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Effect of Solvent Polarizability on the Keto/Enol Equilibrium of Selected Bioactive Molecules from the 1,3,4-Thiadiazole Group with a 2,4-Hydroxyphenyl Function

Arkadiusz Matwijczuk,[*,†] Dariusz Karcz,[§] Radosław Walkowiak,[†] Justyna Furso,[∥] Bożena Gładyszewska,[‡] Sławomir Wybraniec,[§] Andrzej Niewiadomy,[⊥,#] Grzegorz P. Karwasz,[▽] and Mariusz Gagoś[*,○]

[†]Department of Biophysics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

[‡]Department of Physics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

[§]Department of Analytical Chemistry (C1), Faculty of Chemical Engineering and Technology, Krakow University of Technology, Warszawska 24, 31-155 Krakow, Poland

[∥]Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland

[⊥]Institute of Industrial Organic Chemistry, Annopol 6, 03-236 Warsaw, Poland

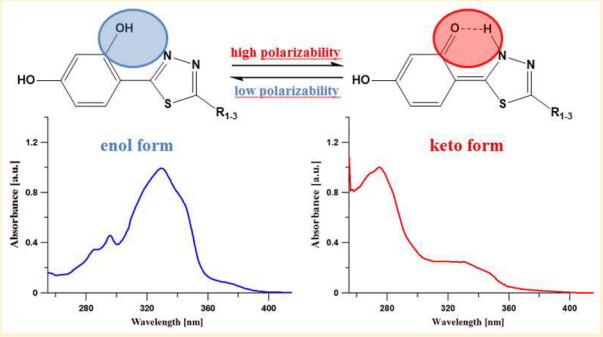
[#]Department of Chemistry, University of Life Sciences in Lublin, 20-950 Lublin, Poland

[▽]Aleksander Jabłoński Institute of Physics, Nicolaus Copernicus University, 87-100 Toruń, Poland

[○]Department of Cell Biology, Institute of Biology, Maria Curie-Skłodowska University, 20-033 Lublin, Poland

Supporting Information

ABSTRACT: Three novel 1,3,4-tiadiazole-derived compounds with biological-activity, i.e., 4-(5-(methylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (MDFT), 4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (PhATB), and 4-(5-(4-chlorophenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (4-ClPhATB) were characterized with the use of several spectroscopic methods. Detailed UV−vis studies revealed keto/enol tautomerism of the examined compounds. The absorption spectra recorded in nonpolar solvents exhibited bands that were characteristic of keto tautomers, while in polar solvents the enol form is predominant. A number of spectra revealed the presence of both tautomeric forms in the solution. The keto/enol equilibria observed were both solvent- and temperature-dependent. The keto/enol equilibrium was also observed using FTIR spectroscopy. A detailed analysis of the spectroscopic data leads to a conclusion that the solvent-induced tautomerism of the selected compounds from the 1,3,4-thiadiazole group does not depend on the electric dipole moment of the solvent but more likely on its average electric polarizability. Additionally, a clear effect of the substituent present in the molecule on the tautomeric equilibrium in the selected 1,3,4-thiadiazole analogues was noted.



INTRODUCTION

The most important challenges of modern medicine include the fight against neoplastic diseases. Literature data indicate that these diseases are currently the leading causes of patients' mortality worldwide, irrespective of their age. Recent estimates predict that at least one out of four inhabitants of highly developed countries will develop a neoplastic disease within the next few years. Therefore, the research on novel anticancer therapies is currently among the most dynamically developing disciplines of medicinal sciences. Yet, the effectiveness of currently used antitumor drugs is still insufficient for various reasons. This creates a need for continuous search for compounds with the desired properties and precisely targeted mechanism of antitumor action. One of the major clinical problems, which often results in failure in the fight against cancer, is the phenomenon of cellular resistance of neoplastic cells to the drugs used in current therapies. A hope for changing this situation lies in synthetic compounds from the 1,3,4-thiadiazole group with a substituted resorcyl fragment. It is worth mentioning that their structure contains small heterocyclic fragments and carbon-heteroatom bond blocks, which is a common trait of compounds with anticancer activity. In this context, 1,3,4-thiadiazoles seem most attractive of the four known thiadiazole systems. These compounds are used as

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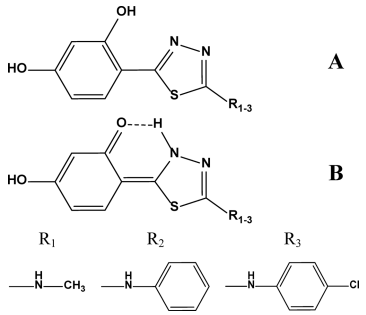
colorants, and metal complexing agents.[1,2] Additionally, 1,3,4-thiadiazoles exhibit antitumor,[3] antifungal,[4] antibacterial,[4] anti-inflammatory,[5] anticonvulsant,[6] antiviral,[7] antituberculosis,[8] antihypertensive,[9] and antidepressant[10] activity.

Three promising 1,3,4-thiadiazole analogues with proven antitumor activity were chosen for investigations of the mechanism of molecular interactions; these were 4-(5-(methylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (MDFT), 4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (PhATB), and 4-(5-(4-chlorophenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol (4-ClPhATB) (Scheme 1A and B, R_1, R_2 and R_3, respectively). It should be strongly emphasized that the structures of these compounds are similar in their main fragment (resorcyl ring and amine group −NH−) but differ in the structure of the substituent: R_1 = $-CH_3$, R_2 = benzene ring, and R_3 = Cl-substituted benzene ring (see Scheme 1, R_1, R_2, R_3). It should also be mentioned that the 1,3,4-thiadiazole compounds and their other analogues selected for the analyses exhibit not only interesting and proven pharmacological properties but also remarkable spectroscopic traits, which may be involved in their biological activity. The spectroscopic effects exhibited by the 1,3,4-thiadiazole group include, e.g., the effect of keto/enol tautomerism induced by changes in medium polarizability,[11−13] polymorphism[14] and solvatomorphism[15] effects of crystals growing in different solvent media, and very interesting effects in model lipid systems.[16,17] A highly interesting effect, although rare in the molecular environment, exhibited by this group of compounds is the dual fluorescence or generation of several fluorescence spectra induced by changes in the pH, temperature, or concentration of the compounds.[18,19] It seems obvious that association of these effects with the pharmacological properties exhibited by thiadiazole systems may contribute to recognition of the mechanisms of action of the analyzed 1,3,4-thiadiazole compound group.

