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Structural investigations of aroylhydrazones derived from nicotinic acid hydrazide in solid state and in solution

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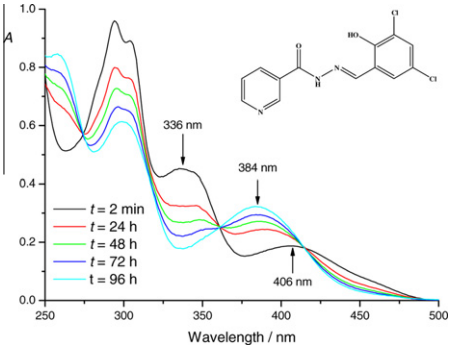
HIGHLIGHTS

- Substituents affected ketoamino–enolimino equilibria of aroylhydrazones.
- Tautomerism was observed for aroylhydrazone with two hydroxyl groups.
- Chloride substituents induced formation of ketoamino tautomers.
- When exposed to daylight *E*/*Z* isomerisation in aqueous solutions occurred.

GRAPHICAL ABSTRACT



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ABSTRACT

Structural forms of aroylhydrazones derived from nicotinic acid hydrazide have been studied in the solid state by FT-IR spectroscopy and in solution by NMR, UV–Vis and ATR spectroscopy. The studied compounds were *N*′-benzylidene-3-pyridinecarbohydrazide (**1**), *N*′-(2,4-dihydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**2**), *N*′-(5-chloro-2-hydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**3**), and *N*′-(3,5-dichloro-2-hydroxymethoxyphenylmethylidene)-3-pyridinecarbohydrazide (**4**). The compound **1** adopted the most stable ketoamine form (form I, —CO—NH—N=C—) in the solid state as well as in various organic solvents. In mixtures of organic solvents with water the UV–Vis and ATR spectra implied intermolecular hydrogen bonding of **1** with water molecules. The presence of both tautomeric forms I and II (form II, —COH=N—N=C—) was proposed for the solid substance and highly concentrated solutions of **2**, whereas form I was detected as the predominant one in diluted solutions. For compounds **3** and **4** a coexistence of forms I and III (form III, —CO—NH—NH—C=C—CO—) was noticed in the solid state and in polar protic organic solvents. The conversion to form III was induced by increasing the water content in the solvent mixtures. This process was the most pronounced for compound **4**. When exposed to daylight, an appearance of a new band was observed during time in the UV–Vis spectrum of **4** in organic solvent/water 1/1 mixtures, which implied that tautomeric interconversion was most likely followed by *E*/*Z* isomerisation.

Introduction

The chemistry of aroylhydrazones has been intensively studied due to their analytical and biological applications. Many compounds of this type possess antimicrobial, antituberculosis and antitumor activities [1–3]. Recent studies have shown that nicotinic acid hydrazones could be considered as anti-inflammatory and analgesic agents [4], and as a novel pharmacophore in the design of anticonvulsant drugs [5].

Aroylhydrazones can be involved in keto-enol tautomeric interconversion (Scheme 1, forms I and II). Ketoamine tautomer (form I) is usually the most stable form in solution and in the solid state

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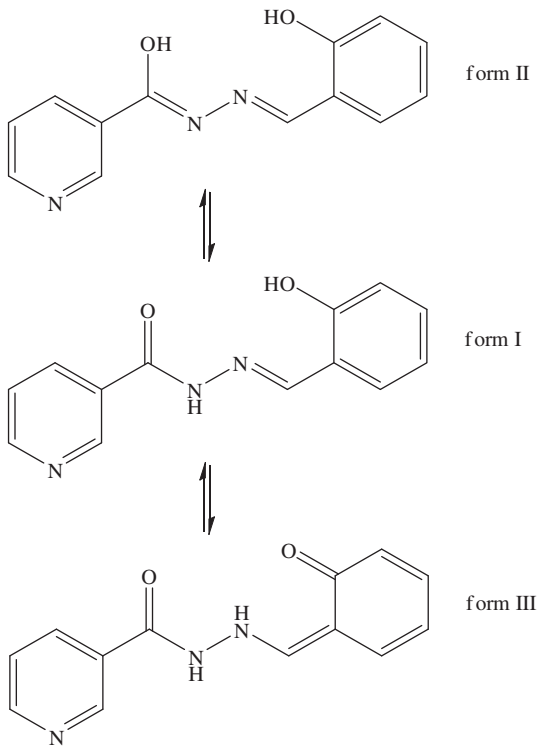
since it is stabilized by strong intermolecular or solute–solvent hydrogen bonds [6–9]. However, upon complexation of metal cations, the tautomeric interconversion into the enolimine (form II) commonly occurs [6–8,10]. If the hydroxyl group is situated in *ortho* position regarding the C=N double bond, tautomeric equilibrium can also involve this part of the molecule (Scheme 1, form III). Detailed investigations of Schiff bases derived from salicylaldehyde and its derivatives have shown that the solvent polarity, as well as the nature of the substituents in the salicylidene part of the molecule affects the electron distribution in the system and the position of the tautomeric equilibrium [11]. The polar solvents cause shift of the interconversion to the ketoamine (form III), and the process is accompanied by an appearance of a new band in absorption spectra with λ_{max} > 400 nm [11,12]. Electron donor groups in *ortho* and/or *para* position (electron withdrawing substituents in *meta* position) with respect to the C=N double bond also shift the equilibrium toward NH tautomer (form III) [11]. Limbach et al. have shown that in Schiff bases derived from pyridoxal, protonation of the pyridine nitrogen shift the tautomeric equilibrium to the ketoamino form in the solid state and in the polar aprotic solvents but only when there is aliphatic substituent on azomethine nitrogen. When the Schiff base nitrogen atom carries an aromatic substituent, protonation of ring nitrogen has little effect on the equilibrium [13–15]. Apart from tautomeric interconversion, an *E–Z* isomerisation of hydrazones due to the solvent effect, pH value or UV irradiation can also occur (Scheme 1, form IV) [16–19]. Since different tautomeric and isomeric forms can have diverse properties, the detailed study of such equilibria is relevant for successful application of these compounds.

Previously we reported on the structural properties of *N′*-salicylidene-3-pyridinecarbohydrazide and *N′*-(2-hydroxy-3-methoxyphenylmethylidene)-3-pyridinecarbohydrazide [10,20,21]. In solution and in the solid phase both compounds appear in form I *E* [20]. *E*/Z interconversion as well as the formation of tautomeric form III was not observed even in the systems containing water [21]. The tautomeric interconversion into the form II occurred upon



Scheme 1. Possible tautomeric forms of aroylhydrazones.

complexation of metal cations. As a consequence, these aroylhydrazones coordinate cations as doubly deprotonated, tridentate *O,N,O* ligands both in the solution and in the solid state [10]. In the present work, we extended the investigations of various equilibria (keto-enol tautomerism, *E*/*Z* interconversion, and hydrolysis) to structurally related aroylhydrazones, derived from nicotinic acid hydrazide and differently substituted benzaldehydes.

