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Characterization of potential NMDA and cholecystokinin antagonists I. Acid–base properties of 2-methyl-4-oxo-3H-quinazoline-3-alkyl-carboxylic acids at the molecular and submolecular levels

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Abstract

The protonation macroconstants (log K) of 4(3H)-quinazolone (**1**) and two 2-methyl-4-oxo-3H-alkyl-quinazoline-3-carboxylic acid derivatives (**2**,**3**) were determined by pH-potentiometry. The acid–base chemistry of compounds **2** and **3**, where proton-bindings take place in an overlapping fashion, was described in terms of protonation microconstants as well. Microspeciation was carried out by two means: UV–pH titration (selective, pH-dependent monitoring of the N_1-binding site), and deductively (using a derivative compound as covalently fixed model of one of the protonation isomers). The microconstant values obtained by the two different methods are in agreement within 0.05 log K units. Microspeciation revealed remarkable differences between the two homologue compounds (**2** and **3**). The microconstant values show that insertion of a second methylene moiety into the aliphatic acid side-chain (1) increases the electron-density and most basicity parameters of both functional groups; (2) significantly modifies the extent of site–site interactions in the molecule; (3) opens new conformational preferences by N_1 ring nitrogen–carboxylic group intramolecular hydrogen bond formation and (4) reverses the predominantly zwitterion-involved protonation pathway into a neutral form-involved pathway. These molecules exemplify that microconstant values allow the comparative prediction and quantitative evaluation of pharmacokinetic behaviour, and signify the fact that microspeciation is a powerful tool in the process of drug development.

Keywords: Quinazolones; Protonation macro- and microconstants; Microspeciation

1. Introduction

Acid–base properties, lipophilicity and conformational preferences are the major molecular characteristics that influence the viability of drug molecules in the body. The generally accepted

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pH-partition theory postulates that the non-ionic (zero-charged, lipophilic) form of a drug is preferred to penetrate the membranes by passive diffusion, whereas the receptor-binding form is predominantly the ionized species, in an appropriate conformation (Block, 1991). The thorough understanding of proton-donor and -acceptor properties is therefore of great importance in drug research. The acidity/basicity of monovalent compounds can be quantitated in terms of the classical, macroscopic $pK_a/\log K$ parameters (macroconstants). Macroconstants in the case of multiprotic compounds, however, characterize the basicity of the molecule as a whole. They refer to the stoichiometric composition of the species, but they fail to provide information on the specific proton-binding sites. Site-specific, submolecular basicities can be obtained when microconstants are determined. The related process, microspeciation (Noszál, 1986) yields, for example, pH-dependent distribution diagram for all the microspecies, including the protonation isomers, such as the zwitterionic and neutral forms of amphoteric compounds. The macroscopic and microscopic basicities provide authentic information on propensities of intermolecular interactions both in the pharmacokinetic and pharmacodynamic stages. These parameters are therefore powerful tools of increasing recognition in drug design (Dearden, 1990).

A general scheme for the protonation/deprotonation pathways of an amphoteric, diprotic molecule, with the relevant macroscopic and microscopic protonation constants are shown in Fig. 1.

The micro- and macroconstants in terms of species concentration are listed in Eqs. (1)–(6):

$$k^{\mathrm{N}} = \frac{[XH^{\pm}]}{[X^{-}][\mathrm{H}^{+}]} \tag{1}$$

$$k^{\mathrm{C}} = \frac{[XH^{0}]}{[X^{-}][H^{+}]} \tag{2}$$

$$k_{\mathrm{N}}^{\mathrm{C}} = \frac{[XH_2^{+}]}{[XH^{\pm}][H^{+}]} \tag{3}$$

$$k_{\mathrm{C}}^{\mathrm{N}} = \frac{[XH_2^{+}]}{[XH^{0}][H^{+}]} \tag{4}$$

$$K_1 = \frac{[XH]}{[X^{-}][H^{+}]} \tag{5}$$

$$K_2 = \frac{[XH_2^{+}]}{[XH][H^{+}]} \tag{6}$$

where k^{N} and $k_{\mathrm{C}}^{\mathrm{N}}$ are microconstants of an amino site, k^{C} and k_{N}^{C} are those of a carboxylate, K_1 and K_2 are stepwise macroconstants. The relationships between macro- and microconstants are as follows:

$$\beta_1 = K_1 = k^{\mathrm{N}} + k^{\mathrm{C}} \tag{7}$$

$$\beta_2 = K_1\,K_2 = k^{\mathrm{N}}\,k_{\mathrm{N}}^{\mathrm{C}} = k^{\mathrm{C}}\,k_{\mathrm{C}}^{\mathrm{N}} \tag{8}$$

