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Tautomerism and Physical Properties of Pyrido[1,2-*a*]benzimidazole (PBI) GABA-A Receptor Ligands

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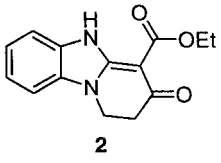
Abstract—Structural features of the pyrido[1,2-*a*]benzimidazole (PBI) chemical series of high-affinity GABA-A receptor ligands were studied by a variety of techniques, including NMR spectroscopy and AM-1 semi-empirical force-field calculations. This analysis revealed that the heterocyclic system exists nearly exclusively in the keto form, with a preference of ca. 8 kcal/mol for the keto over the enol tautomer in the gas phase.

The pyrido[1,2-*a*]benzimidazole (PBI) chemical series represents a novel class of high-affinity ligands for the benzodiazepine binding site on $GABA_A$ receptors.[1,2] Prototype carboxanilide **1**, and many of its analogs, are potent $GABA_A$ partial agonists with possible utility in the treatment of generalized anxiety and related neurological disorders.[1,3] To understand how such ligands might interact with their binding sites on the target receptor protein and to analyze structure–activity data, it is important to understand the physical properties of this heterocyclic system. Since there are plausible keto and enol structures for **1** (shown) that would equilibrate by virtue of a proton shift, we sought to determine the preferred tautomeric form.



The PBI structural type, as the corresponding ethyl ester **2**, was first reported by Ohta and co-workers, who assigned the enolic tautomer as the predominant form based on chemical analysis.[4] By contrast, we have generally presented the PBI structure in the keto form.[1] Through the use of NMR spectroscopy and AM-1 semi-empirical force-field calculations, we have now ascertained that the keto form is indeed the major tautomer for the PBI series and in the closely related compounds that we have studied.

Results and Discussion

Heterocycles often display tautomerism due to the transfer of a proton, such as for keto/enol and imine/amine equilibria.[5–7] The preferences for one form over the other can greatly influence interactions with biological targets such as G-protein coupled receptors (GPCRs) and ion channels. We have studied the PBI system to sort out the issue of keto/enol tautomeric preference, which has been a key factor in analyzing structure–activity relationships of related anxiolytic agents.[8] ^{13}C NMR spectroscopy is a particularly useful tool for the characterization of the keto/enol tautomerism, because the enolic carbon appears upfield when compared with the corresponding keto carbon by ca. 30–40 ppm.[9] The keto/enol tautomerism of enaminones has been evaluated by NMR methods, including ^{17}O and ^{13}C NMR.[10] The keto form is typically favored for compounds such as **3**, although the enol form can be observed depending on structure, choice of solvent, and temperature.[10]

We evaluated a series of PBIs and related compounds by ^{13}C

Keywords: tautomerism; NMR; benzimidazoles; pharmacologically active compounds; dipole moment.

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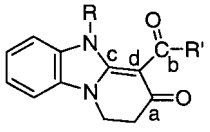
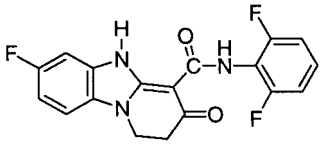
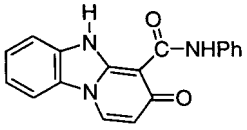
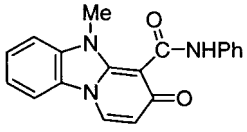
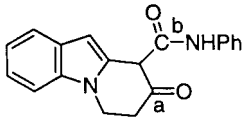
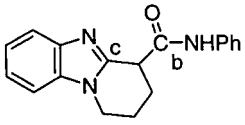
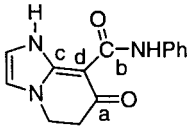
Table 1. Selected ^{13}C NMR chemical shifts for PBIs and related compounds



