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Population, basicity and partition of short-lived conformers. Characterization of baclofen and pregabalin, the biaxial, doubly rotating drug molecules

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ABSTRACT

Populations, protonation constants and octanol-water partition coefficients were determined and assigned specifically to fast interconverting individual conformers, exemplified in baclofen and pregabalin, the GABA-related drug molecules of biaxial, double rotations. Rotamer statuses along both axes in water and octanol were elucidated from ^{1}H NMR vicinal coupling constants. Conformer abundances were obtained by the appropriate combination of the rotamer populations in the two adjacent moieties in the molecule. The bulky aromatic group in baclofen versus the aliphatic side chain of pregabalin explains why baclofen exists mainly in trans-trans conformeric form, throughout the pH range, unlike pregabalin that has no any highly dominant form. Characteristically enough, for pregabalin, the lipophilicity of the conformers is primarily influenced by the conformation state. Conformers in gauche state are of higher lipophilicity. The conformers of the two compounds were ranked by their membrane-influx and –outflow propensities.

1. Introduction

Rotamers and conformers are stereochemical forms of flexible molecules. Most drug molecules exist, react and interact in solution in specific forms of rotamers and conformers. Also, their different forms of conformation are the binding ones upon acting at various subtypes of receptors of a kind. Rotamers are a subclass of conformers which refer to stereoisomers of small molecules having only one or a few rotational axes. Rotamers undergo fast interconversion in solution. The relative residence time of a molecule in a particular rotameric form can also be interpreted as rotamer population. Conformers are, however, often considered to be the few coexisting stereochemical forms of numerous theoretically possible ones with several rotational axes. The structure of such conformers can be stabilized by a number of noncovalent, intramolecular, or solvent-mediated interactions. So far such stereo-specific physico-chemical parameters have only been reported for molecules of one rotational axis only. Determination of rotamer populations for molecules of ABX spin systems has become possible through the measurement of ^{1}H NMR three-bond coupling constants and an appropriate evaluation procedure (Fujiwara et al., 1974; Noszal et al., 1991). Thus, rotamer populations have been published for relatively simple compounds such as amino acids (Fujiwara et al., 1974; Noszal et al., 1991; Noszal and Sandor, 1989), *N*-acetyl-cysteine (Noszal et al., 2000), clenbuterol (Kraszni et al., 2003) and histamine (Kraszni et al., 2002). Rotamer-specific protonation constants can be determined from the rotamer abundance at different pH and the bulk protonation constant (Noszal and Sandor, 1989; Noszal et al., 2000; Kraszni et al., 2002; Kraszni et al., 2004), while rotamer-specific partition coefficient can be calculated if rotamer populations have all been determined in octanol and water and the bulk partition coefficient is known also (Kraszni et al., 2003; Noszal and Kraszni, 2002). Previous results show that order of magnitude differences can occur between the lipophilicity of rotamers (Kraszni et al., 2003). Vistoli et al. have also shown that molecular flexibility is related to lipophilicity of compounds (Vistoli et al., 2009). Forerunners in this field have also shown how conformer-specific lipophilicities can be determined using theoretical calculations (Gaillard et al., 1994), and why the bulk physico-chemical parameters must be viewed in light of the dynamic behavior of the molecules.

In this work our aim was to determine the conformer-specific parameters (populations, protonation constants, lipophilicities) of baclofen and pregabalin drugs around two rotational axes (Fig. 1).

Baclofen ((*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid, Lioresal®) is a γ-aminobutyric acid derivate, used as a centrally acting muscle relaxant. It has proved to be efficacious in the treatment of spasticity resulting from multiple sclerosis, particularly for the relief of flexor spasms and concomitant pain, clonus, and muscle rigidity. Baclofen

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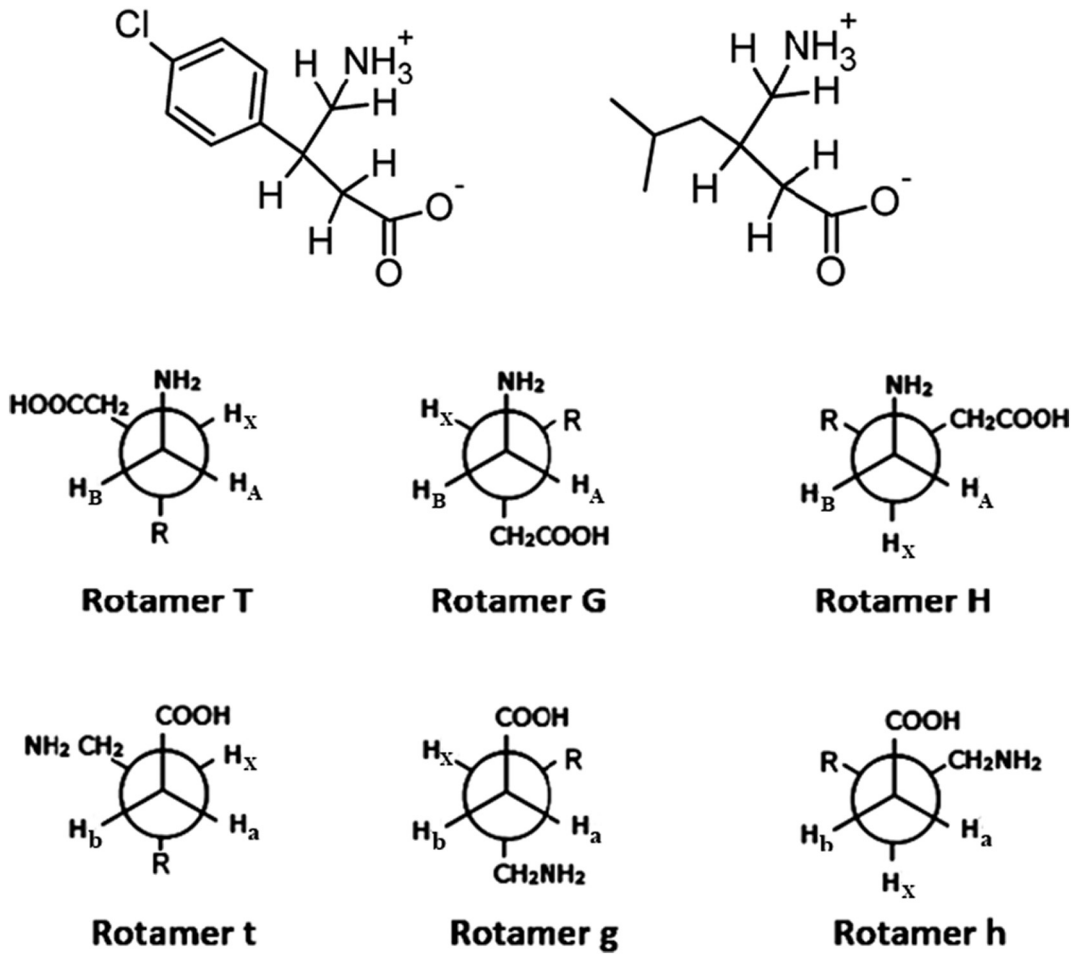