A derivative of Eqs. (1) and (4), as well as Eqs. (2) and (3) is $\Delta \log k_{\mathrm{N-C}}$, the interactivity parameter:

$$\Delta \log k_{\mathrm{N-C}} = \log k^{\mathrm{N}} - \log k_{\mathrm{C}}^{\mathrm{N}} = \log k^{\mathrm{C}} - \log k_{\mathrm{N}}^{\mathrm{C}} \tag{9}$$

The general aspects and theoretical treatment of microspeciation were reviewed by Noszál (1990). The determination of microconstants needs at least two experimental approaches. pH-potentiometry is the ubiquitous one, providing information on the total number of bound protons to the molecule. The second method is typically a molecular spectroscopy (NMR, UV, CD, etc.) that can selectively monitor the pH-dependent protonation stage of one (or some) of the basic groups. The

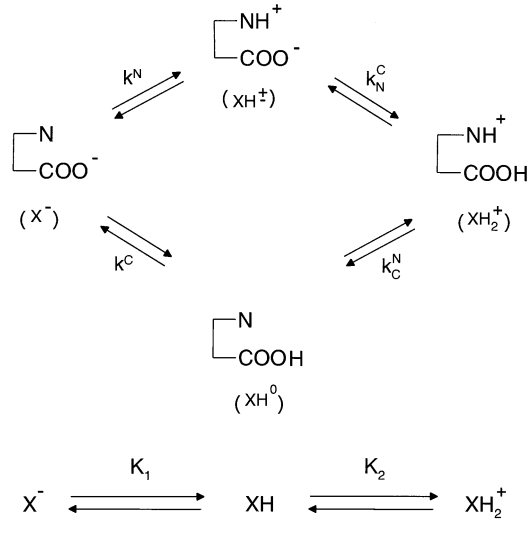


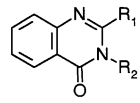
Fig. 1. The protonation/deprotonation macro- and microequilibria of a diprotic ampholyte molecule.

experimental data of the two techniques establish an appropriate system of equations that allows the determination of microconstants for molecules of up to three groups. Such determinations have been made for example for cysteine (Benesch and Benesch, 1955), DOPA (Kiss et al., 1989), oxytocin and vasopressin (Noszál et al., 1992) and fluoroquinolones (Takács-Novák et al., 1990).

When proton-binding of the basic groups takes place in non-overlapping pH range, or site-specific monitoring of protonation is not feasible, deductive methods must be used for the determination of microconstants. These methods include determination of macroconstants for the parent compound, and also, for its derivative of reduced number of functional groups. The related classical work of Ebert (1926) determined the macroconstants of glycine and glycine-methyl ester. In that study the electron-withdrawing effect of the ester group was regarded as identical to that of the protonated carboxylate. Thus, the glycine-methyl ester protonation constant could be introduced into the glycine microequilibrium scheme as k_C^N, the amino basicity of the minor protonation pathway. The analogous principle was used for a number of bio- and drug molecules, such as tetracyclines (Martin, 1985), aspartic acid (Noszál and Sándor, 1989), arginine (Noszál and Kassai-Tánczos, 1991) and piroxicam (Takács-Novák, et al., 1995a).

Here we report the macroscopic and microscopic acid–base characterization of 4(3H)-quinazolone (**1**) and three 2-methyl-4-oxo-3H-quinazoline-3-alkyl-carboxylic acid derivatives **2**–**4** (Fig. 2.)These molecules are synthetic products of a project to develop new, potential NMDA and cholecystokinin antagonist compounds. 4(3H)-Quinazolones cover a wide pharmacological profile and have been studied in numerous works of several aspects. Only a few of them can be cited below.

The relationship between CNS depressant and MAO inhibitory effects of substituted quinazolone 1,3,4-oxadiazoles was investigated by Barthwal et al. (1973). New, 2-substituted quinazolone-3-carboxylic acid derivatives as potential anticonvulsants were prepared by Husain and Singh (1982), and some quinazolone-3-acetic and



1. 4(3H)-quinazolone: R_1= H, R_2= H
2. 2-methyl-4-oxo-3H-quinazoline-3-acetic acid: R_1= -CH_3, R_2= -CH_2-COOH
3. 2-methyl-4-oxo-3H-quinazoline-3-propionic acid: R_1= -CH_3, R_2= -$(CH_2)_2$-COOH
4. methyl-2-methyl-4-oxo-3H-quinazoline-3-acetate: R_1= -CH_3, R_2= -CH_2-$COOCH_3$

Fig. 2. Structure of quinazolone-3-alkyl-carboxylic acid derivatives.

propionic acid derivatives were found to have antiallergic properties (LeMahieu et al., 1983). Substituted quinazolone derivatives were prepared and analyzed by X-ray crystallography as cholecystokinin/gastrin receptor ligands (Yu et al., 1992). Ester derivatives of 2-methyl-4-oxo-3H-quinazoline-3-acetic acid were synthesized and evaluated for H_1-antihistaminic activity (Rao and Reddy, 1993).