Scheme 1. Chemical Structures of the Selected 1,3,4-Thiadiazoles[a]



[a]Key: A, enol form; B, keto form; R_{1-3}, substituent groups of the selected 1,3,4-thiadiazoles.

The aim of the spectroscopic study presented in this paper was to analyze the selected analogues in various solvents and describe the keto/enol tautomerism effects and their close relationship with changes in the polarizability of the solvent.[20,21] Additionally, we refer to available publications on the tautomerism of the analyzed compound group. Besides the description of the keto/enol tautomerism, this paper presents and emphasizes the strong effect of the substituent present in each analogue on the keto/enol equilibrium of the compounds. Using various spectroscopic methods, e.g., electronic absorption spectroscopy and primarily FTIR spectroscopy, we show the complexity of the physical processes that may influence tautomerism effects and their close correlation with the changes in the medium polarizability. The research results presented in this article describe three 1,3,4-thiadiazole analogues, whose structure is composed of the characteristic 1,3,4-thiadiazole system and a resorcyl group as well as an amine group, benzene (PhATB) and chlorobenzene rings (4-ClPhATB), and a CH_3 group (MDFT) (see Scheme 1). Similar to FABT,[11] these compounds are able to form intramolecular hydrogen bonds, which occur between the *ortho* hydroxyl group of the resorcyl ring and the ═N−N═ moiety of the thiadiazole ring (Scheme 1). These characteristic structural features allow intramolecular proton transfer, which are a basis for the keto/enol equilibria occurring in the solution. Moreover, a set of calculations performed allowed determination of the influence of solvent properties, such as average electric dipole polarizability, on the position of the absorption maxima.

Proton transfer is determined by numerous biological, chemical, and physical processes.[22] Depending on the system, numerous intra- and intermolecular proton transfer processes are possible.[23−26] A classic example of intramolecular proton transfer is the keto/enol tautomerism.[26−28] Numerous variants of these processes may occur in molecules that are able to form intramolecular hydrogen bonds.[29] A large number of potential practical applications for compounds with proton transfer abilities increasingly attract researchers' attention.[30,31] It has been reported that various keto/enol tautomers may act as laser dyes, molecular switches, or memory modules.[32] Molecules that can serve as molecular probes (e.g., the analyzed 1,3,4-thiadiazoles) are highly attractive to investigators.[33] Numerous biologically active molecules and therapeutic agents are known to exhibit keto/enol tautomerism.[34]

Numerous reports refer to the solvent polarity as the main factor influencing the keto/enol equilibrium.[35] The strong solvation effect is considered as a main cause of the dominance of the keto form in polar solvents, while in nonpolar solvents the tautomeric equilibrium is usually shifted toward formation of enol forms, most likely due to the formation of internal hydrogen bonds.[33,36,37] On the other hand, studies on some specific Schiff bases revealed an opposite solvent-dependent effect taking place in a range of polar and nonpolar solvents.[38] The present work aimed to determine the influence of polar and nonpolar solvents on the enol/keto equilibrium, using solvents with different electric polarizabilities.

MATERIALS AND METHODS

MDFT (4-(5-(methylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol) $C_9H_9N_3O_2S$, molecular mass 223.25 g/mol (Scheme 1, R_1), PhATB (4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)-benzene-1,3-diol) $C_{14}H_{11}N_3O_2S$, molecular mass 285.32 g/mol (Scheme 1, R_2), and 4-ClPhATB (4-(5-(4-chlorophenylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3-diol) $C_{14}H_{10}ClN_3O_2S$, molecular mass 319.77 g/mol (Scheme 1, R_3) were analyzed. The 1,3,4-thiadiazole derivatives used in this study were synthesized at the Department of Chemistry, University of Life Sciences, Lublin. All syntheses were carried out according to procedures reported previously.[39,40]

All solvents were purchased from Sigma-Aldrich. The purified solvents were found to be free from impurities and were transparent in the spectral region of interest. All the synthesized compounds were recrystallized from 96% methanol prior to use. All compounds were additionally purified by

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HPLC, using the YMC C30 column (250 × 4.6 mm). A mixture of acetonitrile:methanol:H_2O (72:8:3 v/v) was applied as the mobile phase. Excess solvents were removed under reduced pressure. In order to remove solvent residues, the samples were dried under a stream of N_2 and then stored under vacuum for 1.5 h.

Electronic Absorption Measurements. Electronic absorption spectra were recorded using a double-beam UV−vis spectrophotometer Cary 300 Bio from Varian equipped with a thermostated cuvette holder with a 6 × 6 multicell Peltier block. All UV−vis spectra were measured in the spectral range of 200−600 nm at the slit width of 1.5 nm. Temperature was controlled with a thermocouple probe (Cary Series II from Varian) placed directly in the sample. All measurements were performed at a temperature of 23 °C.

FTIR Measurements. All ATR-FTIR background corrected spectra were carried out using a HATR Ge trough (45° cut, yielding 10 internal reflection elements) crystal plate for liquids and were recorded on a Varian 670-IR spectrometer. Typically, 25 scans were collected, Fourier-transformed, and averaged for each measurement. Absorption spectra at a resolution of one data point per 1 cm^{-1} were obtained in the region between 4000 and 400 cm^{-1}. The instrument was continuously purged with argon for 40 min before and during the measurements. The Ge crystal was cleaned with ultrapure organic solvents from Sigma-Aldrich Co. All experiments were carried out at 20 °C. Spectral analysis was performed with Grams/AI 8.0 software from Thermo Electron Corporation. All compounds were dissolved in a range of solvents and transferred onto the surface of the Ge crystal plate. The solvents were then evaporated in the N_2 atmosphere, leaving a thin layer of the compound on the surface of the crystals. The measurement of the FTIR spectrum was performed in the solid phase.