Experimental

Reagents

Compounds **1**–**4** were prepared by mixing equimolar amounts of nicotinic acid hydrazide (Fluka) with aldehyde (benzaldehyde, 2,4-dihydroxybenzaldehyde, 2-hydroxy-4-chlorobenzaldehyde and 3,5-dichloro-2-hydroxybenzaldehyde purchased from Sigma). The reactions were carried out in dry ethanol under argon atmosphere at 85 °C for 20 h. The solvent were evaporated from reaction mixtures and the solids were suspended in CH_2Cl_2 (EtOH was used for **2**), filtered and dryed at 50 °C. Hydrazones were characterised by standard methods. Deionized water and organic solvents of p.a. purity grade (Kemika) were used for spectroscopic measurements.

Apparatus

UV–Vis measurements were carried out using Varian Cary 3 spectrometer. The absorption spectra were recorded in the spectral range from 200 nm to 500 nm. Conventional quartz cells (l = 1 cm) were used throughout.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer in deuterated DMSO. COSY spectra were recorded to enable a complete assignment of signals. TMS was used as an internal standard.

FT-infrared spectra in transmission mode as well as in attenuated total reflection (ATR) mode were acquired on a Bruker Equinox 55 interferometer. Transmission spectra were obtained from solid samples prepared as KBr pellets. For each pellet, 1 mg of the solid sample and 100 mg of KBr were weighted, grounded in an agate mortar and pressed. Spectra were recorded over the spectral range between 4000 and 400 cm^{-1} at 2 cm^{-1} resolution. ATR spectra were measured using the PIKE MIRacle ATR sampling accessory with a diamond/ZnSe crystal plate. A pressure clamp was used during the measurement of the solid samples, whereas ATR spectra of the liquid samples were measured from a drop on the crystal plate. The spectrum of the solvent or the solvents mixture was used as a background while recording the ATR spectrum of the respective hydrazone solution. The spectra were taken in the single reflection configuration, over the spectral range 4000–600 cm^{-1} at 2 and 4 cm^{-1} resolution for solids and liquids, respectively. Thirty two scans were averaged to obtain a final spectrum.

Samples preparation

Stock solutions of compounds **1**–**4** ($c = 1 \times 10^{-3}$ mol dm^{-3}) were prepared in chloroform, dioxan, acetonitrile (MeCN), dimethylsulphoxide (DMSO), and methanol (MeOH). The working solutions for UV–Vis measurements were prepared in a 10.0 mL flask in pure organic solvents as well as in MeCN/water, DMSO/water and MeOH/water mixtures by adding stock solution of **1**–**4** and an appropriate amount of organic solvent or water. Concentration of the hydrazones in the working samples was 5×10^{-5} mol dm^{-3}.

Working samples for measurement of ATR spectra were prepared by dilution of an appropriate volume of the DMSO stock solutions of compounds **1**–**4** (0.40 mol dm^{-3}) with DMSO and water, resulting in the final hydrazone concentration of 0.20 mol dm^{-3}. Solutions of all

studied compounds were prepared in pure DMSO, but in different volume portions of solvents when DMSO was mixed with water. While **1** was soluble in the solvent mixtures in the volume ratios, $V(DMSO)/V(H_2O)$, of 9/1, 8/2, 7/3, 6/4 and 5/5, a poor solubility of **2** and **3** allowed preparation of solutions in the mixed solvents only in ratios $V(DMSO)/V(H_2O)$ of 9/1 and 8/2. At the given concentration compound **4** precipitated from the DMSO solution upon addition of even a small amount of water.

Results and discussion

The structural analysis of *N′*-benzylidene-3-pyridinecarbohydrazide (**1**), *N′*-(2,4-dihydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**2**), *N′*-(5-chloro-2-hydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**3**), and *N′*-(3,5-dichloro-2-hydroxymethoxyphenylmethylidene)-3-pyridinecarbohydrazide (**4**), shown in Scheme 2, was performed by different spectroscopic techniques both in the solid state and in the solution. The solid state structure of compounds **1**–**4** was investigated by FT-IR spectroscopy, whereas the NMR, UV–Vis and FT-IR spectroscopies were used for the structural analysis of aroylhydrazones in solvents of different polarities as well as in the mixtures of organic solvents and water.

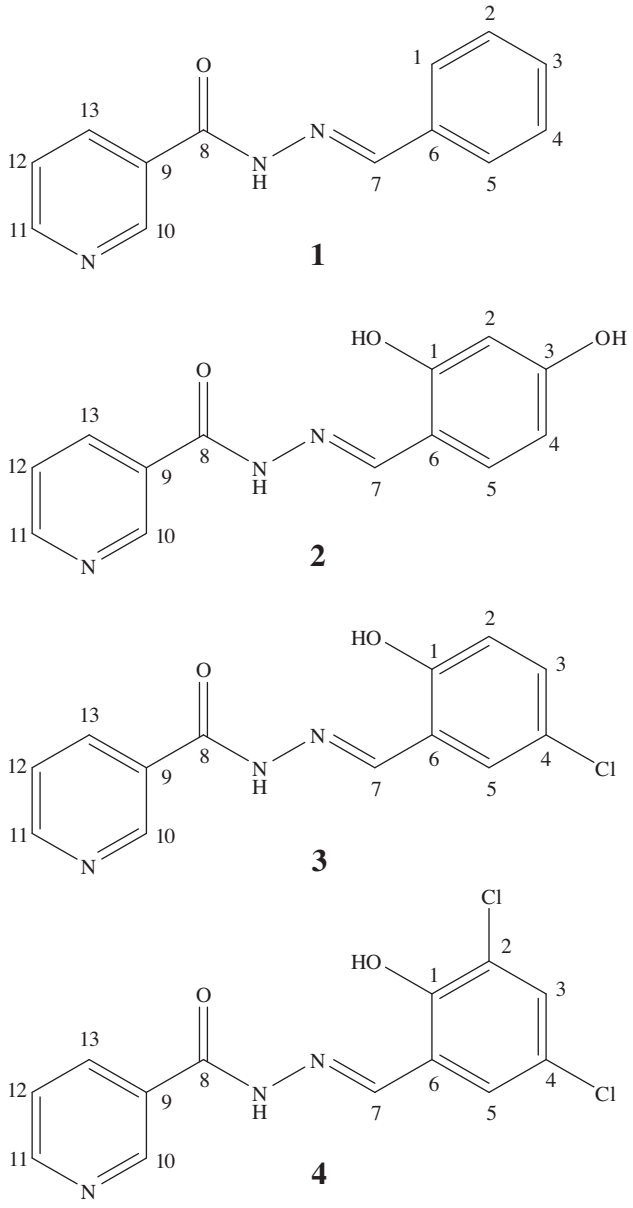
The compound **1**, derived from benzaldehyde with no hydroxyl group in *ortho* position to C=N double bond, was chosen as a model system since no tautomeric interconversion can occur in this part of the molecule (form I to form III). The hydroxyl group situated in *para* position to C=N double bond in **2** can increase the basicity of the nitrogen atom, whereas chlorides in *meta* position (compounds **3** and **4**) can enhance the acidity of phenolic group [11]. As a consequence, both substituents can shift the tautomeric interconversion of aroylhydrazones to form III.

