only one clear absorption maximum at 229 nm in ethanol and acetonitrile. In addition, compound **1** did not absorb UV light in 250–350 nm region in aprotic solvents, such as acetonitrile and dioxane, while in the strongly polar aprotic DMSO, it had a maximum at 314 nm.

It was noticed that oximes **3** and **6** that have a methyl group at the third diazepine ring position differed most distinctively in organic solvents by the shift of the third absorption maxima to longer wavelengths (Fig. 1c). For oxime **3**, the shift reached up to 20 nm in DMSO in comparison to compound **2** and 4 nm for oxime **6** in comparison to compounds **4** and **5**.

In acetate buffer solution, the absorbance spectra maxima were observed at the same wavelengths 225, 255, 290 nm and 229–231, 255–260 and 290 nm, respectively, for compounds **1**–**3** and **4**–**6** (Fig. 1e).

Values of absorption spectra maxima and determined values of extinction coefficient in ethanol and acetate buffer solution are presented in Table II.

TABLE II. Extinction coefficients (ε) for oximes **1**–**6** in ethanol and acetate buffer solution

Solvent	Comp.	λ_1 / nm	ε_1 / mM^{-1} cm^{-1}	λ_2, /nm	ε_2/ mM^{-1} cm^{-1}	λ_3, / nm	ε_3 / mM^{-1} cm^{-1}
Ethanol	**1**	230	20.8	260	6.3	305	2.7
	2	230	18.4	260	6.3	295–300	2.5
	3	233	25.0	264	6.8	315	3.4
	4	235	27.6	262	10.3	295	4.2
	5	237	28.4	262	10.7	296	4.4
	6	235	25.7	262	9.6	295	4.2
Acetate buffer	**1**	225	18.1	255	7.6	290	2.9
	2	225	13.5	255	5.7	290	2.1
	3	225	26.5	255	10.7	290	4.3
	4	229	25.5	255	10.9	290	3.7
	5	231	23.7	260	9.6	290	3.4
	6	229	24.3	255	10.1	290	3.6

The presented results show that the spectral properties of compounds **1**–**6** depend not only on their molecular structure, but also on the nature of the solvent. Absorbance spectra maxima differences in organic solvents for compounds **1** and **2** and **4** and **5** are insignificant (5 nm), whereas for compounds **3** and **6** they diverge, *i.e.*, the absorbance spectra maxima are shifted to the longer wavelength region (red shifted). The utmost shift was observed for third absorbance spectra maxima of compound **3**.

*Fluorescence spectra of compounds **1**–**6** in organic solvents*

Fluorescence properties of the synthesized compounds **1**–**6** in the selected organic solvents and acetate buffer solution were also investigated. It was observed that fluorescence was characteristic to newly synthesized compounds and depended on their structure, as well as on the nature of the solvent.

The excitation spectra of the investigated compounds **1**–**6** were observed in the region 250–340 nm, using an emission wavelength of 370 nm. The emission spectra of compounds were observed in the region 330–450 nm using an excitation wavelength of 290 nm. The maximal intensities of the emission spectra of the

compounds in the organic solvents were observed in the interval 360–380 nm. The fluorescence spectra of the investigated compounds are presented in Fig. 2. Excitation spectra of compounds **1**–**6** had two maxima in the interval 250–340 nm. The values of excitation and emission spectra maxima are presented in Table III.