Fig. 1. The structure of baclofen (top left) and pregabalin (top right), and their component staggered rotamers below. Rotamer states and AB hydrogens in the ABX spin (sub)system on the rotation axis pertaining the amino group are signed by capital letters, while the rotamer states and ab hydrogens in the abX spin (sub)system on the rotation axis pertaining the carboxyl group are signed by lower case letters (T, t = trans; G, g = gauche; H, h = hindered) (Martin and Mathur, 1965), while H_X is contained by both ABX/abX spin (sub)systems. Abbreviation R in the rotamers stands for the *p*-chlorophenyl and isopropyl moiety in baclofen and pregabalin, respectively.

may also be of value in patients with spinal cord injuries and other spinal cord diseases (Honc et al., 1985). Baclofen produces these effects by activating the $GABA_B$ receptor. The bulk lipophilicity value of baclofen has been previously determined by Leisen et al. to be −0.96, which is in good agreement with our result (see Results and discussions) (Leisen et al., 2003). Vaccher et al. have studied the conformational states of baclofen in D_2O with NMR, but only for the two axes separately (Vaccher et al., 1995).

Pregabalin ((*S*)-3-aminomethyl-5-methylhexanoic acid, Lyrica®) is a neurotransmitter γ-aminobutyric acid (GABA) analogue, to treat epilepsy, neuropathic pain, fibromyalgia, and generalized anxiety disorder (Tassone et al., 2007). Pregabalin binds the alpha2-delta subunit protein of voltage-gated calcium channels in central nervous system tissues with high affinity, reducing thus the release of excitatory neurotransmitters. The conformational states of pregabalin have been studied with density functional theory by Sadeghzade et al. (2015).

Both compounds have two main rotational axes, containing one methine and two non-identical methylene protons in ABX/abX ^{1}H NMR spin pattern in every axis. This allows the measurement of vicinal AX/aX and BX/bX couplings, and the subsequent determination of the rotamer populations. The structural differences are also significant, since pregabalin has an aliphatic chain, while baclofen has a bulky aromatic group (Fig. 1).

2. Materials and methods

Baclofen, pregabalin, *n*-octanol, and substituted ethane derivates (isobutyric acid, isovaleric acid, 2,4-dimethylpenthane, isopropylamine, isobutylamine) were obtained from Sigma- Aldrich. Deuterium oxide (D_2O) and methanol were purchased from Merck. Carboxyl-ester derivative of baclofen and 1-chloro-4-isopropylbenzene were synthesized as shown in (Manglik et al., 1980; Seyferth and Cheng, 1973), respectively. All reagents were of analytical grade, obtained from commercial suppliers. The deionized water was prepared with a Milli-Q Direct 8 Millipore system.

2.1. NMR

All NMR measurements were carried out on a Varian VNMRS spectrometer (599.9 MHz for ^{1}H) with a dual 5 mm inverse-detection gradient (IDPFG) probehead.

The NMR-pH titrations were performed at 25.0 ± 0.1 °C at a constant ionic strength (I = 0.15 mol/dm^3, auxiliary electrolyte: KCl) in 5% D_2O–95% H_2O solutions. This deuterium concentration proved to be enough for the spectrometer lock system. Using such a small amount of D_2O, the pH is within the 0.02 isotope shift limit, according to the Gross–Butler–Purlee theory (Glasoe and Long, 1960; Purlee, 1959). The spectra were referenced to internal DSS. 0.005 M solutions of the compounds studied were used for the titrations.

For pH measurement a combined glass electrode (Metrohm 6.0234.110) was used. The pH data are pH meter readings based upon NIST primary standards: 0.05 M potassium tetraoxalate (pH = 1.68), 0.05 M potassium hydrogen phthalate (pH = 4.01), 0.025 M KH_2PO_4 + 0.025 M Na_2HPO_4 (pH = 6.87) and 0.01 M borax (pH = 9.18).