Finding new NMDA and cholecystokinin antagonists of quinazolone structure was an objective of our project as well. Synthetic aspects of the new 35 compounds, results of the pharmacological screening and lipophilicity studies appear elsewhere (Almási et al., in progress)

This paper focuses on the basicity parameters at the molecular and submolecular level. The macroscopic and microscopic basicity data of selected compounds are interpreted in terms of intramolecular electronic and conformational effects, and will also be considered as predictors of pharmacokinetic behaviour.

2. Materials and methods

2.1. Materials

The method of LeMahieu et al. (1983) was used for the synthesis of 4(3H)-quinazolone. 2-Methyl-quinazolone-3-acetic and propionic acids were prepared by the method of Errede and McBrady

(1978). The synthesis of 2-methyl-quinazolone-3-acetic acid methyl ester was carried out according to Barthwal et al. (1973). Melting points were determined on a Boetius hot plate and are uncorrected. Spectral (IR, NMR, UV) and X-ray crystallographic data were obtained for the new compounds and were in agreement with the assigned structures. The purity was controlled by TLC (normal and reverse phases). All reagents were of analytical grade.

2.2. Methods

2.2.1. Potentiometric determination of protonation macroconstans

The details of the instrument used (PCA 101; Sirius, Forest Row, UK) for potentiometric log K determination were described earlier (Avdeef, 1992). Typically, 10 ml of 0.5–10 mM solutions of the samples were pre-acidified to pH 1.8–2.0 with 0.5 M HCl, and were then titrated alkalimetrically with 0.5 M NaOH titrant to some appropriate high pH (maximum 12.0). In the case of 2-methyl-4-oxo-3H-quinazoline-3-propionic acid, which is sparingly soluble in water, 1 ml of 0.5 M NaOH was added before dissolving the substance, then the alkaline solution of pH 10 was titrated with standardized 0.5 M HCl to pH 2. The titrations were carried out at 25 ± 0.1°C, at constant ionic strength ($I = 0.1$ M, NaCl) and under an inert gas atmosphere. The initial estimates of log K values were obtained from Bjerrum difference plots ($\tilde{n}_H$ vs. pH) and then were refined by a weighted non-linear least-squares procedure (Avdeef, 1993). For each molecule, five separate titrations were performed and the average log K values and their standard deviations were calculated.

The four-parameter procedure for electrode standardization was performed in the recommended non-standard way, namely, by titrating 3 mM K_2HPO_4 from pH 1.8 to 12.2 with 0.5 M NaOH titrant. This procedure must be applied with the PCA instrument when determining pK_a values lower than 3 (or higher than 11) and using low sample concentration. Further details can be found elsewhere (Applications and Theory Guide to pH-metric pK_a and log P determination, Sirius Analytical Instruments Ltd., 1993).

2.2.2. UV spectroscopic determination of protonation macroconstant of compound 4

The methyl ester of 2-methyl-quinazolone-3-acetic acid is sparingly soluble in water. A 0.8 mM solution, the minimum concentration for pH-potentiometric log K determination could not be prepared. Due to intense UV activity, the standard UV–pH titration method (Albert and Serjeant, 1984) could be applied in 0.1 mM concentration.

2.3. Determination of protonation microconstants by combination of pH-potentiometry and UV–pH titrations

Fig. 3(a) shows the pH-dependence of the UV spectra of compound **2**. The isosbestic points indicate that two spectrally distinct species exist in the solution only: the N_1-protonated and the N_1 basic forms. In other words: the protonation state of the carboxylate site does not influence the spectrum. Otherwise no isosbestic points could be found. As the N_1 protonation selectively influences the spectral absorption, the fraction of N_1-protonated species (α_{NH^+}) can be written as:

$$\alpha_{NH+(pH)} = \frac{A_{pH} - A_N}{A_{NH^+} - A_N} = \frac{[XH^{\pm}] + [XH_2^+]}{[X^-] + [XH^0] + [XH^{\pm}] + [XH_2^+]} \quad (10)$$

where A_N and A_{NH^+} are absorptions in solutions where N_1 is practically completely unprotonated and protonated, respectively, and A_{pH} is absorption of a solution of known pH where the N_1 protonation is partial. Introducing Eqs. (1)–(6) into Eq. (10) yields:

$$\alpha_{NH+(pH)} = \frac{k^N[H^+] + \beta_2[H^+]^2}{1 + \beta_1[H^+] + \beta_2[H^+]^2} \quad (11)$$