RESULTS AND DISCUSSION

The structures of the 1,3,4-thiadiazole derivatives selected for the study, i.e., MDFT, PhATB, and, 4-ClPhATB, are presented in Scheme 1. Panels A and B illustrate the possible enol and keto forms for the 1,3,4-thiadiazole compounds mentioned above. Each structure consists of a central 1,3,4-thiadiazole ring substituted with a resorcinol moiety at the C carbon on the left side of the 1,3,4-thiadiazole ring. The C4 carbon in each derivative carries additional substituents, which are presented in Scheme 1 as R_{1-3} (all of them have a secondary amine-derived residue (−NH group)).

Figure 1 shows electronic absorption spectra of MDFT (panel A), PhATB (panel B), and 4-ClPhATB (in panel C) recorded in several organic solvents with different polarity (propan-2-ol (dipole moment =1.58D), CCl_4 (dipole moment =0), and *n*-heptane (dipole moment = 0)). All spectra were recorded in a range of 240−450 nm. The spectrum in propan-2-ol is dominated by a strong absorption maximum at ∼330 nm for all the compounds. Although this band is still visible in the spectrum recorded in *n*-heptane, its intensity is significantly lower and accompanied by the presence of another band with an absorption maximum at ∼270−276 nm. No such high energy transition was observed in spectra recorded in polar solvents. Molar extinction coefficients determined for MDFT, PhATB, and 4-ClPhATB in polar solvents are approximately 180−200 times lower compared to these determined in nonpolar solvents.[11,12] The position of the absorption maximum near 270−276 nm together with the relatively low value of the extinction coefficient corresponds to the n → π*

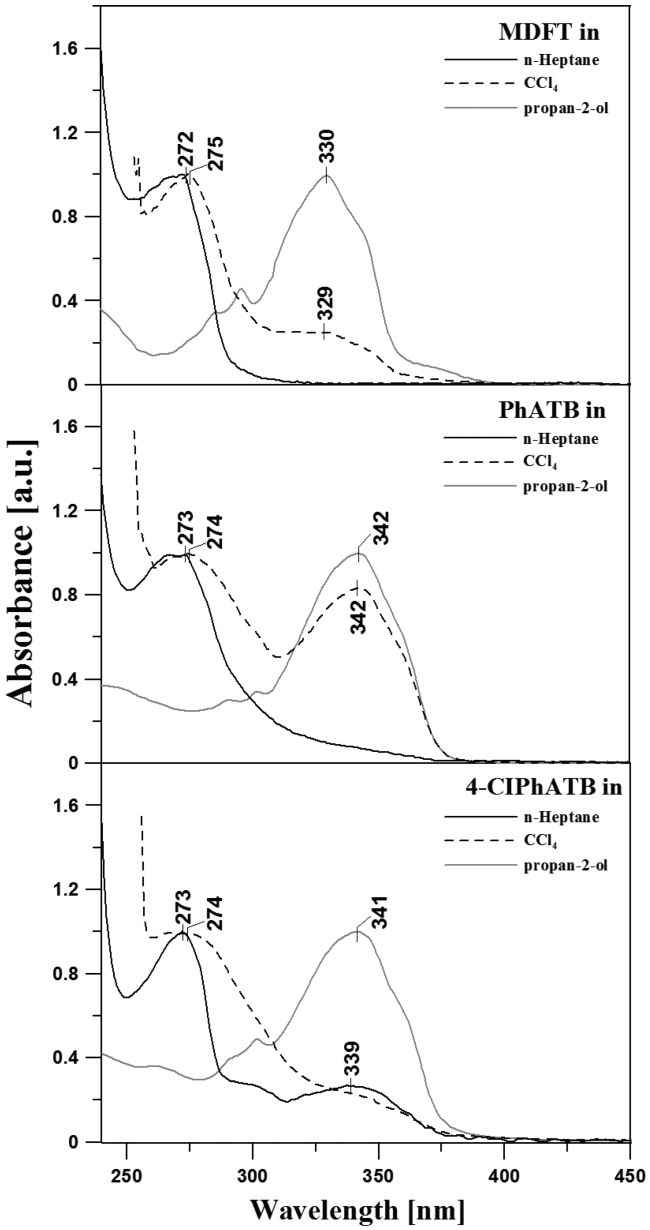

Figure 1. Normalized electronic absorption spectra of the selected 1,3,4-thiadiazoles dissolved in various organic solvents (A, MDFT; B, PhATB; C, 4-ClPhATB). Measurements of all spectra were performed at 23 °C.

electronic transition within the carbonyl group (Scheme 1).[11] This assignment is additionally supported by the results from the FTIR measurements, which confirm the presence of the C═O group in each of the investigated compounds (in the text below). The results obtained indicate an intramolecular proton transfer from the *ortho*-hydroxyl group of the resorcinol moiety to N3 nitrogen of thiadiazole, with the formation of a keto tautomer (Scheme 1B). Numerous structures have been reported to exhibit similar solvent-related effects.[41−44]

The set of electronic absorption measurements consisted of spectra recorded in 17 solvents with varied dipole electric moment and electric polarizability. In the case of the polar solvents such as water, methanol, or 1-butanol, the characteristic band with a maximum at ∼330 nm is clearly visible (Figure 1). The series of spectra revealed a slight bathochromic shift of this band, corresponding to a decrease in the solvent polarity.