Protonation constants were evaluated by using Microcal Origin 8.0 (https://www.originlab.com/). The fundamental relationship for the determination of the macroconstants was as follows:

$$\delta_{obs} = \delta_{L^-}\alpha_{L^-} + \delta_{LH}\alpha_{LH} + \delta_{LH_2^+}\alpha_{LH_2^+} = \frac{\delta_{L^-} + \delta_{LH}K_1[H^+] + \delta_{LH_2^+}K_1K_2[H^+]^2}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (1)$$

where δ_{obs} is the observed chemical shift, L^-, LH, LH_2^+ are the non-protonated, mono-, and diprotonated macrospecies, respectively, α_{L^-}, α_{LH}, $\alpha_{LH_2^+}$ are mole fractions, δ_{L^-}, δ_{LH}, $\delta_{LH_2^+}$ are chemical shifts of the species in subscript.

Vicinal proton-proton coupling values were obtained in water at pH = 1.0, 13.0, and at the isoelectric pH (6.5 for baclofen, 7.2 for pregabalin) where the three distinct protonation states of the compounds investigated exist overwhelmingly. For the determination of conformer-specific lipophilicities the vicinal coupling values were also measured in water-saturated octanol at the isoelectric pH. The solvent resonance was diminished using “wet” pulse sequence (Hore, 1983; Hors, 1983). Since nondeuterated octanol was used for the experiments, we have placed a coaxial insert filled with D_2O into the octanol samples to set the lock signal. All NMR spectra were recorded with a digital resolution of 0.02 Hz. The free induction decay was digitized into 32 K data points. Typically 16 transients were co-added and a 15 s repetition time was used. Coupling constants were evaluated from the spectra at 600 MHz by non-first-order spectral analysis, using Spinworks 4.0 (https://home.cc.umanitoba.ca/~wolowiec/spinworks/) program (Fig. 2).

2.2. Substituent parameters

For the determination of rotamer and conformer populations the appropriate, standard gauche and trans coupling constants are needed, which can be obtained from Altona's generalized Karplus-equation (Altona et al., 1994). In order to obtain latters for each rotamer, determination of the relative group electronegativities (also known as substituent constants) is necessary. This parameter can be calculated from the vicinal coupling constants in monosubstituted and 1,1-disubstituted ethanes, using equation (Altona et al., 1989):

$$^3J_{(HH)} = 7.84 - 0.59(\lambda_1 + \lambda_2) - 0.42(\lambda_1\lambda_2) \quad (2)$$

where $^3J_{(HH)}$ is the observed vicinal coupling constant, λ_1 and λ_2 are group electronegativities. Since the methyl group electronegativity is already known as 0.80 (Seyferth and Cheng, 1973), using the ^1H-^1H NMR coupling constants of the aliphatic protons of isopropylamine, isobutyric acid, isobutyl-amine, isovaleric acid, 1-chloro-4-isopropylbenzene and 2,4-dimethyl-pentane, the group electronegativities can be calculated. The results are summarized in Table 1 corresponding to two different ionization state:

Table 1
Substituent constants (λ) in water at different pH values. The values with * have been determined previously (Altona et al., 1994).

Molecule	pH	Group	$^3J_{HH}$	λ
Isopropylamine	1	$-NH_3^+$	6.62	0.82*
Isopropylamine	13	$-NH_2$	6.35	1.10*
Isobutyric acid	1	-COOH	7.00	0.39*
Isobutyric acid	13	$-COO^-$	6.98	0.42*
Isobutylamine	1	$-CH_2-NH_3^+$	6.80	0.61
Isobutylamine	13	$-CH_2-NH_2$	6.74	0.68
Isovaleric acid	1	$-CH_2$-COOH	6.65	0.78
Isovaleric acid	13	$-CH_2-COO^-$	6.65	0.78
1-chloro-4-isopropylbenzene	1	C_6H_4Cl-	6.95	0.45
1-chloro-4-isopropylbenzene	13	C_6H_4Cl-	6.95	0.45
2,4-dimethylpentane	1	$-CH_2-CH(CH_3)-CH_3$	6.82	0.63
2,4-dimethylpentane	13	$-CH_2-CH(CH_3)-CH_3$	6.82	0.63

The conformer populations in octanol are also necessary constituent data, however the substituent constants may vary under different dielectric constant conditions, which have to be taken into account. Slight changes in the dielectric constant can be considered negligible, therefore we determined the λ values for two extreme cases (namely in water, and in deuterated chloroform) and used the latter value for measurements in octanol. Based on previous results (Altona et al., 1994) the substituent constant of the amino group changes with the dielectric constant, while in $CDCl_3$ that of the carboxyl group is the same as in water. For the non-polar isobutyl and *p*-chlorophenyl groups the λ values are also expected to remain invariant of the dielectric constant (Kraszni et al., 2003). Based on the above, the λ of the carboxymethyl group ($-CH_2COOH$) is assumed to be unaffected by dielectric constant, while the λ of NH_2 (1.19) is taken from a previous work (Altona et al., 1994). The λ of aminomethyl group ($–CH_2NH_2$) was determined using an analogous method described for aqueous medium and was proved to be 0.73 (Fig. 3).