Concerning parameters in Eq. (11), β_1 and β_2 macroconstants can be determined by pH-potentiometry, $\alpha_{NH+(pH)}$ is an experimental data of UV–pH titrations. Thus, k^N microconstant (or any other microconstant, depending on the definition and rearrangement of (10) and (11)-type equations) can be calculated. Once k^N is known, k^C, k_C^N and k_N^C can be calculated from Eqs. (7) and (8).

In order to obtain $\alpha_{(NH^+)}$ values of maximum accuracy, two aliquots of 2×10^{-4} M solution of compounds **2** or **3** were prepared in either 0.1 M HCl or 0.001 M NaOH. The ionic strength was 0.1 M in both solutions adjusted by NaCl. Mixing these acidic and basic solutions in various portions, results in solutions of various, intermadiate pH, and always identical (2×10^{-4} M) analyte total concentration. Their spectra were recorded on a Hewlett-Packard 8452A spectrophotometer, and treated as described above.

The average of $\log k^N$ values was calculated from spectroscopic data obtained at eight different pH values in three parallel measurements (a total of 24 points). The other microconstants were calculated using Eqs. (7) and (8).

2.3.1. Determination of protonation microconstants by deductive method

The methyl ester derivative was used in the indirect analogue approach. The macroscopic $\log K$ of compound **4** was considered and handled as the $\log k_C^N$ microconstant of compound **2**. The arguments are detailed in the following section.