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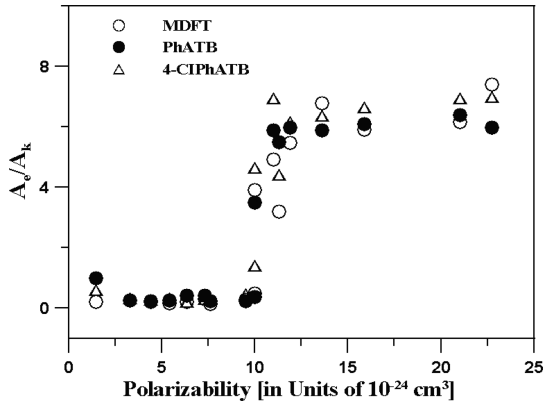

Figure 2. Positioning of the absorption maxima of the selected 1,3,4-thiadiazoles depending on the average electric dipole polarizabilities of solvents used (in units of 10^{-24} cm^3). See the text for further details. A list of all parameters is presented in Table 1.

In the case of the spectra in solvents such as *n*-hexane or *n*-heptane, the band with a maximum at ∼270−276 nm appears. The ∼330 nm band is still visible in the spectra recorded in CCl_4 and cyclohexane, suggesting equilibrium between the two tautomeric forms. Noteworthy, the tautomeric equilibrium in the analyzed molecules is greatly influenced by the presence of various substituents. The substituent group determines the equilibrium form between the observed bands assigned to the respective tautomeric forms. Nearly complete disappearance of the ∼330 nm band was observed in spectra recorded in the solvents with polarizability higher than $\alpha = (10-15) \times 10^{-24}$ cm^3 (Figure 1). A similar effect was reported by Yan et al. in the case of solutions of 3-hydroxy-2-mercaptopyridine. The absorption maximum in the ethanolic solution was present at 340 nm, while 273 nm was reported for the solution in dioxane.[45] Nevertheless, these changes were not considered as clearly solvent-dependent and were not discussed in terms of solvent polarizability[45] or substituent effects. On the other hand, the position of the absorption maxima of 2-(*N*-methyl-α-iminoethyl)-4,6-dichlorophenol varied depending on solvent polarity and was associated with the formation of keto/enol tautomers. Very similar effects have been observed in other 1,3,4-thiadiazole analogues, such as FABT[11] and NTBD,[12] where a strong relationship between the keto/enol equilibrium and changes in polarity and, mainly, polarizability of the medium used have been reported. Furthermore, these molecules had different forms of the substituent groups, which greatly influenced their tautomerism. FABT (2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole) has an amine group as a substituent and the so-called fluorobenzene, while NTBD has a CH_2 group and a naphthalene ring. Clearly, in the case of these two analogues, there was a difference in the equilibrium between the keto and enol forms of the compound. In the case of FABT, there was a more pronounced transition from one form to another, which more strongly depended on the changes in the polarizability of the solvent than in the case of NTBD. The impact of the substituent changes on the observed effects seems to be evident enough to be examined in detail in subsequent studies. Moreover, the so-called azo dyes are a very important group of compounds exhibiting the keto/enol tautomerism. Similar to 1,3,4-thiadiazoles, they demonstrate a great impact of solvent polarity on the tautomeric equilibrium.[46] The keto form (with a carbonyl group in the structure of these compounds) dominates in nonpolar solvents, whereas polar solvents are dominated by the enol form (with the −OH group).

The fluorescence measurements preformed using the different solvents revealed the solvent-dependent keto/enol tautomerism of the investigated compounds (Figure 1S in Supporting Information). All compounds exhibited two emission maxima upon 270−276 nm excitation (in a range characteristic of the keto form of the compounds), which corresponded to two tautomeric forms of thiadiazoles. For all the compounds, the emission maxima associated with the keto forms were present at a range of 310−330 nm, and the signals

Table 1. Position of Maxima in the Absorption Spectra for MDFT, PhATB, and 4-ClPhATB Compared to the Average Dipole Molecular Polarizability, Dielectric Constant ε, Index of Refraction *n*, and Dipole Moment μ of the Solvents[a]

			λ [nm]									
			MDFT		PhATB		4-CTPhATB					
		solvents	enol	keto	enol	keto	enol	keto	polarizability [in units of 10^{-24}cm^3]	ε	*n*	μ (D)
polar	1	H_2O	323	–	329	–	326	–	1.45	80.1	1.3333	1.855
	2	methanol	327	–	339	–	339	–	3.29	33	1.3265	1.700
	3	acetonitrile	328	–	339	–	339	–	4.40	36.64	1.3416	3.925
	4	ethanol	330	–	341	–	341	–	5.41	25.3	1.3594	1.690
	5	acetone	339	–	341	–	341	–	6.33	21	1.3587	2.880
	6	DMSO	332	–	343	–	340	–	7.30	47.24	1.4773	3.960
	7	2-propanol	330	–	342	–	342	–	7.61	20.18	1.3772	1.580
	8	chloroform	329	–	342	–	342	–	9.50	4.81	1.4429	1.040
nonpolar	9	pentane	328	270	327	271	331	272	9.99	1.84	1.3575	0.130
	10	benzene	341	–	343	–	342	–	10.00	2.28	1.5011	0
	11	cyclohexane	335	271	336	272	335	272	11.00	2.02	1.4262	0
	12	tetrachloromethane	329	275	342	275	339	275	11.30	2.24	1.4631	0
	13	*n*-hexane	340	274	341	273	338	275	11.90	1.89	1.3723	0
	14	*n*-heptane	–	274	342	273	339	274	13.60	1.92	1.3876	0
	15	octane	–	280	–	280	–	280	15.90	1.948	1.3947	0
	16	undecane	–	282	–	281	–	282	21.03	1.997	1.4147	0
	17	dodecane	–	281	–	281	–	282	22.75	2.012	1.4186	0

[a]The solvents are ordered following the rising value of the polarizability.