Using group electronegativities, the standard coupling constants can be calculated as follows (Altona et al., 1994):

$$^3J(HH) = 14.63\cos^2(\phi) - 0.78\cos(\phi) + 0.60 + \sum_i \lambda_i\{0.34 - 2.31\cos^2[s_i(\phi) + 18.4\,|\lambda_i|]\} \quad (3)$$

where ϕ stands for the dihedral angle, which was taken +60°, −60° and + 180° for the staggered conformers according to the gauche and trans positions, s_i is the ‘sign factor’ +1 or −1, depending on the position of the substituents relative to the coupling protons. As Table 1 shows, protonation influences the group electronegativities, the standard coupling constants therefore have to be calculated for each rotameric and protonation state. The results are shown in Table 2:

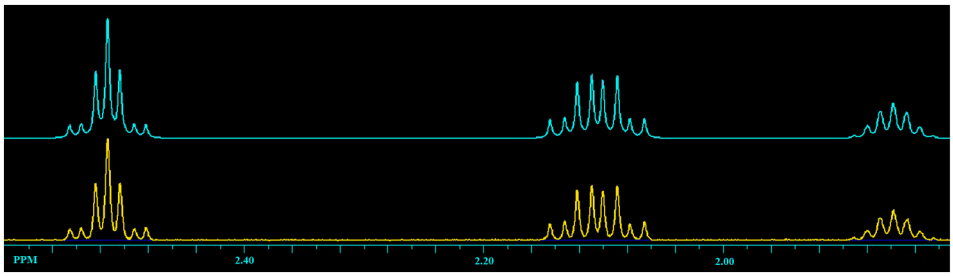

Fig. 2. The experimental (bottom, yellow) and the simulated (top, blue) NMR spectra of pregabalin at pH = 13. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

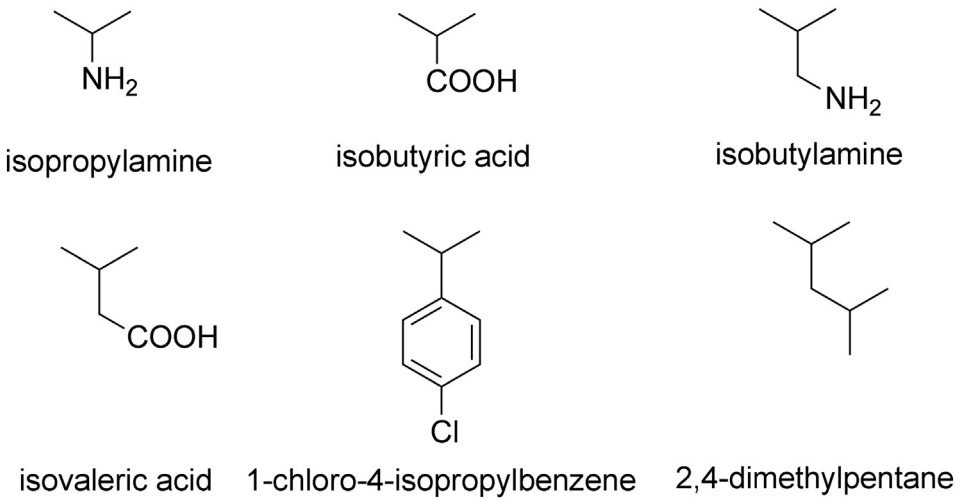

Fig. 3. The structures (in uncharged form) of the ethane derivatives presented in Table 1.

Table 2
Standard coupling constants in Hz, obtained from Eq. (3), to be used in Eqs. (4) and (5) for baclofen and pregabalin for both axes, at three different pH values in water and in octanol, respectively. The pH values in the first column refer to the aqueous experiments, while the indication 'octanol' refers to the experiments in octanol.

Baclofen						
pH	J_{AX_T}	J_{AX_G}	J_{AX_H}	J_{BX_T}	J_{BX_G}	J_{BX_H}
1	3.42	12.23	3.08	12.23	3.91	2.60
6.5	3.42	12.23	3.08	12.23	3.91	2.60
13	3.56	11.86	2.63	11.86	4.06	2.14
octanol	3.61	11.76	2.46	11.76	4.11	1.97
pH	J_{aX_t}	J_{aX_g}	J_{aX_h}	J_{bX_t}	J_{bX_g}	J_{bX_h}
1	3.47	13.24	3.49	13.24	3.68	3.28
6.5	3.47	13.21	3.47	13.21	3.68	3.26
13	3.39	13.09	3.50	13.09	3.71	3.18
octanol	3.41	13.16	3.51	13.16	3.70	3.22
Pregabalin						
pH	J_{AX_T}	J_{AX_G}	J_{AX_H}	J_{BX_T}	J_{BX_G}	J_{BX_H}
1	3.47	11.91	2.90	11.91	3.73	2.65
7.2	3.47	11.91	2.90	11.91	3.73	2.65
13	3.62	11.54	2.45	11.54	3.88	2.19
octanol	3.71	11.49	2.26	11.49	3.91	2.06
pH	J_{aX_t}	J_{aX_g}	J_{aX_h}	J_{bX_t}	J_{bX_g}	J_{bX_h}
1	3.53	12.93	3.31	12.93	3.50	3.34
7.2	3.53	12.89	3.29	12.89	3.50	3.32
13	3.45	12.77	3.32	12.77	3.53	3.24
octanol	3.47	12.84	3.33	12.84	3.52	3.28

2.3. Determination of logP values by stir flask methods

The bulk partition coefficient was calculated from the absorbance or peak area ratios of the investigated molecules before and after partitioning at several octanol/water phase ratios (3–100) at isoeletric pH (Brooke et al., 1990; Hersey et al., 1989). For concentration determination of baclofen and pregabalin UV absorbance and HPLC-MS measurements were used, respectively (see next chapter). The total concentration before partioning was 2.5×10^{-4} M. Partitioning experiments were performed at 25 °C.

HPLC-MS method for the determination of pregabalin.

The pregabalin concentration was determined by HPLC–MS in scan mode. HPLC analysis was performed by an Agilent 1260 Infinity LC system in conjunction with an Agilent 6460 triple-quadrupole mass spectrometer (Agilent, Waldbroon, Germany). Chromatography was carried out using a Zorbax Eclipse Plus C18 (100 X. 46 mm, 3.6 µm particle size) column with a mobile phase of methanol/0.1% formic acid in water (10/90 V/V), delivered with 0.5 ml/min flow rate at 30 °C. The mass spectrometer was operated in conjunction with a JetStream electrospray ion source in positive ion mode. Data were processed using Agilent MassHunter B.04.00 software.