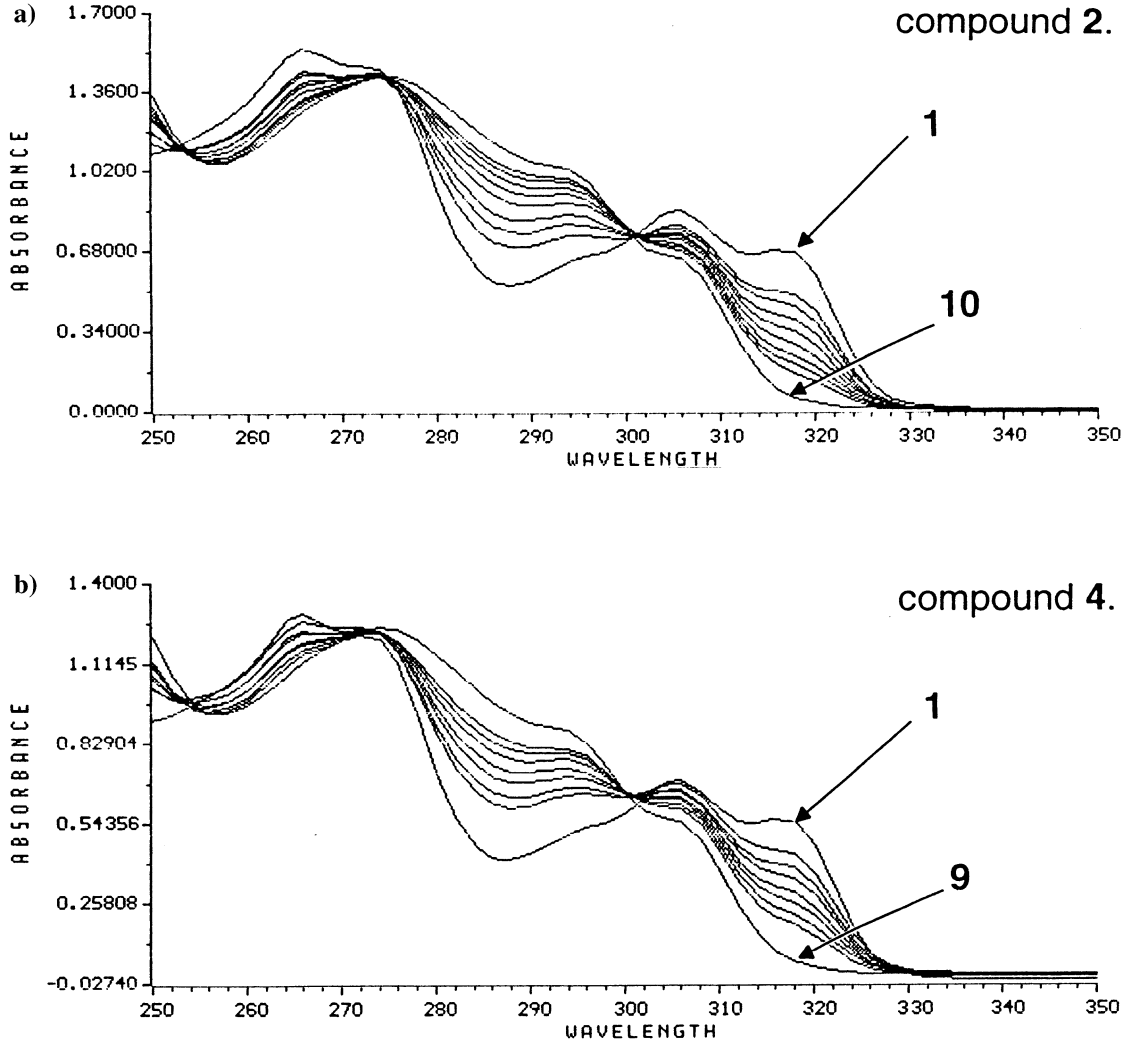


Fig. 3. The pH-dependence of spectra of compounds **2** and **3**.

Table 1
Protonation macroconstants of 4-oxo-3H-quinazoline-3-alkyl-carboxylic acid derivatives

Compound	Log $K_1 \pm \sigma$	Log $K_2 \pm \sigma$
1. 4(3H)-Quinazolone	9.62 ± 0.00	2.29 ± 0.01
2. 2-Methyl-4-oxo-(3H)-quinazoline-3-acetic acid	3.30 ± 0.01	2.25 ± 0.04
3. 2-Methyl-4-oxo-(3H)-quinazoline-3-propionic acid	3.93 ± 0.01	2.45 ± 0.02
4. Methyl-2-methyl-4-oxo-(3H)-quinazoline-3-acetate	–	2.79 ± 0.09[a]

[a] Log K value of N_1 obtained by UV spectroscopy.

2.3.2. Quantum chemical calculations

The starting geometries of the uncharged (XH^0) form of compounds **2** and **3** were obtained using standard bond lengths and angles. They were subsequently optimized with the molecular mechanical MM + method and then the semiempirical AM1 method using the PC SPARTAN molecular modeling software package on a Pentium (Intel, 90 MHz) PC.

3. Results and discussion

Table 1 contains the stepwise protonation macroconstants determined by pH-potentiometry (compounds **1–3**) or UV spectroscopy (compound **4**). Precision of the data are reflected by the standard deviations (Table 1).

The data show remarkable differences in the acid–base properties of the molecules. The more than seven log K unit difference between log K_1 and log K_2 for 4(3H)-quinazolone (**1**) is apparently due to the different character of the N_1 and N_3 (lactame) nitrogens. Beside their obvious, different electron densities, the large $\log K_1 - \log K_2 = 7.33$ value is also a consequence of the close proximity of the N_1 and N_3 nitrogens, that makes their interactions intense. Once one of them protonates, basicity of the other one dramatically drops, which is the case here. The macroconstant values and their comparison with the N_3 carboxylic acid derivatives clearly indicate that this molecule predominantly exists in its neutral form in the 6 ± 3 pH range, as ordinary ampholytes do (Albert and Serjeant, 1984).

The ionization properties of the quinazolone derivatives undergo significant changes when the N_3 hydrogen is substituted by an alkyl-carboxylic acid moiety. The $\log K_1 - \log K_2$ differences of compound **2** and **3** are much smaller than that of compound **1**. This indicates that acid–base equilibria of compound **2** and **3** can be site-specifically described, in terms of microconstants only. Fig. 3 shows the characteristically identical spectral behaviour of 2-methyl-quinazolone-3-acetic acid and its methyl ester derivative. Also, the isosbestic points in Fig. 3(a) verify that the UV–pH titration selectively monitors the N_1 protonation. Thus, the evaluation method described in the experimental section can be used.

The microconstants of compound **2** were also determined by the deductive method. The high spectral resemblance of compound **2** and its methyl ester derivative (**4**) (Fig. 3) proves that the methyl ester is similar to the parent compound in electronic, steric, and conformational aspects. A significant difference is, however, the lack of dissociation property of the carboxylic moiety. Thus, the use of methyl ester as model of the XH^0 and XH_2^+ microspecies, justifies the use of the deductive method. Accordingly, log K of compound **4** was introduced into the microspeciation scheme of compound **2** as $\log k_C^N$, that furnished also the basis to the calculation of all the respective microconstants. The microconstants obtained by UV–

Table 2
Protonation microconstants of 4-oxo-3H-quinazoline-3-alkyl-carboxylic acid derivatives