FT-IR spectra of the solid samples

Infrared spectra of the solid samples **1**–**4** were taken in attenuated total reflection (ATR) mode as well as in transmission mode (KBr pellets). The spectra obtained by two IR techniques were in a very good agreement, and small spectral differences, if observed, were attributed to the preparation procedures of the measured samples (Figs. S1–S4). Given the resemblance between the spectra obtained by two IR techniques, and the following study of the DMSO/water solutions in the reflectance mode, a preliminary assignment of vibrational bands is given in Table 1 only for ATR spectra [9,16,21–25].

A strong band at 1649 cm^{-1} in the spectrum of **1** was assigned as the amide I band, originating mainly from the stretching vibration of the C=O group as well as from the C—N stretching and NH bending to a lesser extent (Fig. 1a). A weak band at 1665 cm^{-1} implied that some carbonyl groups were not involved in interactions with adjacent molecules [21]. In addition to vibrational modes of the amide group, the NH deformation and the N—C=O stretching contributed to the intense amide II band at 1551 cm^{-1}, whereas the C—N stretching and the NH bending gave rise to the weak amide III band at 1355 cm^{-1}. The deformation of the NH group produced a strong band observed at 1283 cm^{-1}. Stretching mode of the C=N bond and stretching vibrations of the phenyl and pyridyl rings contributed to a band of moderate intensity at 1602 cm^{-1}. Other bands in the range from 1600 to 1300 cm^{-1} were mostly attributed to the stretching of the aromatic moieties in the molecular structure of **1**.

Unlike the well defined vibrational bands in the spectrum of **1**, clearly indicating the presence of form I, a pattern of the overlapping bands between 1650 and 1500 cm^{-1} in the ATR spectrum of **2** implied existence of various molecular forms (Fig. 1b). Although



Scheme 2. Structures of compounds **1**–**4**.

a band at 1635 cm^{-1} was assigned to the stretching vibration of the C=O group present in the molecular structure of form I, disappearance of the N—H stretching band from the high wavenumber region, appearance of the amide II band as a shoulder (1554 cm^{-1}) and broadening of the amide III band (1350 cm^{-1}) pointed to a change of the amide group. A broad band peaking at 1608 and 1600 cm^{-1}, associated with the stretching modes of the aromatic rings and C=N bond in hydrazones, indicated a change in the central part of the molecule most likely due to a conversion of keto-amine (form I) to enolimine (form II) [16]. Moreover, a broad irregularly shaped band of moderate intensity at 1229 cm^{-1}, associated with the bending of the enol OH group, supported presence of form II, beside form I, in the solid state of compound **2**.

Interestingly, in the ATR spectra of the solids **3** and **4** two well separated bands were obtained in the spectral range of the carbonyl group stretching (Fig. 1c and d). Intense bands at 1669 cm^{-1} and 1657 cm^{-1} were assigned to the C=O stretching of the amide group in compounds **3** and **4**, respectively, whereas those observed at the higher wavenumbers, 1688 cm^{-1} (**3**) and 1695 cm^{-1} (**4**), implied an

Table 1
Preliminary assignment of the vibrational bands in the ATR spectra of the solid samples **1**–**4** and their solutions in dimethylsulphoxide in the spectral range 1700–1100 cm^{-1}.

Wavenumber (cm^{-1})								Assignment
Solid				DMSO solution				
1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	
		1688 m	1695 m					ν C═O (form III)
1665 w		1669 s	1657 s	1679 s	1673 s	1677 s	1680 s	ν C═O + ν C—N + δ NH (amide I)
1649 s	1635 s							ν C═O + ν C—N + δ NH (amide I)
					1630 m			ν C═N conjugated (form II)
	1608 m	1610 w						ν C═N; ν CC/CN (pyridyl); ν CC (phenyl)
1602 w	1600 w	1599 w	1596 w	1603 w	1608 s	1603 w	1601 sh	ν C═N; ν CC/CN (pyridyl); ν CC (phenyl)
1588 m	1577 m	1590 w	1590 w	1589 m	1591 m	1591 m	1590 m	ν CC/CN (pyridyl); ν CC (phenyl)
1551 s	1554 sh	1542 m	1544 m	1562 m	1561 w	1558 m	1558 m	δ NH + ν N—C═O (amide II)
	1508 m				1513 m			ν CC (phenyl)
1492 w				1492 w				ν CC (phenyl)
	1476 w	1480 s	1484 w	1481 w		1481 m		ν CC (phenyl)
1473 w	1463 w				1473 w			ν CC (phenyl, pyridyl)
			1449 s		1464 w		1452 m	ν CC (phenyl)
1447 w	1427 w	1432 w	1431 m	1450 w			1434 w	ν CC (phenyl, pyridyl)
1421 w	1416 w	1419 w	1420 m	1416 w	1417 w	1422 m	1418 w	ν CC (pyridyl)
		1369 w	1379 w			1374 w	1377 w	ν CC (phenyl, pyridyl)
1355 w	1350 w	1336 s	1344 m	1366 m	1356 w	1349 m	1349 m	ν C—N + δ NH (amide III)
1330 w	1324 w				1326 w			ν CC (pyridyl)
	1295 m		1298 m	1285 s	1287 s	1286 s	1294 s	δ NH; δ OH
1283 s		1280 vs	1284 m					δ NH
		1271 s	1277 m		1265 sh	1271 sh	1274 m	δ OH
	1229 m				1223 m			δ OH (form II)
1222 w		1214 w	1218 m	1229 w			1223 m	δ_{ip} CH
1203 w	1200 w	1187 m	1197 w	1195 w	1178 w	1185 w	1184 m	ν C—N
1176 w	1169 w		1179 m		1167 w			δ_{ip} CH
1154 w		1150 m	1164 m	1149 m	1153 w	1154 w	1161 w	δ_{ip} CH
1131 m	1136 w	1118 m			1127 w	1117 w		ν C—O

Abbreviations: sh, shoulder; w, weak; m, medium; s, strong; vs, very strong; ν, stretching; δ, deformation; ip, in plane.