3. Results and discussion

3.1. Determination of protonation macro- and microconstants

The compounds studied are diprotic ones, with amino and carboxylate protonation sites. The macro- and microscopic protonation schemes are depicted in Fig. 4.

The protonation macroconstants were determined using NMR-pH titrations, while protonation microconstants of baclofen were determined with deductive method using baclofen methyl ester, introducing the K of the ester into k_C^N of baclofen. For the determination of the pregabalin microconstants the $E_{C\text{-}N}$ carboxylate-amino interactivity parameter of baclofen was used, since the pair interactivity parameter is the most inert and best transferable parameter between molecules having analogous moieties (Szakács and Noszál, 1999). The log$E_{C\text{-}N}$ value was found to be 0.76 ± 0.05.

The protonation macro- and microconstants are summarized in Table 3.

Basicity of an amino site is well-known to be significantly higher than that of a carboxylate. The difference between the two logK_1 values can well be explained by the structures. The aromatic group in baclofen has an electron withdrawing effect, whereas the aliphatic chain in pregabalin is electron donating. Using the microscopic protonation constants, the pH-dependent distribution of baclofen and pregabalin microspecies can be calculated. Concerning the protonation isomers, abundance of the zwitterionic form exceeds that of the uncharged form by some five orders of magnitude in both compounds (Fig. 5).

3.2. Conformer analysis

Rotamer populations can be calculated using the observed vicinal ^{1}H-^{1}H NMR coupling values for ABX spin systems. The observed vicinal coupling values are shown in Table 4. The geminal coupling values (J_{AB}) and (J_{ab}) have also been obtained and applied in the simulation process (typical values were 12.5–16.5 Hz), however this parameter is not needed for further calculations, therefore it is not shown in Table 4.

Because of rapid interconversion among rotamers, the observed couplings are the weighted sums of the various gauche and trans

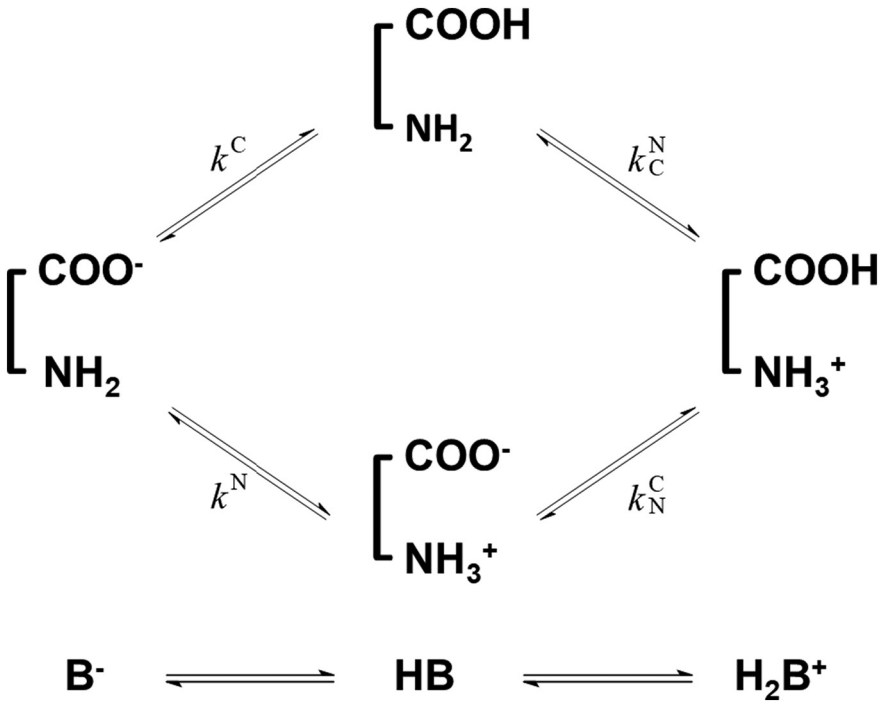

Fig. 4. Macroscopic (bottom) and microscopic (top) protonation schemes of baclofen and pregabalin.

Table 3
Macroscopic and microscopic protonation constants of baclofen and pregabalin in log units.

Parameter	Baclofen	Pregabalin
$\log K_1$	9.82 ± *0.01*	10.37 ± *0.01*
$\log K_2$	3.88 ± *0.01*	3.97 ± *0.01*
$\log k^N$	9.82 ± *0.01*	10.37 ± *0.01*
$\log k^C$	4.64 ± *0.05*	4.73 ± *0.05*
$\log k_C^N$	9.06 ± *0.05*	9.61 ± *0.05*
$\log k_N^C$	3.88 ± *0.02*	3.97 ± *0.01*

coupling constants of individual rotamers, where weighting factors are the appropriate mole fractions. The following relationships encompass the parameters:

$$^3J_{AX} = f_T J_{AX_T} + f_G J_{AX_G} + f_H J_{AX_H} \quad (4)$$

Table 4
Observed vicinal coupling values in Hz for baclofen and pregabalin at three different pH values in water and also in octanol. The pH values in the first column refer to the aqueous experiments, while the indication 'octanol' refers to the experiments in octanol.

pH	$^3J_{AX}$	$^3J_{BX}$	$^3J_{aX}$	$^3J_{bX}$
Baclofen				
1	4.90	10.65	5.95	9.15
6.5	5.10	10.20	6.65	8.65
13	5.30	9.30	6.50	9.30
octanol	5.90	8.05	3.65	10.40
Pregabalin				
1	6.30	6.30	5.85	7.03
7.2	5.40	6.90	5.90	7.35
13	5.70	5.85	7.15	7.45
octanol	3.25	8.45	3.00	9.45