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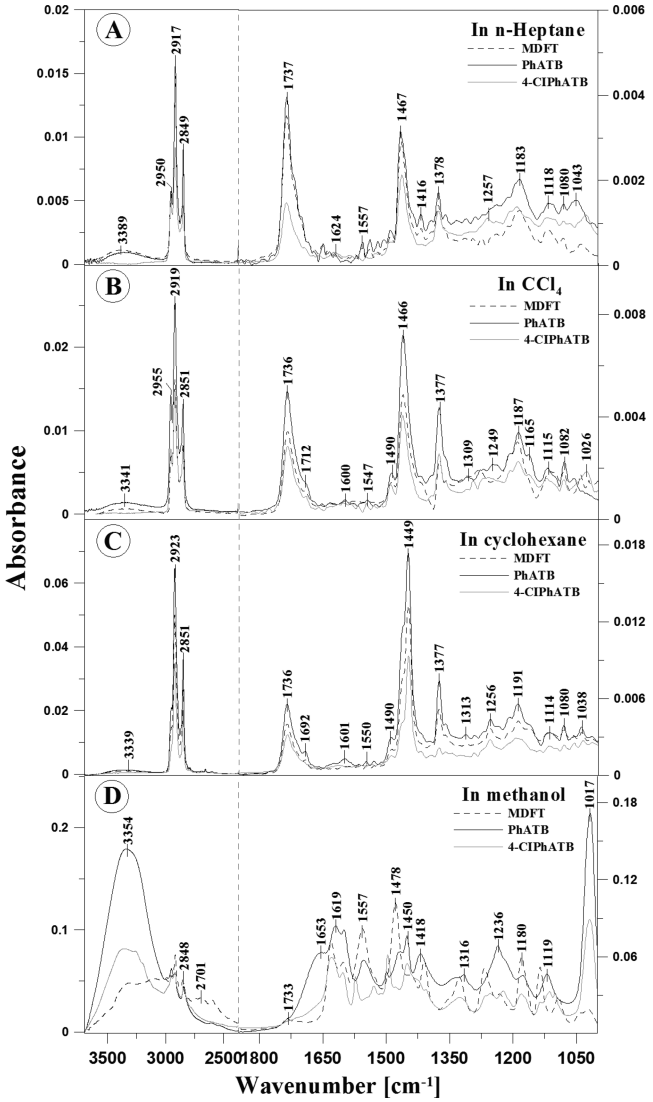

Figure 3. ATR-FTIR absorption spectra of MDFT, PhATB, and 4-ClPhATB dissolved in *n*-heptane (A), CCl_4 (B), cyclohexane (C), and methanol (D), respectively. Measurements were carried out on the solvents using a trough HATR Ge crystal plate for liquids at the temperature of 23 °C.

observed at a range of 390−420 nm were assigned to the respective enol tautomers. Because of the low fluorescence quantum yield of the n → π^* transitions in the C═O group, bands assigned to the keto forms are significantly lower than these assigned to enols. In the case of excitation with wavelengths corresponding to the absorption maxima of enols, only one emission band with the maximum at a range of 310−330 nm was observed. Such significant differences in both the positioning and intensity of emission maxima are clearly related to changes in the chemical equilibrium between the keto and enol tautomers. Compared to the previously reported studies on FABT, the fluorescence spectra of MDFT, PhATB, and 4-ClPhATB distinctly reveal tautomeric equilibria. In all cases, the fluorescence features depend on the polarizability of the solvent.[47] Similar solvent-dependent effects were observed for 2-(2′-hydroxyphenyl)benzoxazole, which exhibited dual fluorescence in the methanolic solution. The fluorescence of this compound in *n*-hexane was assigned to the keto tautomer, while the enol form was predominant in DMSO.[48]

Additional calculations were performed using data obtained from the UV−vis measurements. First, the enol to keto ratios (A_e/A_k where A_e is the band in a region approximately 330 nm and A_k is the band in a region approximately 274 nm) were determined based on the relevant absorption intensities. The A_e/A_k was then stacked against three solvent parameters, namely the dielectric constant-related Kirkwood function ε ($(\varepsilon-1)/(2\varepsilon+1)$) (Figure 2S in Supporting Information), the refractive index-related Lorentz−Lorentz electric polarizability n ((n^2-1/n^2+2)) (Figure 2S in Supporting Information), and the average electric dipole polarizability α—Figure 2—for all compounds. The plots of the A_e/A_k versus both ε and n did not exhibit any regular correlations, while a clear trend was observed in the case of α. It was noticed that A_e/A_k changed rapidly, once near the α of ∼(9−10) × 10^{-24} cm^3. In solvents with polarizability other than that value, the changes in A_e/A_k are negligible; this means that we see only a slight spectral shift. In the case of solvents with polarizability of approximately ∼(9−10) × 10^{-24} cm^3, such as CCl_4 or cyclohexane, two absorption maxima are visible, which indicates the presence of both tautomeric forms (Figure 2, Table 1). Figure 2 clearly shows the role of the substituent group in the analyzed compounds.

The temperature dependence of the keto/enol equilibrium in all the compounds was extensively studied using electronic absorption spectroscopy. A similar effect was reported for FABT[11] as well as for a number of other molecules.[36,38,42] The temperature effect was not observed in most polar solvents where the keto/enol equilibrium was strongly shifted toward the enol and hence the keto form was probably below the detection level of our equipment.