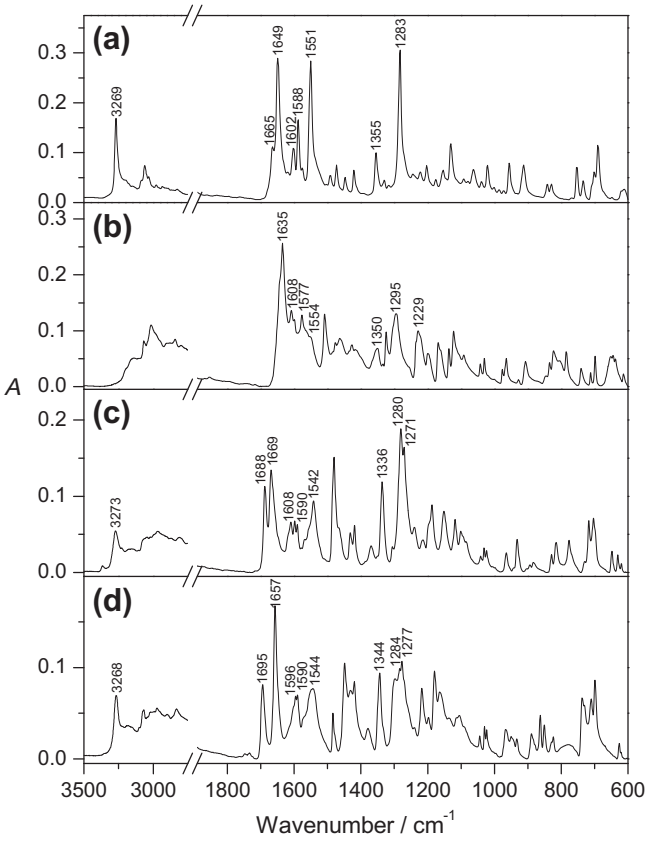


Fig. 1. ATR spectra of the solid substances **1** (a), **2** (b), **3** (c) and **4** (d).

additional carbonyl group in the molecular structure. Given the possibility of tautomeric conversion in the phenylmethylidene part

Table 2
^{1}H NMR spectra of **1**–**4** in dimethylsulphoxide.

Proton	δ (ppm)			
	1	**2**	**3**	**4**
H (C1) (O)	7.762 (dd)	12.064 (s)	12.316 (s)	12.648 (s)
H (C2)	7.4731 (m)	6.405 (dd)	6.967 (dd)	–
H (C3) (O)	7.4731 (m)	9.987 (s)	7.321 (dd)	7.679 (d)
H (C4)	7.4731 (m)	6.376 (d)	–	–
H (C5)	7.762 (dd)	7.354 (d)	7.689 (d)	7.608 (d)
H (C7)	8.484 (s)	8.531 (s)	8.644 (s)	8.582 (s)
H (C10)	9.104 (s)	9.094 (s)	9.111 (s)	9.118 (s)
H (C11)	8.779 (d)	8.769 (d)	8.782 (d)	8.806 (d)
H (C12)	7.576 (dd)	7.567 (dd)	7.578 (dd)	7.598 (t)
H (C13)	8.281 (d)	8.276 (d)	8.291 (d)	8.302 (d)
H (N)	12.021 (s)	11.358 (s)	11.185 (s)	12.362 (s)

of the aroylhydrazone molecules having a hydroxyl group at the *ortho* position with respect to the C═N bond, it was very likely that form III was formed. Owing to the electron withdrawing ability, chloride substituents on the phenyl moiety facilitated transfer of the hydrogen atom from the hydroxyl group to the nitrogen atom of the C═N group. Nevertheless, strong bands at 1271 cm^{-1} (**3**) and 1277 cm^{-1} (**4**), assigned to the OH deformation modes, indicated the presence of the form I.

^{1}H and ^{13}C NMR spectra

NMR spectral data of compounds **1**–**4** in DMSO are given in Tables 2 and 3. A complete assignment of signals corresponding to aromatic protons was enabled by 2D (COSY) ^{1}H NMR spectra (data not shown). In the ^{1}H NMR spectra of **2**–**4** strong deshielding of hydroxyl protons (δ > 12 ppm) due to intramolecular hydrogen bonds was observed. The similar downfield shifts of the —OH signals were recorded for analogues aroylhydrazones derived from nicotinic

Table 3
^{13}C NMR spectra of **1**–**4** in dimethylsulphoxide.

C atom	δ (ppm)			
	1	**2**	**3**	**4**
1	129.317	161.553	156.531	152.731
2	127.666	103.163	118.702	123.443
3	130.714	161.40	131.402	130.819
4	127.666	108.284	123.521	121.997
5	129.317	131.767	127.959	128.832
6	134.614	110.937	121.107	121.133
7	148.949	149.01	146.741	147.907
8	162.195	160.005	162.049	162.084
9	129.668	129.304	129.075	128.528
10	152.741	152.739	152.926	153.132
11	149.066	150.104	149.135	149.178
12	124.043	124.048	124.053	124.066
13	135.896	135.804	135.927	135.987

acid hydrazide and salicylaldehyde or *o*-vanillin [20,21]. The signals of NH protons in compounds **1**–**4** were observed at (11.2–12.4) ppm, indicating hydrogen bond formation between solvent molecules and amide proton. The signals at (8.5–8.6) ppm, assigned to azomethine protons, pointed out that all investigated aroylhydrazones in DMSO adopted the most stable conformation, form I *E* [8,20,21]. The signals of azomethine protons in aroylhydrazones in a *Z* configuration would be at lower field, approximately around 8.32 ppm, as in the salicylaldehyde benzoyl hydrazone, due to increased electron density of the HC=N double bond [16]. Although the intramolecular OH—N=C bond was noticed, that was not the case with the tautomeric interconversion leading to form III, since no splitting of methine proton signal was observed in the spectra of **2**–**4**.

The presence of form I for compounds **1**–**4** in DMSO was confirmed by ^{13}C NMR spectra. The signals observed at ≈160 ppm are typical for C=O moiety of amide group (form I), while the signals of the same carbon atom (C8) in the tautomeric form III should be at ≈100 ppm [9,17]. In addition, the signals at ≈180 ppm typical for C1 atom in form III were missing in the spectra of aroylhydrazones **2**–**4**.