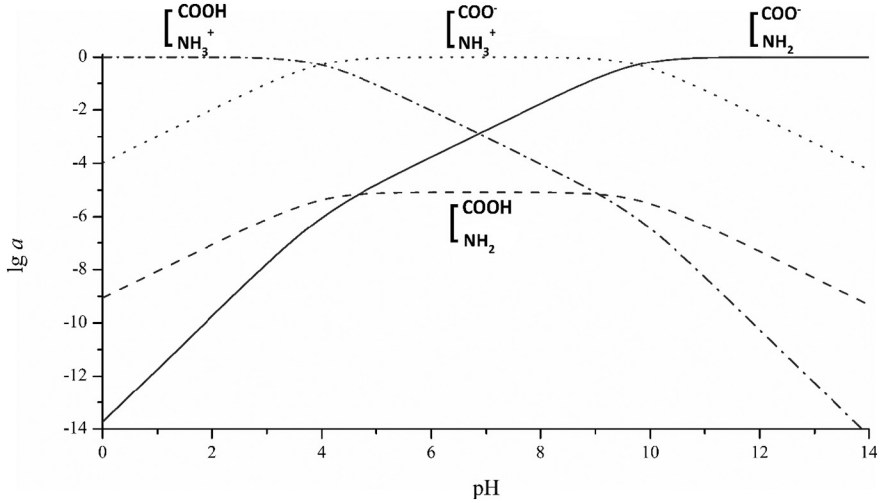

Fig. 5. Distribution of baclofen microspecies (α is the mole fraction of the species indicated) as a function of pH, in log units. The analogous diagram of pregabalin is of highly similar character.

$$^3J_{BX} = f_T J_{BX_T} + f_G J_{BX_G} + f_H J_{BX_H} \quad (5)$$

$$f_T + f_G + f_H = 1 \quad (6)$$

where f_T, f_G, f_H are mole fractions of the respective T, G, H rotamers while J_{AX_T}, J_{AX_G} and J_{AX_H} are the standard coupling constants between hydrogen 'A' and 'X' in the trans, gauche and hindered rotamers, respectively (Noszal and Sandor, 1989). Eq. (5) is built for hydrogens 'B' and 'X' and the related parameters under identical principles. Analogous equations can be applied for $^3J_{aX}$ and $^3J_{bX}$ observed coupling values for the calculation of f_t, f_g and f_h mole fractions, using the appropriate standard coupling values from Table 2.

The conformer populations for molecules with two rotational axes can be calculated as the product of the two rotamer populations pertaining the two rotational axes. For example, a conformer in which the rotamers are in T and t position:

$$f_{Tt} = f_T f_t \quad (7)$$

where fractions with single-letter subscript belong to rotamers, while the fraction with two-letter subscript identifies the conformer. Since three staggered rotamers of appreciable life time exist along the two rotational axes, the number of conformers is 9. The calculated conformer populations are in Table 5.

3.3. Determination of conformer-specific protonation constants

Since concentration of the zwitterionic form exceeds that of its uncharged counterpart by more than five orders of magnitude, contribution and parameters of the uncharged form can well be neglected in these calculations. Total concentrations ([L^-], [LH] and [LH_2^+]) are composed of the conformer concentrations at every pH. Subscript(s) (if any) of the conformers indicate the site(s) protonated:

$$[L^-] = [Tt] + [Tg] + [Th] + [Gt] + [Gg] + [Gh] + [Ht] + [Hg] + [Hh] \quad (8)$$

$$[LH] = [Tt_N] + [Tg_N] + [Th_N] + [Gt_N] + [Gg_N] + [Gh_N] + [Ht_N] + [Hg_N] + [Hh_N] \quad (9)$$

$$[LH_2^+] = [Tt_{N,C}] + [Tg_{N,C}] + [Th_{N,C}] + [Gt_{N,C}] + [Gg_{N,C}] + [Gh_{N,C}] + [Ht_{N,C}] + [Hg_{N,C}] + [Hh_{N,C}] \quad (10)$$

As an example, protonation of the Tt conformer at its amino site can be quantified in terms of the $k_{1_{Tt}}$ corformer- and site-specific protonation constant:

$$k_{1_{Tt}} = \frac{[Tt_N]}{[Tt][H^+]} \quad (11)$$

Table 5
Percentage conformer populations of baclofen and pregabalin at three distinctive pH values (high sd values result from the Gaussian propagation of uncertainty for conformers of low abundance).

pH	Tt	Tg	Th	Gt	Gg	Gh	Ht	Hg	Hh
Baclofen									
1	47%	21%	14%	10%	4.3%	2.8%	1%	1%	0.3%
sd	*4%*	*2%*	*4%*	*2%*	*0.9%*	*0.9%*	*3%*	*1%*	*0.8%*
6.5	45%	24%	7%	11%	6%	1.8%	3%	1%	0.4%
sd	*4%*	*2%*	*3%*	*2%*	*1%*	*0.9%*	*3%*	*1%*	*0.5%*
13	42%	22%	6%	13%	7%	2%	5%	3%	0.7%
sd	*3%*	*2%*	*3%*	*2%*	*1%*	*1%*	*3%*	*2%*	*0.5%*
Pregabalin									
1	13%	9%	13%	14%	9%	13%	11%	7%	11%
sd	*2%*	*1%*	*2%*	*2%*	*1%*	*2%*	*2%*	*1%*	*2%*
7.2	18%	11%	14%	10%	7%	8%	13%	8%	10%
sd	*2%*	*1%*	*2%*	*1%*	*1%*	*1%*	*2%*	*1%*	*2%*
13	14%	13%	6%	14%	13%	5%	15%	14%	6%
sd	*2%*	*2%*	*2%*	*2%*	*1%*	*2%*	*3%*	*2%*	*2%*

17 other related conformer- and site-specific constants ($k_{1_{Tg}}$, $k_{1_{Th}}$, …, $k_{2_{Hh}}$) are defined analogously. Subscripts in the $k_{2_{Gh}}$ for example, indicate that this is the second protonation constant of the Gh conformer, in which the product is protonated both at the amino and carboxylate sites.