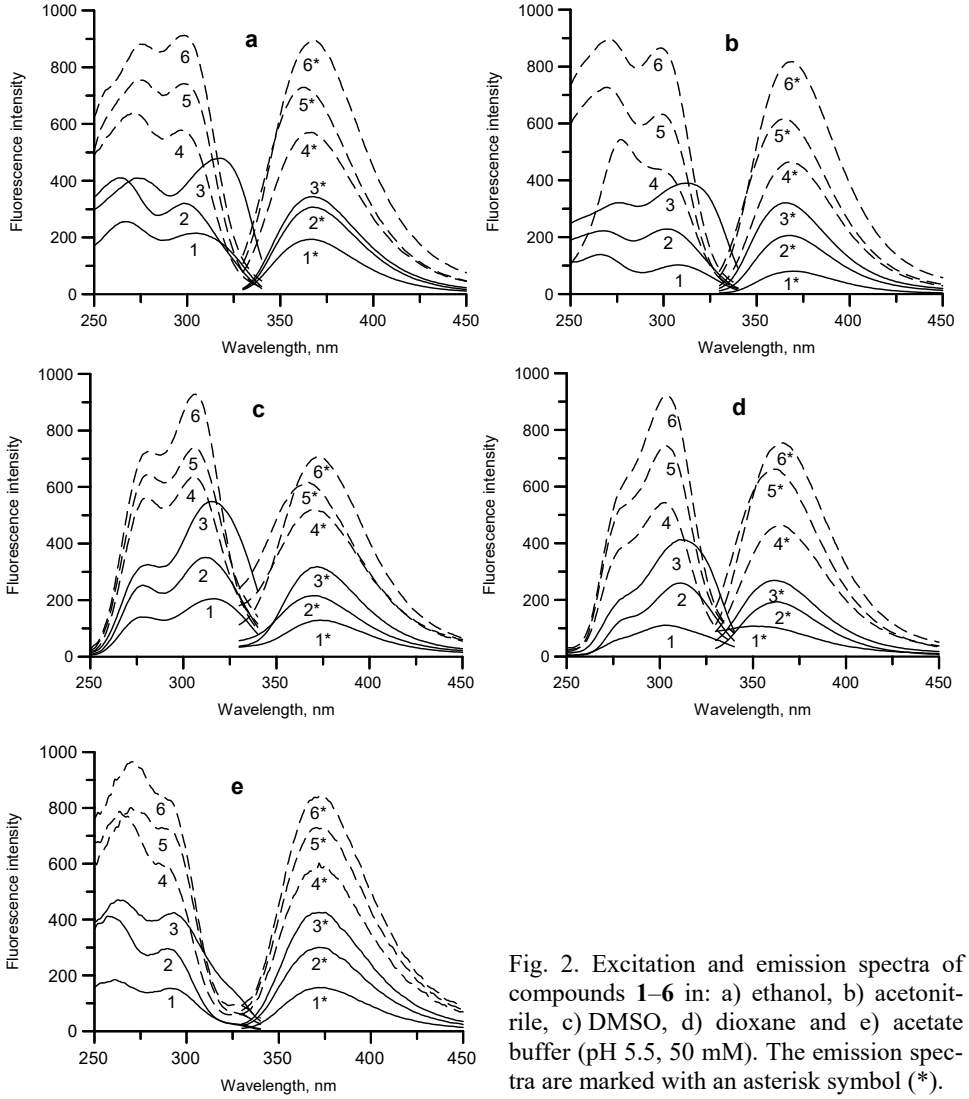


Fig. 2. Excitation and emission spectra of compounds **1**–**6** in: a) ethanol, b) acetonitrile, c) DMSO, d) dioxane and e) acetate buffer (pH 5.5, 50 mM). The emission spectra are marked with an asterisk symbol (*).

In acetate buffer, differences in the excitation spectra of compounds **1**–**3** and **4**–**6** were observed at the position of the first spectra maxima, whereas in all organic solvents at the second one. In the latter case, the second excitation spectra maxima of compounds **1**–**3** were registered in the 363–368 nm interval (aceto-

nitrile and ethanol), 310–315 (DMSO) and 305–312 nm (dioxane), while for compounds **4**–**6**, at 295 (ethanol), 298–301 (acetonitrile), 305 (DMSO) and 300 nm (dioxane).

TABLE III. Excitation and emission spectra maxima of compounds **1**–**6** in various solvents (λ_{ex} and λ_{em}, nm)

Compd.	Acetate buffer		Ethanol		Acetonitrile		DMSO		Dioxane	
	λ_{ex}	λ_{em}	λ_{ex}	λ_{em}	λ_{ex}	λ_{em}	λ_{ex}	λ_{em}	λ_{ex}	λ_{em}
1	260 290	371	265 305	364	265 308	368	275 315	373	280[a] 305	355
2	258 288	371	260 295	366	267 301	366	275 310	368	280[a] 309	360
3	262 290	371	270 315	366	– 313	363	278 315	370	280[a] 312	360
4	262 288	371	270 295	364	275 301	366	278 305	368	280[a] 300	362
5	270 288	371	273 295	360	268 298	362	278 305	364	280[a] 300	360
6	268 286	371	273 295	366	268 298	367	278 305	372	280[a] 300	363

[a]Poorly defined maximum, "shoulder"

It should be pointed out that all compounds **1**–**6** are capable of forming intramolecular hydrogen bond between the hydrogen of NH and the adjacent oxygen atom of the N-OH group. Moreover, compounds **1**–**3** are capable of forming hydrogen bonds between hydrogen at the 5th nitrogen atom in the diazepine ring and hydrogen bond donors, such as protic solvent molecules, whereas in compounds **4**–**6**, this position is occupied by a methyl group. This structural feature influenced the observed spectral properties.

Significant differences were observed in the spectra of compound **3** in all solvents, except acetate buffer solution: the second absorption band was strongly red shifted to the 312–315 nm region in comparison to the other investigated compounds. Both, the UV–Vis and fluorescence spectra showed that the maxima of compound **3** were shifted to longer wavelengths, which means that the molecular structure of the compound determined the observed bathochromic effects.

As was mentioned above, the oximes were arranged in the following order **3**>**1**>**2, 4**–**6** based on the wavelengths of their UV–Vis absorbance spectra in the strongly polar aprotic solvent DMSO. A similar trend was reproduced in their excitation spectra in DMSO.