UV–Vis spectra

The absorption electronic spectra of **1**–**4** were acquired in solvents of different polarities as well as in the mixtures of organic solvents with water (Table 4). The spectra were recorded immediately after the preparation of solutions and during the time, up to several days. The bands at ≈220 nm, attributed to the $\pi \rightarrow \pi^*$ transition of the amide C=O group in investigated hydrazones, was also

Table 4
UV–Vis spectra of **1**–**4** in different solvents.

Solvent	λ (nm) (10^{-4} ε/L mol^{-1} cm^{-1})			
	1	**2**	**3**	**4**
Chloroform		*	342 (0.96)	343 (0.92)
			299 sh (1.42)	304 sh (1.85)
	292 (1.99)		290 (1.74)	294 (2.22)
			244 (1.35)	242 (1.47)
Dioxan		*	337 (1.18)	343 (1.03)
			298 (1.66)	304 (1.88)
	293 (2.14)		289 (1.98)	294 (2.20)
				242 (1.49)
Acetonitrile (MeCN)		*	336 (1.09)	338 (1.01)
			297 (1.79)	301 (2.22)
	293 (2.15)		286 (2.08)	291 (2.53)
	217 (1.53)		239 sh (1.49)	242 sh (1.61)
			220 (1.92)	
DMSO			[b]	455 (0.09)
		336 (2.46)	339 (1.48)	338 (1.19)
	302 (2.18)	304 (1.34)	301 (1.51)	307 (1.97)
		294 (1.08)	291 (1.69)	296 (2.08)
MeOH		400 (0.05)[a]	400 (0.03)[a,c]	400 (0.12)[a,c]
		334 (2.40)	339 (1.21)	339 (1.02)
	300 (2.33)	303 (1.47)	299 (1.60)	303 (1.97)
	218 (1.61)	246 (1.25)	289 (1.87)	292 (2.23)
		216 (1.93)	219 (2.04)	221 (2.27)
MeCN/H_2O 1/1		400 (0.05)[a]	400 (0.03)[a,c]	400 (0.12)[a,c]
		331 (2.58)	335 (1.12)	337 (1.00)
		302 (1.69)	298 (1.75)	302 (2.12)
	296 (2.36)	244 (1.40)	286 (2.02)	292 (2.39)
	217 (1.51)	216 (1.99)	239 sh (1.46)	222 (2.14)
			220 (1.96)	
DMSO/H_2O 1/1		[b]	400 (0.01)[a,c]	407 (0.22)[a,c]
		333 (2.49)	339 (1.28)	337 (0.99)
	300 (2.44)	302 (1.53)	298 (1.71)	304 (1.91)
		290 sh (1.10)	290 (1.94)	294 (2.10)
MeOH/H_2O 1/1		400 (0.05)[a]	400 (0.02)[a,c]	400 (0.33)[a,c]
		332 (2.58)	337 (1.23)	337 (0.91)
	298 (2.61)	302 (1.69)	298 sh (1.84)	302 (1.86)
	218 (1.63)	243 (1.37)	289 (2.13)	293 (2.06)
		215 (1.95)	218 (2.09)	222 (2.09)

* Not soluble.
[a] The bands corresponding to the absorption of the form III.
[b] The bands corresponding to the absorption of the form III appeared during the time.
[c] The *E*/*Z* photoizomerization occurred.

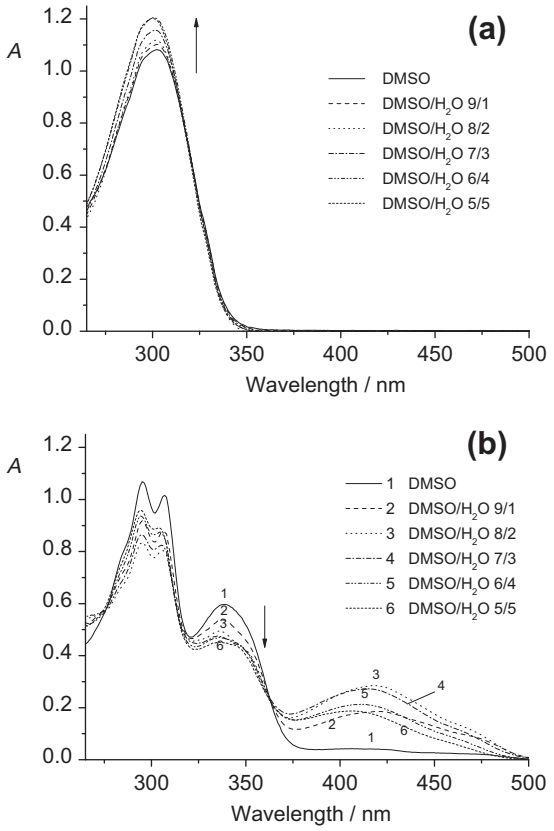


Fig. 2. UV–Vis spectra of **1** (a) and **4** (b) in DMSO and DMSO/H_2O mixtures: V(DMSO)/V(H_2O) 9/1, 8/2, 7/3, 6/4 and 5/5 acquired immediately after preparation of solutions. $c(\mathbf{1}) = c(\mathbf{4}) = 5 \times 10^{-5}$ mol dm^{-3}. Arrows show the change in absorbance with the increase in water content in DMSO/water mixtures.

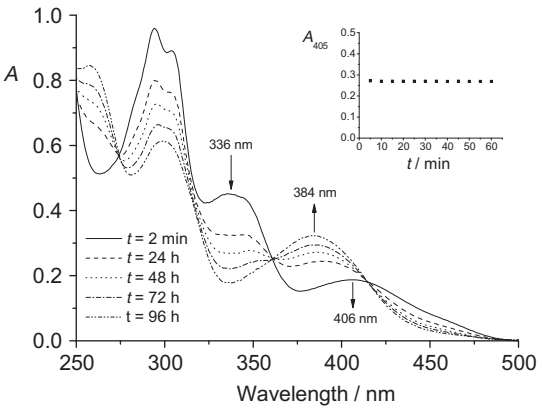


Fig. 3. UV–Vis spectra of **4** in DMSO/H_2O 1/1 mixture during the time when solution was exposed to light. Inset: The absorbance at 405 nm during the time when solution was kept in the dark. $c(\mathbf{4}) = 5 \times 10^{-5}$ mol dm^{-3}.