Since f_{Tt_N}, the fraction of the N-protonated Tt conformer among all the N-protonated species is $f_{Tt_N} = [Tt_N]/[LH]$, and an analogous relationship holds for the non-protonated one, $k_{1_{Tt}}$ can be expressed as follows:

$$k_{1_{Tt}} = \frac{f_{Tt_N}[LH]}{f_{Tt}[L^-][H^+]} = \frac{f_{Tt_N}}{f_{Tt}} K_1 \quad (12)$$

In practice, taking further the example of the Tt species, the conformer-specific protonation constants can be obtained as shown below:

$$\log k_{1_{Tt}} = \log K_1 + \log\left(\frac{f_{Tt_N(pH=iep)}}{f_{Tt(pH=13)}}\right) \quad (13)$$

$$\log k_{2_{Tt}} = \log K_2 + \log\left(\frac{f_{Tt_{N,C}(pH=1)}}{f_{Tt_N(pH=iep)}}\right) \quad (14)$$

Table 4 shows that significant, but typically not dramatic changes took place in populations of the conformers as a function of pH. In baclofen, the dominant conformer at every pH value is Tt, due to the steric effect of the bulky aromatic group, while the hindered rotameric state has a minimal abundance only. The rotation of the axis pertaining to the primary amino group has a greater influence on the conformer population compared to the carboxylate-containing axis. In pregabalin, highly dominant conformeric forms do not occur, due to the moderate steric effect of the aliphatic side chain.

The conformer-specific protonation constants calculated by equations, like (13) and (14) are summarized in Table 6:

Data in Table 6 reveal that the conformer-specific amino protonation constants decrease when the axis pertaining to the amino group rotates from trans to gauche state (e. g. Tt to Gt). In this phenomenon the amino group departs from the carboxylate site, and approaches the hydrophobic aromatic moiety or the aliphatic chain, becoming thus less accessible to water molecules. Rotation to hindered state or the rotation of the carboxylate-containing axis result in less significant changes. Since the protonating carboxylate oxygen atom is one bond more remote of the rotational axis than a protonating amino nitrogen, the conformer-specific carboxylate constants are less sensitive to the change of the conformeric states. For baclofen the very low abundance of the Ht, Hg and Hh conformers at every pH results in high errors in the calculated conformer-specific constants, so the difference in these parameters is not significant. Using the macroscopic, microscopic and the conformer-specific protonation constants, the distribution of species at different conformer and protonation states can be calculated (Fig. 6).

3.4. Conformer-specific liphophilicity

To obtain conformer-specific partition coefficients, D, the bulk partition coefficients and the rotamer populations both in the octanol and aqueous phases have to be known (Noszal and Kraszni, 2002). Taking further the example of the Tt conformer, its partition coefficient is as follows:

$$d_{Tt} = \frac{[Tt]_o}{[Tt]_w} = \frac{f_{Tt_o}}{f_{Tt_w}} D \quad (15)$$

where o and w indices refer to the octanol and aqueous phases, d_{Tt} and D are the conformer-specific and bulk partition coefficients, respectively. The bulk partition coefficients are shown in Table 7:

Using the coupling values in octanol phase (Table 4), conformer populations have been determined analogously to the method in water, and are collected in Tables 8 and 9.

Upon trans to gauche rotation of the baclofen carboxyl group (e. g.

Table 6
Conformer-specific protonation constants, and their standard deviations in italics for baclofen and pregabalin.

Conformer	Tt	Tg	Th	Gt	Gg	Gh	Ht	Hg	Hh
Baclofen									
logk_1	9.86	9.85	9.94	9.76	9.75	9.84	9.52	9.52	9.60
sd	*0.05*	*0.06*	*0.32*	*0.10*	*0.10*	*0.33*	*0.53*	*0.53*	*0.61*
logk_2	3.90	3.82	4.15	3.81	3.73	4.07	3.48	3.40	3.74
sd	*0.05*	*0.06*	*0.23*	*0.12*	*0.12*	*0.26*	*1.27*	*1.27*	*1.29*
Pregabalin									
logk_1	10.46	10.29	10.75	10.26	10.09	10.55	10.32	10.15	10.61
sd	*0.09*	*0.08*	*0.15*	*0.08*	*0.07*	*0.15*	*0.11*	*0.10*	*0.16*
logk_2	3.85	3.87	3.94	4.08	4.11	4.17	3.89	3.92	3.98
sd	*0.09*	*0.09*	*0.11*	*0.08*	*0.07*	*0.10*	*0.12*	*0.11*	*0.13*

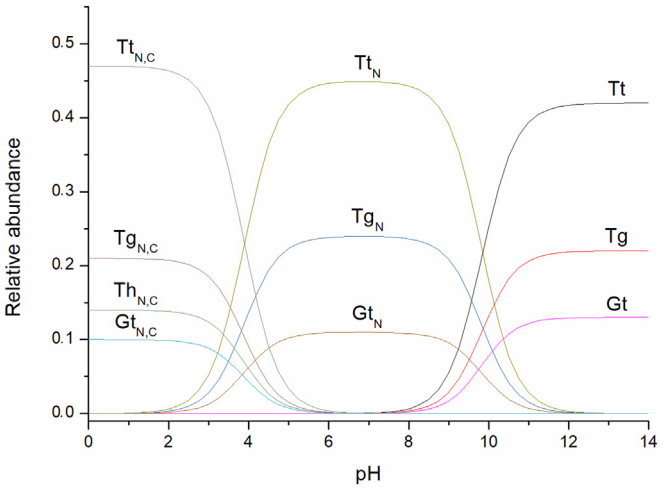

Fig. 6. Distribution of the dominant baclofen conformer species at different protonation states. The baclofen conformers that did not reach 0.1 relative abundance were omitted to improve clarity of the figure.