The emission spectra maxima of compounds **1**–**6** along with the dielectric constants of the solvents are presented in Table IV. The maxima values in acetate buffer and DMSO were registered in the 364–371 nm interval. In the other organic solvents, the maxima values were shifted to the shorter wavelength region, depending on polarity of organic solvent used, which is in good agreement with various studies of the effect of the solvent on electronic absorption and fluorescence spectra.[13] As is known from the literature, the fluorescence band maxima are largely red-shifted with increasing solvent polarity compared to the corresponding absorption band under the same conditions. This fact indicates an increase in dipole moment of the excited state compared to ground state.[15]

TABLE IV. Dielectric constants (ε, 20 °C) of various solvents and emission spectra maxima of compounds **1**–**6**

Solvent	ε[16]	Compound					
		1	**2**	**3**	**4**	**5**	**6**
Acetate buffer	79.7[a]	371	371	371	371	371	371
DMSO	46.6	373	368	370	368	364	372
Acetonitrile	37.5	368	366	363	366	362	367
EtOH	22.4	364	366	366	364	360	366
Dioxane	2.2	355	360	360	362	360	363

[a]Dielectric constant of the buffer solution was assumed equal to that of water at 20 °C

A comparison of the emission spectra maxima of compounds **1**–**6** registered in DMSO, acetonitrile and ethanol with the spectral maxima in the least polar dioxane showed a bathochromic shift of 10 nm.

Unexpectedly, the emission spectra maxima of compound **5** in DMSO, acetonitrile and ethanol were shifted to shorter wavelengths in comparison to the other investigated compounds.

Along with studies in various organic solvents, the fluorescence and absorption spectra of compounds **1**–**6** were registered in different Britton–Robinson buffers of different pH values. Variation of the pH values influenced insignificantly the absorption and emission spectra, but strongly influenced the fluorescence intensity, which decreased at acidic pH values

The pK_a values of compounds **1**–**6** were determined using their spectrophotometric and spectrofluorimetric data. The fluorescence quantum yield (Φ_f) data of the synthesized compounds are presented in Table V.

TABLE V. The values of pK_a and fluorescence quantum yields (Φ_f) of compounds **1**–**6**

Compound	Absorbance pK_a	Fluorescence pK_a	Quantum yields Φ_f
1	4.9±0.1	4.56±0.04	0.016
2	4.4±0.1	4.60±0.03	0.019
3	4.4±0.1	4.51±0.03	0.014
4	4.1±0.1	4.21±0.05	0.016
5	4.4±0.2	4.41±0.03	0.015
6	4.1±0.2	4.26±0.03	0.010

The pK_a values determined by both methods are similar. Fluorescence quantum yields (Φ_f) for synthesized compounds in buffer solution were calculated. The fluorescence quantum yields (Φ_f) of compounds **1**–**6** were determined *via* the comparison method, using tryptophan as a standard sample in 0.01 M potassium phosphate buffer solution, pH 7.2.[14]

Absolute values were calculated using standard samples that have a fixed and known fluorescence quantum yield, according to the following equation:

$$\Phi_f = \Phi_{st} (F_f/F_{st}) (\eta^2_f/\eta^2_{st}) \quad (1)$$

where the subscripts st and f denote standard and test respectively, Φ is the fluorescence quantum yield, F the gradient from the plot of the integrated fluorescence intensity *vs*. absorbance, and η is the refractive index of the solvent.

The observed fluorescence efficiency of the investigated oximes **1**–**6** was low suggesting that these compounds poorly absorb photons which could start fluorescence.

CONCLUSIONS

The novel 1,3,4,5-tetrahydro-2*H*-1,5-benzodiazepin-2-one oximes **1**–**3** were obtained by refluxing mixture of the appropriate thiolactam **1a**–**3a**, hydroxylamine hydrochloride and sodium acetate in anhydrous ethanol. The structure of compounds **1**–**3** was proved by the methods of ^{1}H- and ^{13}C-NMR, IR and elemental analysis.

The influence of the nature of the organic solvent on the absorbance and fluorescence spectra of compounds **1**–**6** was investigated in 5 solvents. Noticeable differences in absorption spectra appeared in organic solvents that were determined not only by the structures of the title compounds but also by polarity of the solvent. Significant differences were observed in both the UV–Vis and the fluorescence spectra of compound **3** in all solvents. The molecular structure of this oxime determined the observed bathochromic effects.

The 1,5-benzodiazepine oxime derivatives **1**–**6** showed fluorescence in solutions. The fluorescence intensity and quantum yield values of the investigated compounds depended on their structure, as well as on the nature of solvent used.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

СИНТЕЗА И СПЕКТРАЛНА КАРАКТЕРИЗАЦИЈА НОВИХ 1,5-БЕНЗОДИАЗЕПИСКИХ ОКСИМСИХ ДЕРИВАТА

LINA REKOVIC[1], LIDIJA KOSYCHOVA[1,2], IRINA BRATKOVSKAJA[1] и REGINA VIDZIUNAITE[1]

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Синтетисана су три нова 1,3,4,5-тетрахидро-2*H*-1,5-бензодиазепин-2-он-оксима и окарактерисани су ^{1}H-, ^{13}C-NMR и ИЦ спектрима и елементарном анализом. Нова једињења су, заједно са претходно описаним једињењима која имају једну додатну метил-групу на 5. атому азота, окарактерисана UV–Vis и флуоресцентном спектроскопијом, у различитим растварачима. Испитан је и дискутован утицај органског растварача на спектроскопске особине испитиваних једињења.

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