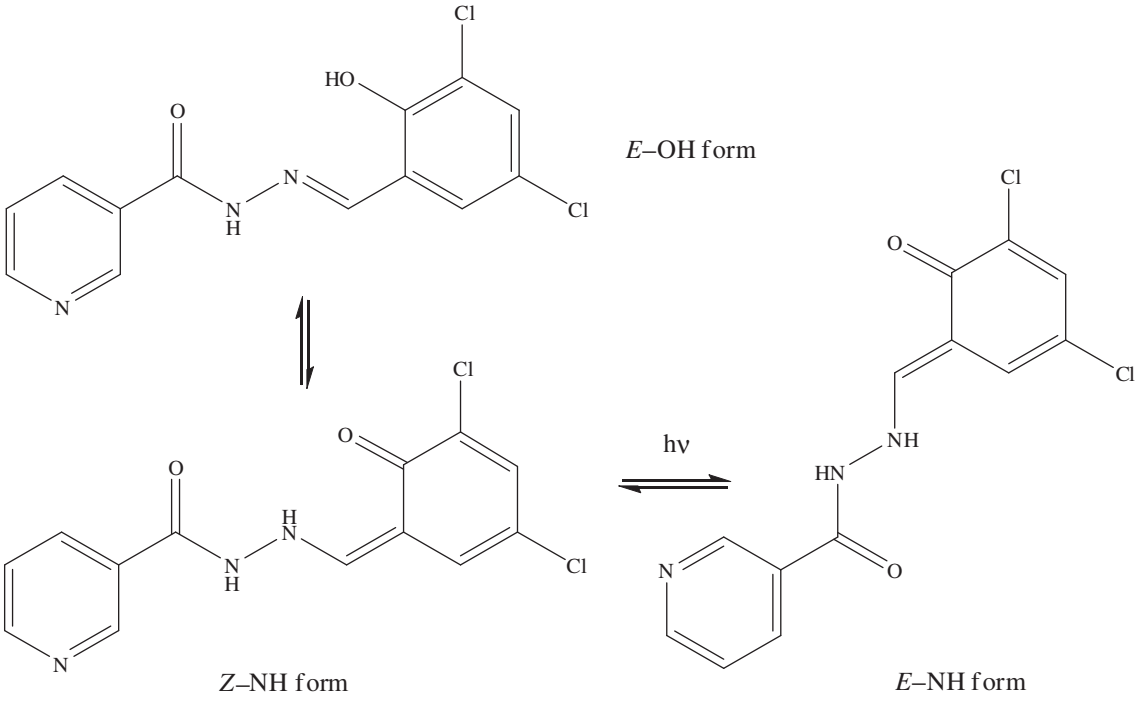
noticed in the UV spectra of nicotinic acid hydrazide. The bands in the spectral region (286–307) nm can be assigned to the azomethine C═N bonds and aromatic rings [26], while the bands above 330 nm to the part of the aroylhydrazones originated from aldehydes [10,21]. That was confirmed by recording the absorption spectra of the starting aldehydes under the same experimental conditions.

On the basis of the comparison of NMR data for **1**–**4** in DMSO, and absorption data in various solvents (Table 4), it was concluded that in solution all investigated aroylhydrazones predominantly adopted the most stable configuration (form I *E*). As expected, in the UV–Vis spectra of compound **1** in solvents of different polarities, no absorption above 400 nm was observed since there is no possibility of tautomeric interconversion to form III. Also, no hydrolysis in solvents containing water was recorded. In the absorption spectra of **2**–**4** in polar, protic solvents and in the mixtures of organic solvent with water, the band centred around 400 nm appeared, indicating the presence of form III. The effect was most pronounced in the electronic absorption spectra of **4** as there were two chloride atoms in *meta* positions, influencing the tautomeric equilibrium. As for **1**, the hydrolysis was not observed for **2**–**4**.

The influence of water content in the mixed organic solvent–water system on keto-enol tautomeric interconversion and possible *E*/*Z* isomerisation of **1**–**4** was studied more detailed in the case of DMSO and acetonitrile. The volume ratio (V(organic solvent)/V(water)) was changed from 9/1 to 5/5. The addition of water into the system caused increase in absorbance at 303 nm (DMSO) and at 297 nm (MeCN) in the case of **1** for approximately 0.12 units (Fig. 2a). These spectral changes can be assigned to the hydrogen bond formation between the —NH protons and solvent molecules [21]. No change in the absorption spectra of **1** in above mentioned solvents was observed during the time. Small increase in absorbance at 336 nm with increase of water content in DMSO/H_2O mixtures in UV spectra of **2** also indicated the formation of hydrogen bonds. The appearance of a new band centred around 400 nm, assigned to form III, was recorded in 1/1 organic solvent/water mixtures. Slight increase in absorbance, (≈0.03 after 24 h and ≈0.09 after 120 h) at 400 nm was noticed in MeCN/water mixture, whereas the increase in absorbance in DMSO/water system at same wavelength was even lower, ≈0.02 after 120 h. The shift of tautomeric equilibrium to form III, by adding water to the system, was very pronounced for compound **4** (Fig. 2b), and in much less extent for **3**. In the absorption spectra of **4** a new, broad band in the spectral region (380–500) nm appeared. It should be pointed out that for **4** (and less for **3**) the prominent change in the absorption spectra were observed in solvents containing water during the time, but only if the solutions were exposed to light. As far as the solutions were kept in the dark, no time dependence of absorbance was noticed. As an example, the UV–Vis spectra of **4** in the DMSO/H_2O 1/1 system are shown in Fig. 3. The absorbance at 294 nm decreased, the band with λ_{max} = 336 nm disappeared as well as the one with λ_{max} = 406 nm, whereas the new band (λ_{max} = 384 nm) appeared. These spectral changes, accompanied by the occurrence of isosbestic points at 274 nm and 361 nm, can be assigned to the *E*/*Z* photoisomerisation of aroylhydrazone as suggested on Scheme 3. The *E*/*Z* photoisomerisation was already reported for ketoenamines including hydrazones [27]. Although the formation of form III was noticed in spectra of **2**, the light induced additional changes in the absorption spectra were not. Presumably that can be a consequence of the presence of two hydroxyl groups in **2** which probably stabilize *E* form forming additional hydrogen bonds in the system [11].

ATR spectra

A tentative assignment of the ATR spectra of compounds **1**–**4** in DMSO (0.20 mol dm^{-3}) is given in Table 1 [9,16,21–25]. In the spectra of all solutions the intense bands around 1680 cm^{-1} and 1290 cm^{-1} were dominant. The former band was assigned to the C═O stretching of the amide group (amide I) and the latter to the mixed NH and OH bending vibrations. Both bands indicated the presence of form I of the studied hydrazones (Fig. 4). However, by comparing the ATR spectrum of **2** with the spectra of other studied aroylhidrazones, broader and weaker amide II (1561 cm^{-1}) and amide III (1356 cm^{-1}) bands were observed, accompanied by the appearance of a strong band at 1630 cm^{-1}



Scheme 3. Possible mechanism of *E*/*Z* isomerisation of **4**.