Compd #	R	R′	Chemical shift assignments[a]				
			a	*b*	*c*	*d*	NMR solvent
2	H	OEt	185.1	167.8	154.3	85.9	$CDCl_3$
4	Me	OEt	185.4	165.7	155.1	86.4	$CDCl_3$
1	H	NHPh	185.8	164.5	152.7	87.1	DMSO-d_6
5	Me	NHPh	187.3	163.5	152.0	89.9	$CDCl_3$
6	H	NH(4-pyridyl)	186.9	165.2	151.8	87.8	DMSO-d_6
7	H	NH(4-pyridyl) HCl	188.2	164.9	152.9	97.8	DMSO-d_6
			191.1	166.4	153.4	90.1	D_2O
8	Me	NH(4-pyridyl)	188.6	163.3	153.4	87.1	DMSO-d_6
9			185.6	165.9	155.1	87.4	$CDCl_3$
			186.5	164.1	153.4	87.1	DMSO-d_6
10			176.3	165.8	148.0	97.7	$CDCl_3$
			176.0	164.6	146.6	96.8	DMSO-d_6
11			177.8	162.7	148.7	100.7	$CDCl_3$
			176.2	162.5	147.1	100.6	DMSO-d_6
12			189	171			$CDCl_3$
13				166.4	149.8		$CDCl_3$
14			183.5	164.8	146.2	87.0	$CDCl_3$

[a] ^{13}C NMR chemical shifts reported downfield of TMS, and spectra were obtained at a concentration of 25 mg in 0.8 mL of solvent.

NMR, and the assignments for selected carbons are shown in Table 1. The chemical shifts for the ketone carbonyls (carbon a, entries 1–11) are at δ 175.9–191.1, which are typical values for ketone carbonyls. No minor peaks were observed in the spectra, so that we did not see a discreet, minor enolic tautomer. It is possible that the two tautomers interconvert sufficiently rapidly so that the observed resonances are averaged values of both forms. Even though **1** is found in the keto form in the solid state,[1a] it is possible that it could equilibrate in solution, depending also on solvent, temperature, and other factors.[10] Although we do not have an enolic PBI as a reference, compounds in which the imidazole nitrogen is alkylated (**4**, **5**, **8**, and **11**) are fixed into the keto form because the alternative enol would be a charge-separated zwitterion of much higher energy. The chemical shifts of the ketone carbonyls (carbon *a*) of esters **2** and **4**, and amides **1** and **5**, differing by virtue of *N*-methylation, are essentially identical. The chemical shifts for **7** and **8**, and **10** and **11**, were very also very similar. The keto carbon of the N–H derivatives (**2** and **1**) appear only 0.3–1.5 ppm upfield, possibly representing at most 1–5% of enolic contribution, based on the estimation of a ca. 30 ppm difference between the carbon *a* resonances for the keto and enol tautomers. The chemical shifts for carbons *a* of **1**, **2**, and **4**–**11** were largely invariant of the solvent used ($CDCl_3$, DMSO-d_6, or D_2O), suggesting an internally hydrogen-bonded structure without substantial intermolecular solvation. The spectrum of the HCl salt of the

4-pyridyl compound (**7**) in D_2O was the most divergent. The ketone carbonyl appeared 3–6 ppm downfield of the same carbons in the other compounds, and carbon *d* was also 2–4 ppm downfield. Compound **7** was heated to 100°C in DMSO-d_6 without an appreciable change in the spectrum. Additional related compounds were examined in order to probe the keto/enol tautomerism. Unsaturated derivative **10** had a grossly similar ^{13}C NMR spectrum in $CDCl_3$ as **1**, with an upfield shift of carbons *a* (9.5 ppm) and *c* (4.7 ppm). The upfield shift of carbon *a* of **10** probably does not reflect significant enolization, however, since the corresponding resonance for *N*-methyl derivative **11** appears only 0.2–1.5 ppm downfield.

Indole **12** differs from **1** by virtue of the replacement of the imidazole NH with a CH. This compound is clearly in the keto form shown, as the two carbonyls appeared at δ 171 and 189. Further, the ^1H NMR spectrum did not display a 2H singlet that would be characteristic of the benzylic methylene if the double bond had moved into conjugation with the two carbonyls. Des-keto derivative **13** was found to be in the benzimidazole form by ^1H NMR (data not shown). The keto form was predominant for desbenzo derivative **14** as well, as the chemical shift for the ketone carbonyl (δ 183.5) was very similar (δ 185.8) to that for **1**.