Compound	Log k^N	Log k^C	Log k_N^C	Log k_C^N	Δ Log k	Method
2.	3.17 ± 0.02	2.72	2.39	2.83	0.34	UV spectroscopy
	3.16	2.76	2.39	2.79 ± 0.09	0.37	Deductive
3.	3.49 ± 0.09	3.73	2.89	2.65	0.84	UV spectroscopy

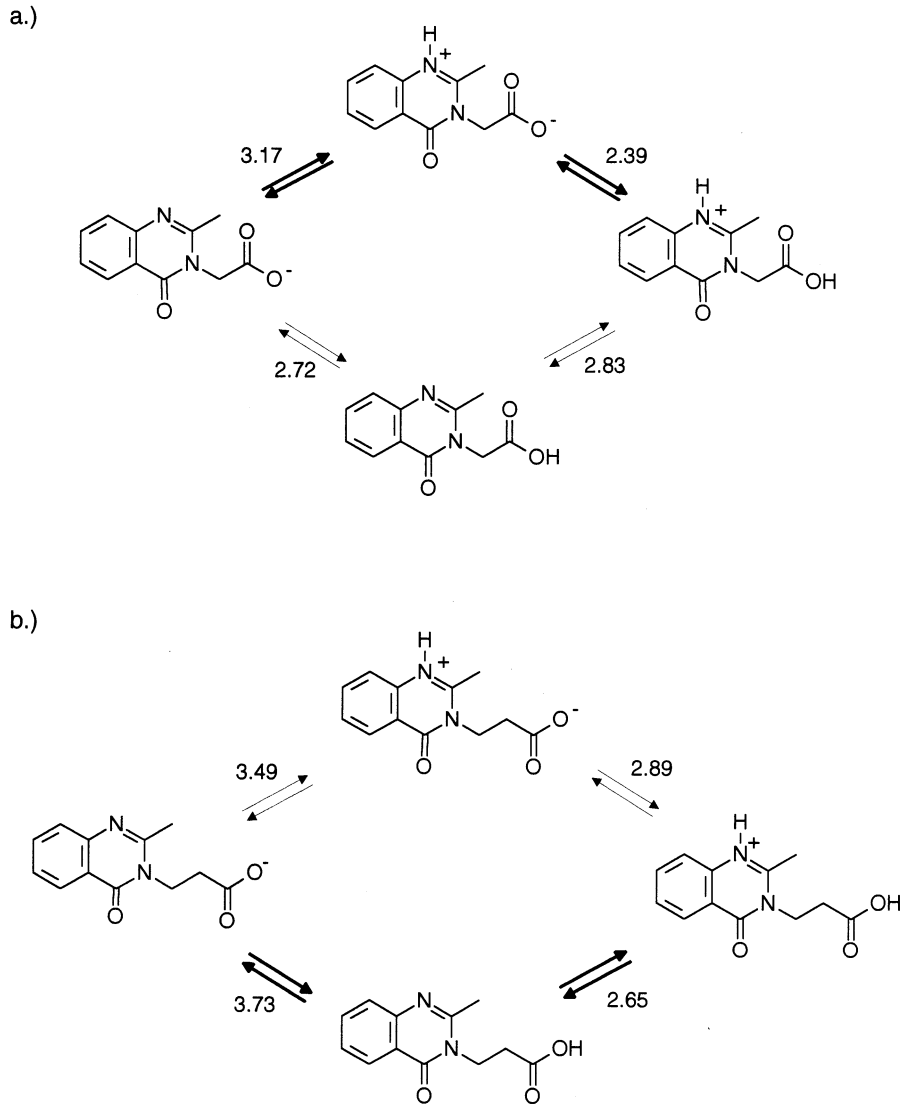


Fig. 4. The protonation/deprotonation microequilibria of compounds **2** and **3**.

pH titrations and deductively are in excellent agreement (Table 2). Microconstants, their standard deviations and the interactivity parameters are listed in Table 2.

The protonation pathways and the pertinent microconstant values are depicted in a visual manner for compounds **2** and **3** in Fig. 4.

The pertinent conclusions are as follows.

(1) Insertion of an extra methylene group into the N_3-aliphatic acid moiety, increases the electron-density of the N_1-site and the carboxylate site, as manifested by the k^C, k_N^C, k_N microconstant values. This increase is understandably more enhanced at the carboxylate site, which is in close proximity to the electron-sending methylene group. The apparent contradiction that k_C^N values do not reflect this phenomenon, can be eliminated when interactivity parameters and conformational preferences are taken into account below.