An additional study on the solvent-dependent intramolecular proton transfer in the selected compounds from the 1,3,4-thiadiazole group was carried out using FTIR spectroscopy. Figure 3 presents the results for MDFT, PhATB, and 4-ClPhATB. Series of FTIR spectra were recorded in the range of 900−3600 cm^{-1}. Assignment of the main FTIR signals for MDFT, PhATB, and 4-ClPhATB is shown in Table 2. Figure 3 presents examples of ATR-FTIR spectra of MDFT, PhATB, and 4-ClPhATB samples treated with various solvents. In each spectrum, the region of 1680−1760 cm^{-1} carried the most significant information regarding the keto/enol tautomerism of the examined compounds. All samples prepared with the use of nonpolar solvents, such as *n*-heptane, gave spectra with a strong, sharp band at ∼1737 cm^{-1}, assigned to the C═O stretching vibrations of the ketone carbonyl.[49,50] The ketone band at ∼1732−1739 cm^{-1} was also present in samples prepared with the use of CCl_4 and cyclohexane, while in the spectra of samples pretreated with higher-polarity solvents it was positioned at ∼1710−1705 cm^{-1}. Signals observed at ∼1630 and 1590 cm^{-1} were assigned to the C═N stretching vibrations of the 1,3,4-thiadiazole ring. These signals were particularly sharp and intense in samples pretreated with polar solvents, such as ethanol, and were considered characteristic of the enol forms of the examined compounds. Spectra recorded for samples pretreated with nonpolar solvents exhibit C═N stretching bands, but the observed signals exhibited notably lower intensities. The intensities of bands assigned to the vibrations of both C═O (keto) and C═N (enol) varied depending on the ratio between the two tautomers, which in

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Table 2. Position of FTIR Vibrations for MDFT, PhATB, and 4-ClPhATB[a]

FTIR band position [cm^{-1}]												
n-heptane			tetrachloromethane			cyclohexane			methanol			
MDFT	PhATB	4- ClPhATB	MDFT	PhATB	4- ClPhATB	MDFT	PhATB	4- ClPhATB	MDFT	PhATB	4- ClPhATB	vibration[b]
3389	3286		3341	3341		3339	3335	3341	3322	3336	3354	ν(O−H)
–	2950		–	2955		–	2923		3108	2949	3261	
	2917			2919			2851			2916		ν(N−H)
	2849			2851						2848		
2956		2956	2953		2955	–			2957		2950	
2918		2918	2918		2918	2925		2923	2915		2919	ν_{s+as}(C−H)
2850		2850	2849		2850	2852		2850	2849		2849	
–			–			–			2695			ν(N−H)
–			–			–			2596			
1737	1736	1737	1736	1736	1737	1736	1737	1737	1733	1701	1713	ν(C═O)
–			–			–			1716			
–			1694			1692			–			
1650	1687	1624	–	1692	1600	–	1692	1616	1631	1653	1629	
											1602	
1557	1651		–			1566	1601		1557	1619	1572	ν(C═N)
										1599	1529	
–	1488	1492	1492	1490	1492	–	1492	1490	1478	1499	1496	ν (C═C)
1467	1467	1466	1466	1465	1466	1449	1449		1440	1466	1479	
–	1416	1441	–	1416		–		1449	1410	1450	1450	δ (C−H) + ν(C═C)
1378	1377	1378	1377	1376	1377	1377	1377	1377	–			
–		1362	1362		–	1362		1325	1338			ν(C−N) in C═N−C δ(C−H)
–		1309		1364	–			–	1316	1326		ν(C−O)
1295	1257	1287		1272	1257	1256	1292	1268	1236	1254		
							1256					δ(N−H) + ν(C−N)
–	1210	1211	1210	1207	1211	1210	1209	1232		1222		
1183	1183	1188	1192	1190	1187	1191	1191	1192	1181	1177	1180	δ(C−C) in C−(C═O)−C
–			–	1163	1165	1166			1136		1136	
1118	1107	1124	1115	1124	1125	1110	1110	1114	–	1119	1113	ν(C−N)
1080	1082	1080	1083	1082	1082	1082	1082	1080	1092	–	1100	ν(C−O−C)
1043	1062	1064	1038	1060		1038	1038	–	1043	1017	–	ν_{as}(C−N) or az δ(C−H)
–	1025	1026					–	1038	1021		1018	

[a]The asterisk symbol denotes both the solvent and molecule band. [b]Key: ν, valence vibration; δ, deformation; s, symmetric; as, asymmetric.

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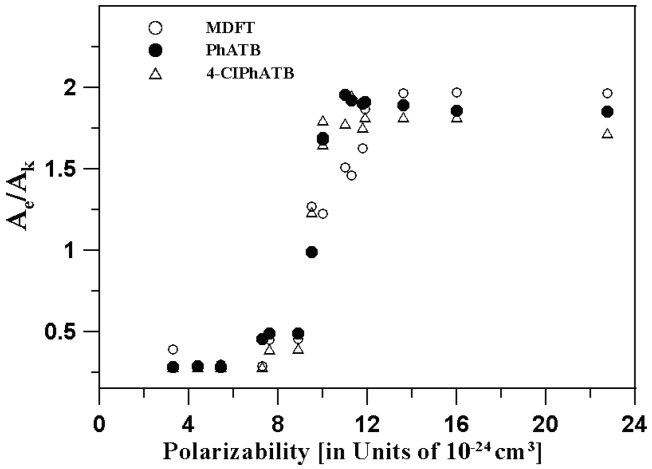

Figure 4. Ratios of A_I/A_{II} absorbance of MDFT, PhATB, and 4-ClPhATB as a function of average electric dipole polarizabilities (in units of 10^{-24} cm^3) (I, absorbance maximum from the region between 1710 and 1716 cm^{-1}; II, absorbance maximum from the region between 1720 and 1742 cm^{-1}). See the text for further details.

turn was related to the polarizability of solvents used for the preparation of thin films.

In order to gain better insight into the relationships of the keto/enol equilibrium in the analyzed 1,3,4-thiadiazoles, the dependence between the position of the band with a maximum at ca. 1730−40 cm^{-1} to a maximum at ca. 1710−15 cm^{-1} and changes in solvent polarizability were assessed for the FTIR spectra.