Table 7
Bulk partition coefficients of the investigated molecules at isoeletric pH.

Molecule	log*D*
Baclofen	−1.04 ± *0.03*
Pregabalin	−1.83 ± *0.01*

Tt to Tg or Gt to Gg), log*d* decreases by one unit. Rotations in the amino-containing axis cause smaller changes in the conformer-specific partition coefficients than those in the other axis. In a gauche to trans rotation of the axis pertaining to the carboxyl group the two polar groups in the molecule get closer. As it can be seen from Fig. 7 the differences in the log*d* values (e.g. the 2 log unit difference between the values of the Tg and Hh conformers) can be explained with the 3D structure of the conformers: in the Tg conformer the polar groups are far apart and exposed to the hydrogen bonding water molecules, while in the Hh conformer these groups are inbound and less exposed.

Pregabalin conformers of high ambiguity or insignificantly low value in octanol are rounded to zero. As a consequence, no real log*d* values were calculated for the Tg, Gt, Gg, Gh, Hg conformers. We can estimate, however, that their log*d* values are below −3. The conformers without any gauche state (Tt, Th, Ht, Hh) have the log*d* value near −1.5 which means that differences between the pregabalin log*d* values exceed those of baclofen. The conformers of gauche states – regardless of the axis – have very little affinity to the non-polar media. In the trans and hindered states, the amino and carboxylate groups are in proximity, while these two groups in gauche state get remote, minimizing the formation probability of hydrogen bonds.

The conformer-specific partition coefficients of pregabalin also indicate that conformers Tt, Th, Ht, Hh can well be assumed to be the favorable influx species in passive membrane transport processes, whereas conformers with gauche state are preferred in membrane-outflow phenomena.

Table 9
Populations in octanol and partition coefficients of the pregabalin conformers.

Conformer	Tt	Tg	Th	Gt	Gg	Gh	Ht	Hg	Hh
Pregabalin									
Population in octanol	45%	0%	26%	0%	0%	0%	20%	0%	12%
sd	*4%*	*2%*	*4%*	*2%*	*1%*	*1%*	*3%*	*1%*	*2%*
log*d*	−1.43	–	−1.56	–	–	–	−1.65	–	−1.78
sd	*0.07*	–	*0.10*	–	–	–	*0.11*	–	*0.13*

4. Conclusions

The conformation populations of two CNS drugs were determined along two rotational axes. The NMR-based method and mathematical formulation can be extended to other compounds, even with larger number of rotational axes, since the conformational populations are a direct result of the combinatoric product of rotamer populations along each axis. With the knowledge of conformer-specific basicity and lipophilicity values the pharmacokinetic characteristics of pregabalin and baclofen are better understood. The transfer of these methods to other compounds can lead to improved rational drug design.

Table 8
Populations in octanol and partition coefficients of the baclofen conformers.

Conformer	Tt	Tg	Th	Gt	Gg	Gh	Ht	Hg	Hh
Baclofen									
Population in octanol	41%	2%	13%	22%	0.9%	7%	11%	0.4%	3%
sd	*3%*	*2%*	*3%*	*2%*	*0.9%*	*1%*	*3%*	*0.4%*	*1%*
log*d*	−1.08	−2.18	−0.79	−0.75	−1.85	−0.46	−0.44	−1.53	−0.14
sd	*0.06*	*0.42*	*0.22*	*0.09*	*0.42*	*0.24*	*0.49*	*0.64*	*0.54*

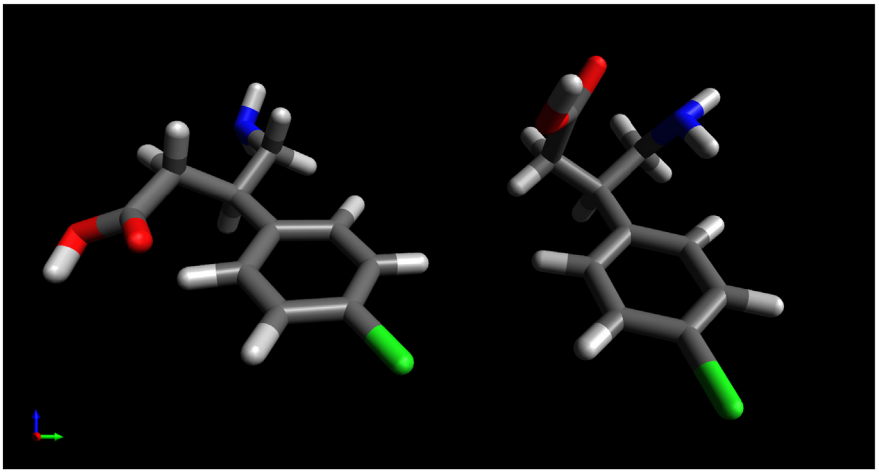

Fig. 7. The 3D structures of baclofen Tg (left) and Hh (right) conformers created with Avogadro (https://avogadro.cc/).

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