(2) Proton-binding at one site decreases the basicity of the other site, as expected. The $k^N/k_C^N = k^C/k_N^C$ values are 2.2 and 6.9 for compounds **2** and **3**, respectively. In other words, the carboxylate basicity diminishes by a factor of 2.2 when the N_1 site protonates, and vice versa. The analogous decreasing factor in compound **3** is 6.9. On the basis of the number of chemical bonds between the N_1 and carboxylate sites, the opposite interactivity order would be reasonable. It indicates that the methylene insertion (a) enhances the electron-densities of the basic sites by through-bond interactions (great increase in k^N and k^C), and (b) significantly modifies the intramolecular, through-space interactions and the concomitant proton-accessibility of the sites (moderate increase in k_N^C, decrease in k_C^N and large interactivity parameter).

In order to interpret the remarkably enhanced interaction in compound **3**, through-space connectivities had to be hypothesized. This assumption was supported by theoretical calculations. Fig. 5 shows the optimized 3D structures of the neutral form of **2** and **3** in two conformer forms. The more flexible side chain of **3** assumes favourable interactions between the N_1 atom and the carboxylic group through the space. The formation of direct intramolecular N_1–HOOC H-bond (2.27 Å) or the inclusion of a water molecule may further stabilize this conformation in compound **3** (Fig. 5).

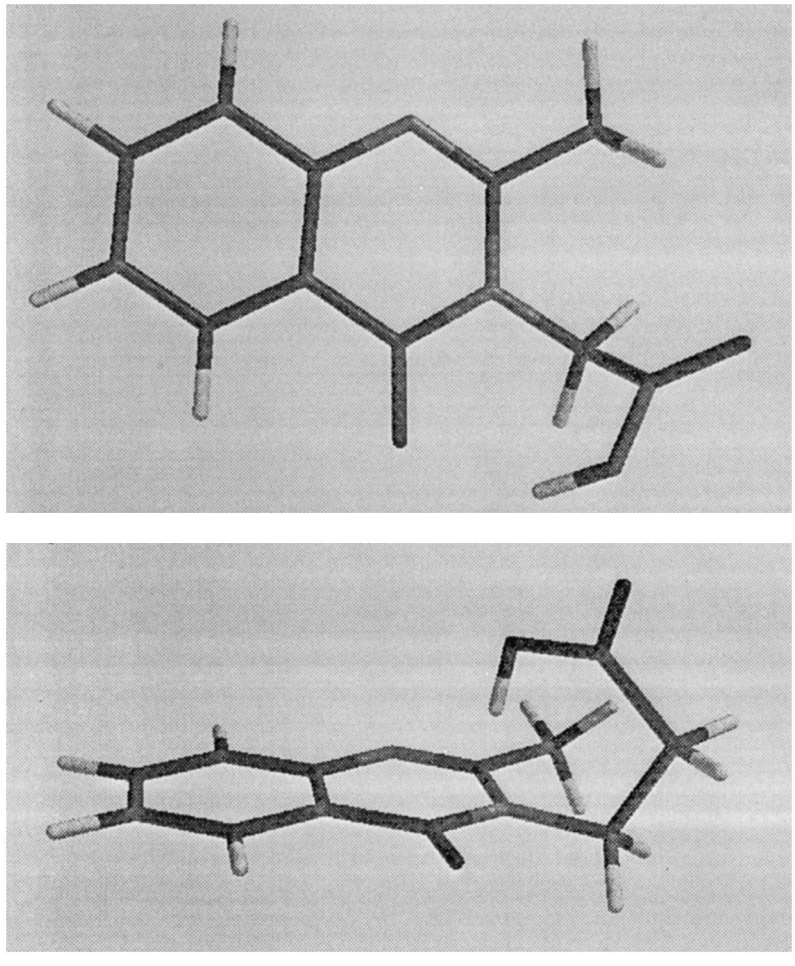


Fig. 5. Optimized conformations of compounds **2** and **3**.