The A_e/A_k ratios (A_e, the band in a region approximately 1710−15 cm^{-1}, and A_k, the band in a region approximately 1730−40 cm^{-1}) for each compound were recalculated based on the FTIR data. The resulting graph (Figure 4) exhibited a trend that clearly indicated a strong relationship between the polarizability of the solvent and the intensity of the C═O stretch signal. Moreover, similar to the previously examined UV−vis data (Figure 2), the FTIR-based calculations revealed that the tautomeric transformation took place in solvents with polarizability of ∼(9−11) × 10^{-24} cm^3. The low intensity of the C═O stretch in solvents with low polarizability (but high permanent dipole moments) is most probably associated with the possibility of hydrogen bonding between thiadiazole and solvent molecules, which stabilizes the enol tautomer. Nonpolar solvents with high dipole polarizability values do not form hydrogen bonds with thiadiazoles, which promotes the internal hydrogen bonding between the *ortho*-hydroxyl group of the resorcinol moiety and N3 nitrogen. This, in turn, favors the formation of a keto tautomer, which results in increased intensity of the C═O stretch band. These findings were consistent with the results obtained in the UV−vis spectroscopic experiments. The relationship shown in Figure 4 demonstrates that the substituent change in the analyzed 1,3,4-thiadiazole molecule exerts an evident effect on the keto/enol equilibrium in these compounds.

The influence of solvent polarity/polarizability on the keto/enol equilibrium in 1,3,4-thiadiazoles was also investigated using mixtures of polar/nonpolar solvents (see Figure 5). The experiments consisted in ATR-FTIR spectroscopic measurements of samples dissolved in a range of mixtures of 2-propanol/*n*-heptane for all selected compounds (presented for MDFT, PhATB, and 4-ClPhATB in panels A, B, and C, respectively) and evaporated on the surface of a Ge crystal plate

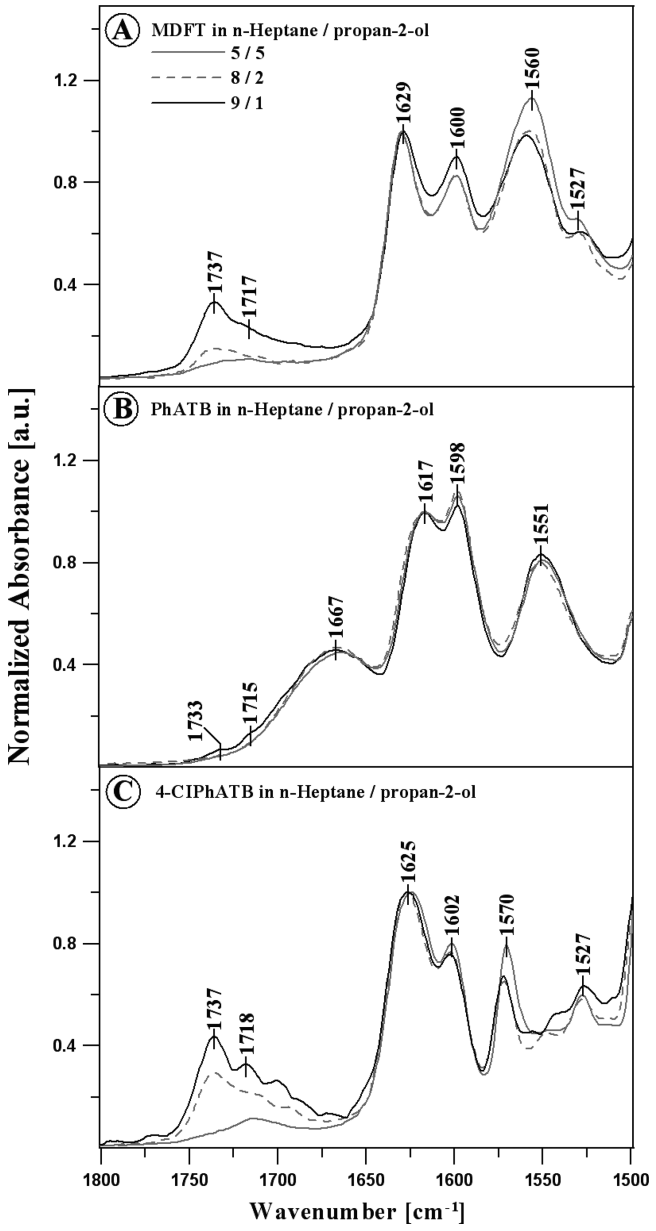

Figure 5. Normalized ATR-FTIR spectra performed for various ratios of *n*-heptane/propan-2-ol at the temperature of 23 °C.

to yield thin films. The ratio between the polar and nonpolar solvent varied from 1:9 to 9:1 (v/v) in the series. For better clarity, all spectra were normalized at ∼1730 cm^{-1}. The spectra of samples pretreated with mixtures containing an increased fraction of *n*-heptane revealed high intensity of ketone C═O stretching vibrations at ∼1737 cm^{-1}. The intensity of the C═O stretch decreased proportionally to the polarity of the solvent mixture used and was accompanied by an increase in the intensity of the enol band at ∼1715 cm^{-1}. The first predominance of the enol form was observed for samples prepared in mixtures of 4:6 (v/v) 2-propanol/n-heptan. In this case, the strong dependence of the keto/enol equilibrium on changes in polarizability resulting from changes in the ratio of the solvents used is particularly noteworthy. Second, there is a more pronounced shift of the tautomeric equilibrium toward the polar solvents with a low polarizability value α. To investigate further the dependence of the keto/enol equilibrium in the analyzed compounds on the different substituents in the

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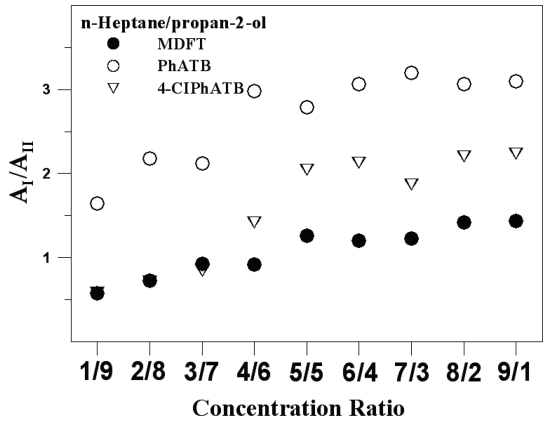

Figure 6. Ratios of C═O bands in MDFT, PhATB, and 4-ClPhATB (~1710−15/1730−40 cm^{-1}) as a function of the concentration ratio of *n*-heptane:propan-2-ol.

molecule, Figure 6 presents the dependence of the ratio of the intensity of the band characteristic of enol form to the band characteristic of the keto form on the n-heptan:propan-2-ol solvent ratio (derived from the spectra presented in Figure 5, above). It is evident that the tautomeric equilibrium in the analyzed analogues clearly depends on the substituent present in the compound. Depending on its type, the transition between the two forms is more or less fluent but always significantly shifted toward the enol form of the compound in the case of a solvent mixture with higher polarity.