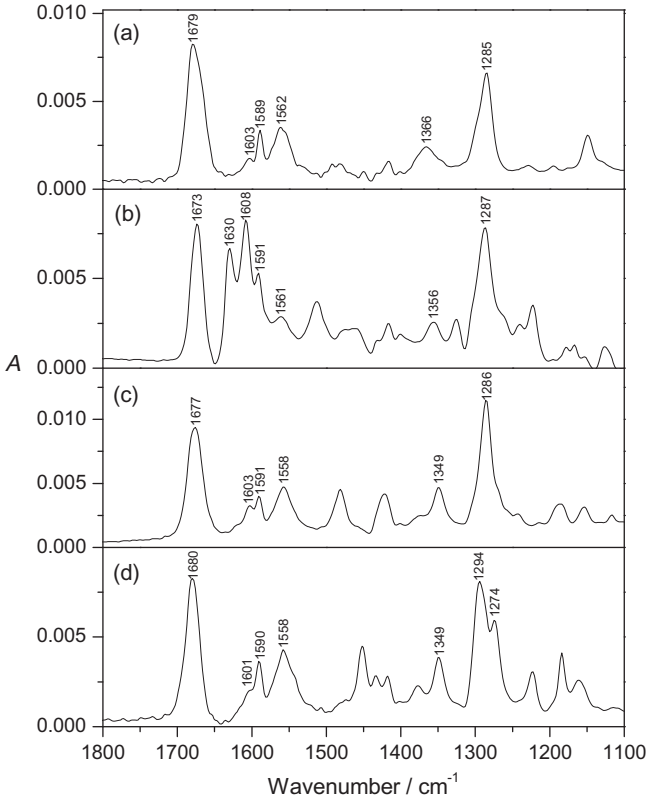


Fig. 4. ATR spectra of **1** (a), **2** (b), **3** (c) and **4** (d) in DMSO. c = 0.20 mol dm^{-3}.

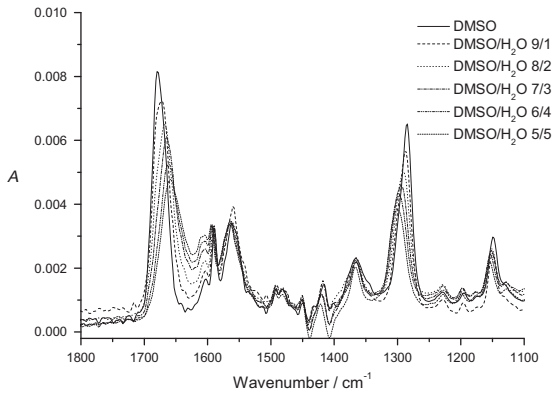


Fig. 5. ATR spectra of **1** in DMSO and DMSO/water mixtures: V(DMSO)/V(H_2O) 9/1, 8/2, 7/3, 6/4 and 5/5. c(**1**) = 0.20 mol dm^{-3}.

(Fig. 4b). The new band was assigned to the stretching of the C=N bond in the molecular structure of the enolimine tautomer, conjugated with the nearby pyridine ring and the hydrazone C=N bond [9,25]. The existence of form II was also suggested by the FT-IR spectrum of **2** in the solid state, but was not observed either in the UV–Vis spectrum or in the NMR spectra of the respective DMSO solution. Discrepancy between the electronic spectrum and the vibrational spectrum was attributed to the different concentrations, 5×10^{-5} mol dm^{-3} and 0.20 mol dm^{-3}, used in UV–Vis and ATR measurements, respectively. It was suggested that in the diluted solution solute–solute intermolecular interactions, which otherwise stabilize enolimine molecules, weakened, enabling interconversion of the molecules to the most stable ketoamine form I. In addition, a detection of only form I by NMR spectroscopy, contrary to the observation of forms I and II by IR spectroscopy, was associated with different time scales of these two spectroscopic methods. Ketoamine–enolimine equilibrium was most likely fast on the NMR time scale, and slow on the IR time scale [16]. Although forms I and III were present in the solid state, new bands indicative of form III were not observed in the spectra of DMSO solution of hydrazones **3** and **4** having chloride substituents on the benzene ring (Fig. 4c and d).

Given that water can induce interconversion of tatutomeric and/or isomeric forms of the hydrazone molecules [16,19], which was observed for the compounds **2**–**4** in organic solvent/water mixtures by UV–Vis absorption spectroscopy, the ATR spectra of aroylhydrazones (0.20 mol dm^{-3}) in DMSO/water mixtures were recorded. Thereby, DMSO/water solutions of **1** were prepared in the volume ratios V(DMSO)/V(H_2O) 9/1, 8/2, 7/3, 6/4 and 5/5, while compounds **2** and **3** were soluble only in the mixed solvents of V(DMSO)/V(H_2O) 9/1 and 8/2. Moreover, the addition of water caused precipitation of the compound **4** from the DMSO solution even at concentration of 0.02 mol dm^{-3}, preventing a structural study of **4** in DMSO/water mixtures by infrared spectroscopy.

In the ATR spectrum of the DMSO solution of **1** the prominent bands at 1679 cm^{-1} and 1285 cm^{-1}, assigned to the carbonyl group stretching and the amino group bending, respectively, changed the most significantly when water was added in the system. By increasing the water content in the solvent mixture, both bands decreased in intensity, got broader and shifted (Fig. 5). With regard to the spectrum of **1** in pure DMSO, a downward shift of 21 cm^{-1} for the C═O stretching band and an upward shift of 15 cm^{-1} for the NH bending band were observed in the spectrum of the mixture containing equal volumes of DMSO and water. The obtained spectral changes indicated an involvement of **1** in interactions with water through the amide group [21,28]. The broadening of the band assigned to the stretching of the C═N bond (1603 cm^{-1}) also pointed to predominant hydrogen bonding of **1** with water molecules, which was consistent with the findings based on the UV–Vis spectra. The intensities of the amide II (1562 cm^{-1}) and the amide III (1366 cm^{-1}) bands were not affected by the water content increase, confirming the stability of the ketoamine form I of **1** in the DMSO/water mixtures.