The FT-IR spectra of **1**, **2**, **4**, and **5** were examined dissolved in 1:1 $CHCl_3$/MeOH, as well as **7** in 1:1 MeOH/H_2O. The carbonyl region absorptions were: **1**, 1647, 1621/1589; **2**, 1608, 1558/1545; **4**, 1684, 1532; **5**, 1652/1647, 1525; and **7**, 1683, 1652, 1558 cm^{-1}. Those values at lower wavenumber were assigned to the ketone carbonyls which provide further support for the keto form. The higher wavenumber absorptions were attributed to the ester or amide. The noteworthy difference between the esters of **2** (1608 cm^{-1}) and **4** (1684 cm^{-1}) is attributed to an in-plane conformation for **2** facilitated by intermolecular hydrogen bonding which allows for conjugation and delocalization.

We also studied the properties of amide **1** in more detail. AM-1 force field calculations were conducted on both the keto and enol forms. Such calculations give information not only on the preferred conformations, but also on the alternatives that can be adopted. The degree to which various low-energy conformations contribute can be readily ascertained by inspection of the calculated energies. Results from AM-1 calculations for the keto and enol forms of **1** are given in Table 2. As can be seen, the keto form is ca. 7 kcal/mol more stable than the enol form in the gas phase. The keto form is predicted to have a higher dipole moment by ca. 2.75 D. The dipole moment of **1** was determined experimentally and found to be 5.41±0.1 D (benzene, 25°C). The shape of the absorption curve for a'' versus the ln of angular frequency indicated rigidity in which relaxation occurred primarily through the molecular structure itself. Although this experimental value was intermediate between the calculated values by AM-1 for the keto and enol forms other factors, such as the presence of solvent, complicate comparison of the experimental and calculated results.

Table 2. Results of AM-1 force-field analysis of **1**

	Enol-**1**	Keto-**1**
Heat of formation (kcal mol^{-1})	34.6	27.6
Ionization potential (eV)	8.63	8.23
Dipole moment (D)	4.07	6.83

The keto form for compound **1** and related derivatives is the only one supported by spectroscopic measurements, and is preferred based on semi-empirical molecular mechanics calculations. This result corrects the literature in which the enolic form had been proposed.[4] Based on the work reported here, we have analyzed the interaction of our anxiolytic series with the $GABA_A$ binding site based solely on the keto form.[1d]

Experimental

General procedures

^1H NMR spectra were recorded on either Varian 390 (90 MHz) or a Bruker AC-300 (300 MHz) instruments. The ^{13}C NMR spectra were taken on Bruker AM-360 (90.56 MHz) and Varian EM-390 (90 MHz) spectrometers using DEPT to determine carbon type. In the NMR experiments, tetramethylsilane (TMS) was used as the internal standard. Solvents and reagents were purchased and used without further purification. The AM-1 geometrization, force field, and dipole moment calculations were conducted using the Sybyl software package (Tripos, St. Louis, Missouri).

Dipole moment determination

Repeated dielectric measurements of an accurately prepared solution of 0.0002973 mole fraction of the compound were conducted in benzene. The static dielectric constant (ϵ_0) was measured at a radio frequency of 0.001 GHz, and dielectric contants ϵ', and losses ϵ'', were determined at frequencies of 0.74, 1.17, 2.28, 9.13, 25.5, and 146 GHz. For all measurements, temperature was maintained at 25±0.01°C. Mole fractions for the static dielectric constant (a_0), the microwave dielectric constants (a'), and losses (a'') were fitted to Debye dispersion and absorption equations to obtain the relaxation time (τ=151 ps) and infinite frequency intercept ($a_{inf.}$=5.00). The dipole moment, μ, was evaluated by employing the Debye equations modified for dilute solution, with the following form:

$$\mu = \frac{1}{2(\epsilon_1 + 2)}\sqrt{\frac{27\mathrm{k}TM_1(a_0 - a_{\mathrm{inf.}})}{\pi N d_1}}$$

where k, *N*, and M_1 are the Boltzmann constant, Avogadro's number, and the molecular weight of the solvent, respectively, and ϵ_1 and d_1 are the values of the dielectric constant and density of the solvent.

Materials investigated

Compounds **1**, **2**, **3**–**11** were obtained as previously described.[1] Compounds **12**–**14** were prepared by related synthetic methods, and analyzed for chemical purity using combustion analysis (C, H, N) and 300 MHz ^1H NMR.

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