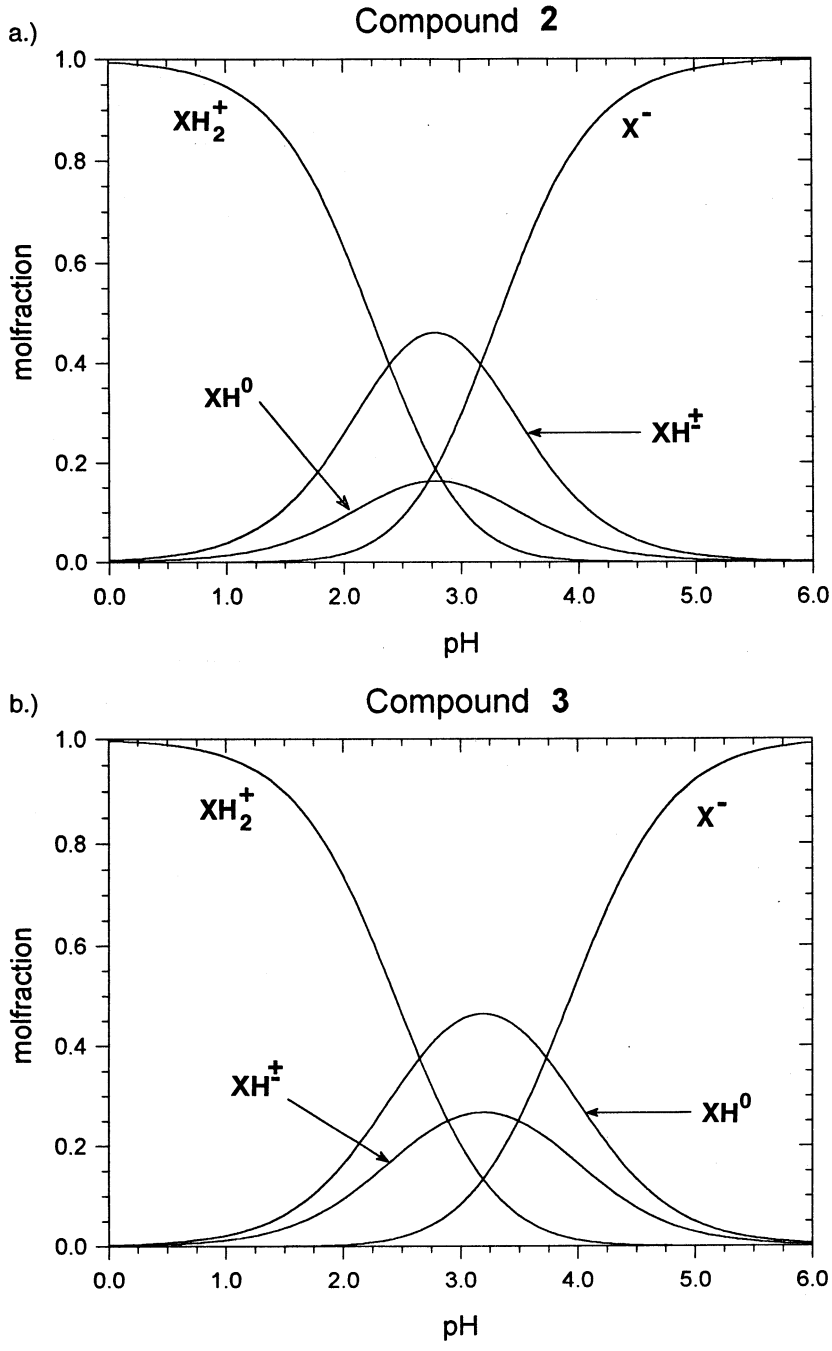


Fig. 6. Distribution of microspecies of compounds **2** and **3**.

(3) The fact that $k^N > k^C$ for compound **2**, but $k^C > k^N$ for compound **3**, suggests that the major protonation pathway is anion → zwitterion → cation for compound **2**, whereas the pathway is anion → neutral species → cation for compound **3**. The corresponding pH-dependent relative concen-

trations and distribution curves are shown in Fig. 6.

These distribution curves indicate two reasons why the bioavailability of compound **3** is presumably higher than that of compound **2**. Firstly, the isoelectric point of **3** belongs to higher pH. Thus, in neutral medium, such as blood, a relatively higher proportion of **3** exists in membrane-penetrable form (Nevertheless, the anionic form near pH 7 highly predominates). Secondly, the $[XH^0]/[XH^{\pm}]$ species ratio is 0.35 and 1.74 for compound **2** and **3**, respectively. The exclusive partition of the neutral microspecies from water into octanol has recently been reported (Takács-Novák et al., 1995b). In view of this, the above parameters predict compound **3** has a 5-fold better chance for partition into the lipophilic phase.

4. Conclusion

Potential NMDA and cholecystokinin antagonist 2-methyl-quinazolone-3-carboxylic acid derivatives show many-sided acid–base properties at the molecular and submolecular levels. Elongation of the aliphatic carboxylic acid side-chain (a) has significantly modified the electron-density of the ring system, (including its basic site) and especially the carboxylate functional group; (b) has brought about intramolecular interactions (presumably via H-bond formation) that has promoted the anion → neutral species → cation alternative protonation pathway to the predominant one, and XH^0, the neutral microspecies, is the major protonation isomer.

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