The ATR spectra of **2** and **3** in DMSO and in solutions containing water did not differ significantly. For both **2** and **3** the same trend as for **1** was observed concerning the bands originated from the C═O stretching as well as the NH and OH bending (spectra not shown). Along with decrease in intensity and broadening, the amide I band at 1673 cm^{-1} (**2**) and 1677 cm^{-1} (**3**) was shifted towards lower wavenumbers for 7 cm^{-1} (**2**) and 5 cm^{-1} (**3**), whereas the band corresponding to the polar groups deformation at 1287 cm^{-1} (**2**) and 1286 cm^{-1} (**3**), appeared at wavenumbers higher for 3 cm^{-1} in the spectra of compounds in DMSO/water mixture, V(DMSO)/V(H_2O) 8/2. Regardless of water content in the studied mixtures, significant changes either in position or in intensity of the bands assigned to the C═N stretching modes in forms I and II for **2** and in form I for **3**, were not observed. It was assumed that at high aroylhydrazone concentration the water content up to 20% did not affect tautomeric equilibria in the studied systems, but rather induced hydrogen bonding with water molecules, stabilizing in that way the present forms in the solutions.

Conclusion

Structural forms of aroylhydrazones derived from nicotinic acid hydrazide were studied in solid state by FT-IR spectroscopy and in solution by means of NMR, UV–Vis and ATR spectroscopy. The compound with an unsubstituted phenyl moiety existed as ketoamine form I *E* in solid state and in solution, stabilized through π–p conjugation between π-electrons of the C═N bond and the lone electron pair of the nitrogen of the C═N—N group. In aqueous organic solutions, intermolecular hydrogen bonds with water contributed to the formation of the hydrated ketoamine molecules. Despite hydroxyl groups in *ortho* and *para* positions on the benzene ring, tautomeric interconversion with respect to this part of the molecules was not observed. However, presence of enolimine form II, involving the carbonyl group on the pyridine ring, was noticed in the solid substance and at high concentration in solution. On the other hand, chlorine atoms in *meta* positions on the phenyl ring enhanced acidity of the hydroxyl group in *ortho* position, resulting in formation of ketoamine form III. In diluted solutions form III was favoured in polar organic solvents and in mixtures with water. In aqueous environment it was stable in the dark, but was subjected to an additional equilibrium when exposed to daylight. There were strong indications that *E*/*Z* photoisomerisation occurred.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2013.01.028.

References

[1] L.N. Suvarapu, Y.K. Seo, S.O. Baek, V.R. Ammireddy, E-J. Chem. 9 (2012) 1288–1304.
[2] P. Kumar, B. Narasimhan, P. Yogeeswari, D. Sriram, Eur. J. Med. Chem. 45 (2010) 6085–6089.
[3] S.N. Khattab, Molecules 10 (2005) 1218–1228.
[4] A. Moradi, L. Navidpour, M. Amini, H. Sadeghian, H. Shadnia, O. Firouzi, R. Miri, S.E.S. Ebrahimi, M. Abdollahi, M.H. zahmatkes, A. Shafiee, Arch. Pharm. Chem. Life Sci. 9 (2010) 509–518.
[5] R. Sinha, U.V. Sing Sara, R.L. Khosa, J. Stables, J. Jain, Med. Chem. Res. 20 (2011) 1499–1504.
[6] A. Trzesowska-Kruszynska, Struct. Chem. 22 (2011) 525–535.
[7] N.M. Hosny, J. Mol. Struct. 923 (2009) 98–102.
[8] B.N. Bessy Raj, M.R. Prathapachandra Kurup, E. Suresh, Spectrochim. Acta Part A 71 (2008) 1253–1260.
[9] A. Manimekalai, N. Saradhadevi, A. Thiruvalluvar, Spectrochim. Acta Part A 77 (2010) 687–695.
[10] N. Galić, M. Rubčić, K. Magdić, M. Cindrić, V. Tomišić, Inorg. Chim. Acta 366 (2011) 98–104.
[11] V.I. Minkin, A.V. Tsukanov, A.D. Dubonosov, V.A. Bren, J. Mol. Struct. 998 (2011) 179–191.
[12] N. Galić, Z. Cimerman, V. Tomišić, Spectrochim. Acta Part A 71 (2008) 1274–1280.
[13] S. Sharif, D.R. Powell, D. Schagen, T. Steiner, M.D. Toney, E. Fogle, H.-H. Limbach, Acta Cryst. B62 (2006) 480–487.
[14] S. Sharif, D. Schagen, M.D. Toney, H.-H. Limbach, J. Am. Chem. Soc. 129 (2007) 4440–4445.
[15] S. Sharif, G.S. Denisov, M.D. Toney, H.-H. Limbach, J. Am. Chem. Soc. 129 (2007) 6313–6327.
[16] C. Cordier, E. Vauthier, A. Adenier, Y. Lu, A. Massat, A. Cossé-Barbi, Struct. Chem. 15 (2004) 295–307.
[17] G.N. Ledesma, M. Gonzalez Sierra, G.M. Escandar, Polyhedron 17 (1998) 1517–1523.
[18] S. Uchiyama, M. Ando, S. Aoyagi, J. Chromatogr. A 996 (2003) 95–102.
[19] P. Kovařiková, K. Vávrová, K. Tomalová, M. Schöngut, K. Hrušková, P. Hašková, J. Klimeš, J. Pharm. Biomed. Anal. 48 (2008) 295–302.
[20] N. Galić, B. Perić, B. Kojić-Prodić, Z. Cimerman, J. Mol. Struct. 559 (2001) 187–194.
[21] N. Galić, A. Dijanošić, D. Kontrec, S. Miljanić, Spectrochim. Acta Part A 95 (2012) 347–353.
[22] P. Koczoń, J.Cz. Dobrowolski, W. Lewandowski, A.P. Mazurek, J. Mol. Struct. 655 (2003) 89–95.
[23] C. Colonna, J.P. Doucet, A. Cossé-Barbi, Specttrosc. Lett. 27 (1994) 1153–1163.
[24] M. Boczar, M.J. Wójcik, K. Szczeponek, D. Jamróz, S. Ikeda, Int. J. Quantum Chem. 90 (2002) 689–698.
[25] G. Frison, G. van der Rest, F. Tureček, T. Besson, J. Lemaire, P. Maitre, J. Chamot-Rooke, J. Am. Chem. Soc. 130 (2008) 14916–14917.
[26] Y.H. Lu, Y.W. Lu, C.L. Wu, Q. Shao, X.L. Chen, R. Bimbong, Spectrochim. Acta Part A 65 (2006) 695–701.
[27] A.D. Dubonosov, V.A. Bren, Russ. Chem. Bull., Int. Ed. 54 (2005) 512–524.
[28] N.S. Myshakina, Z. Ahmed, S.A. Asher, J. Phys. Chem. B 112 (2008) (1877) 11873–11877.