It should be strongly emphasized here that not in all solvents can strong bands derived from enol or keto forms in the UV−vis range be observed. Importantly, in case of the observed keto/enol tautomerism effect in the analyzed 1,3,4-thiadiazoles, a state of equilibrium between both tautomeric forms can be observed. Upon examination of the FTIR spectra (both in pure solvents and in mixtures thereof, see Figure 3 and 5), it is evident that vibrations of the C═O group yielding a band at ~1735 cm^{-1} in the FTIR spectrum can clearly be seen, even if there is no band in the UV−vis spectroscopy assigned to the keto form. In UV−vis spectroscopy, the values of the molar extinction coefficient for the n → π^* transition in the carbonyl group have very low values of ca. 100 M^{-1} cm^{-1} (from ca. 100 to, less frequently, 500 M^{-1} cm^{-1}). Therefore, depending on the solvent (and its features), the transition may not be visible within an energy range, hence several spectroscopic techniques are used for investigation of these effects.

The pH-metric measurements allowed determination of the p*K* value for the *ortho* hydroxyl group in the thiadiazole derivatives. The p*K* value of 8.55 suggests deprotonation of the −OH group, which takes place in polar solvents. This, in turn, allows proposing a hypothesis that the keto/enol equilibria of thiadaizoles in polar solvents may be partially pH-dependent. The high polarizability and induced dipole moment of the nonpolar solvents, such as *n*-hexane or *n*-heptane, are factors influencing the keto/enol equilibria of the examined 1,3,4-thiadiazoles.

CONCLUSIONS

1,3,4-thiadizoles are widely reported to exhibit a wide range of biological activity and are considered as a potential new class of therapeutic agents.[39] Their characteristic structural features ensure internal proton transfer, which is relevant to their biological activity. More specifically, the keto/enol tautomerism of 1,3,4-thiadizoles may be associated with interactions between 1,3,4-thiadizoles and biological membranes.[16]

Spectroscopic studies carried out in this work suggest strong solvent-dependence of the keto/enol equilibria in 1,3,4-thiadiazole derivatives. In particular, the absorption spectra recorded in solvents with low molar polarizability values revealed the predominant character of enol tautomers, with the characteristic absorption maxima at approximately 330 nm. A change in the solvent polarizability results in a shift of tautomeric equilibria toward formation of keto isomers, absorbed at approximately 270−276 nm. This is particularly clearly visible in spectra recorded in solvents with high molar polarizability and low polarity values, such as *n*-hexane, *n*-heptane, or undecane. The results obtained revealed that the changes in electronic absorption depend mainly on the average electric dipole polarizability rather than the dielectric constant ε (Kirkwood function) or the refractive index n (Lorentz−Lorentz model). These findings were confirmed by additional experiments, including FTIR spectroscopy measurements.

It is worth emphasizing that the keto tautomer was a predominant form observed in the hydrophobic environment such as *n*-hexane. This allows an assumption that there is a possibility of similar behavior of 1,3,4-thiadiazoles upon the interactions with lipid membranes or proteins. As the keto tautomers exhibited hydrophobicity higher than that of corresponding enols, it is expected that transport through the biological membranes proceeds more efficiently in the case of 1,3,4-thiadiazoles in the keto form. Moreover, the favored formation of keto tautomers in the hydrophobic environment may influence the nucleophilic addition-related interactions of the C═O group, which may theoretically lead to further enhancement of the biological activity. In this context, the investigation of the solvent-dependence of the tautomeric equilibria of MDFT, PhATB, and 4-ClPhATB is particularly valuable, and may be extended onto most 2,4-dihydroxyphenyl-substituted 1,3,4-thiadiazoles. It is worth emphasizing that the keto/enol equilibrium is characteristic of the analyzed 1,3,4-thiadiazole compounds. Furthermore, it is strictly dependent on changes in the polarizability of the solvent used rather than on changes in its polarity.

In the case of other molecules with the ability to form intramolecular hydrogen bonds, usually the keto form dominates in polar solvents, in which it is surrounded (solvated) by the solvent molecules. In turn, the enol form dominates in nonpolar solvents, in which it is stabilized by the formation of intramolecular hydrogen bonding. However, this effect in the presented 1,3,4-thiadiazoles is probably opposite, with a more polarized keto form dominating in nonpolar solvents, characteristic of a high polarizability value. This substituent effect seems important and will be a subject of more in-depth studies in the future.

The tautomerism of these compounds can significantly contribute to clarification of the wide spectrum of the pharmacological and biological activities of these compounds.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpca.6b08707.

Figure S1,examples of a fluorescence emission spectrum for PhATB in propan-2-ol, and Figure S2, the relationship between the maximum absorption characteristic for

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the enol form and the maximum characteristic for the keto form in relation to the Kirkwood function (PDF)

AUTHOR INFORMATION

Corresponding Authors

*(A.M.) Fax: +(48 81) 4456684. Telephone: +(48 81) 445 69 37. E-mail: arkadiusz.matwijczuk@up.lublin.pl.

*(M.G.) Telephone: +(48 81) 537 59 04. E-mail: mariusz.gagos@poczta.umcs.lublin.pl.

ORCID

Arkadiusz Matwijczuk: 0000-0003-2630-120X

Notes

The authors declare no competing financial interest.

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