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Physicochemical properties of the fluoroquinolone antimicrobials. II. Acid ionization constants and their relationship to structure

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Summary

The quinolone antimicrobials contain one acidic and one basic functional group and at physiological pH they exist as a mixture of the neutral and zwitterionic forms. The ratio of the neutral to zwitterionic species for a given compound is important because it is likely to determine the distribution properties of the drug in vivo. The present study was conducted to determine the relationship between the dissociation constants of the quinolones and their chemical structures. Regression analyses indicated that the apparent pK_a value associated with the carboxylic acid function was influenced by the number of fluorines in the molecule, while the pK_a value associated with the piperazinyl nitrogen was influenced mainly by the presence of an *N*-methyl substituent.

In a recent paper, Ross and Riley (1990) reported the dissociation constants of nine fluoroquinolone antimicrobials. However, very little attempt was made to relate the dissociation constants to structure. Such quantitative relationships may prove useful in drug-design studies and in the explanation of the biopharmaceutical properties of the fluoroquinolones because all the compounds in this series are zwitterionic with a p*I* value of 6.8–7.8. Therefore, at physiological pH the dominant Bjerrum species are the zwitterion ($HQ^{\pm}$) and the neutral form (HQ^0) of the drug. This may be important because small differences in dissociation constant will make large differences in the total fraction of the zwitterion and the neutral species present at physiological pH. Furthermore, the difference in the pK_a for a given compound will determine the ratio of the zwitterion to the neutral species at any pH. The zwitterionic species and the neutral species are likely to have different distribution properties and therefore to fully understand and interpret differences in bioavailability and the structure-activity relationships of these compounds, it is necessary to consider their microscopic dissociations (Scheme 1).

The proton-binding ability of an individual functional group is described by the microscopic

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dissociation constants (k_{11}, k_{12}, k_{21}, and k_{22}), which are related to the macroscopic constants by Eqns 1 and 2:

$$K_1 = k_{11} + k_{21} \quad (1)$$

$$1/K_2 = 1/k_{12} + 1/k_{22} \quad (2)$$

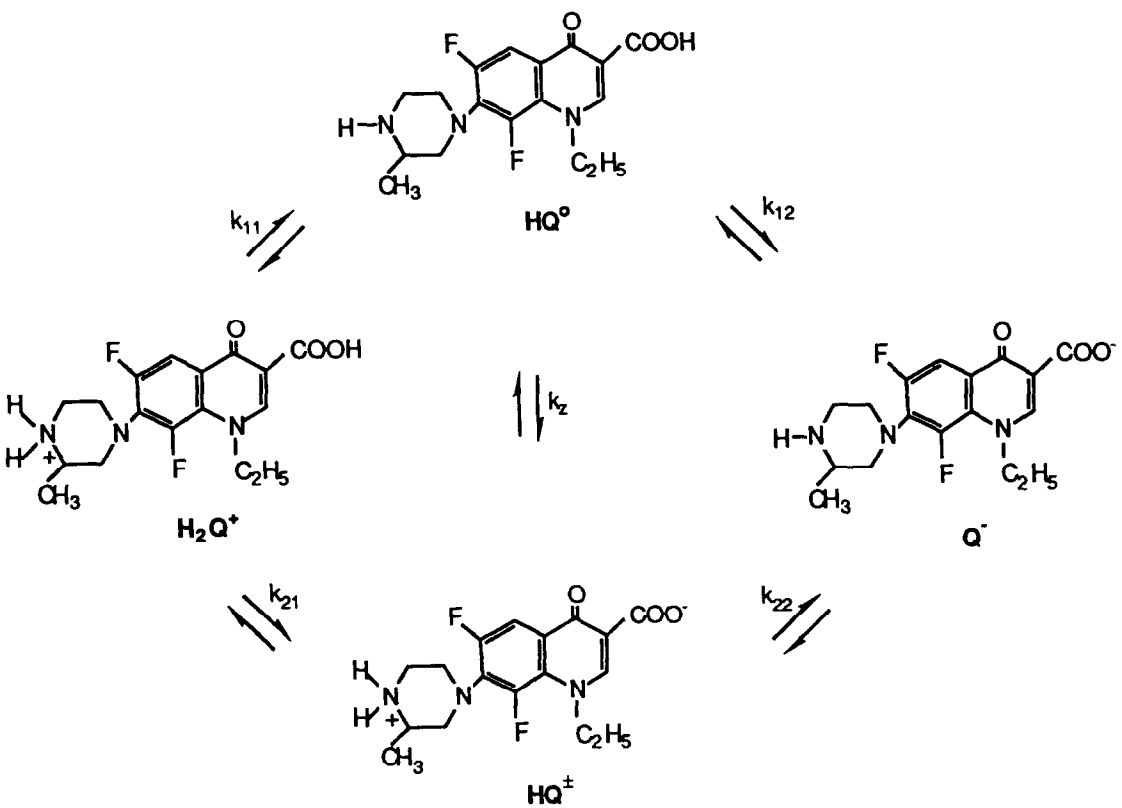
Since the ionizable groups on the fluoroquinolones are spatially separated, it is reasonable to assume that the charge on one functional group will not significantly affect the dissociation constant of the other ionizable function. Evidence for this assumption was obtained from the pK_a value of nalidixic acid, which contains only the carboxylic acid group. Therefore, it may be stated that $k_{11} = k_{22}$ and $k_{21} = k_{12}$, making it possible to solve for the values of k_{11} and k_{12} through rearrangement of Eqns 1 and 2:

$$k_{12}^2 - K_1 k_{12} + K_1 K_2 = 0 \quad (3)$$

The value for k_{12} can be determined using the macroscopic dissociation constants and solving the quadratic Eqn 3. The value for k_{11} can then be calculated from the relationship:

$$k_{11} = \frac{K_1 K_2}{k_{12}} \quad (4)$$

The resulting values for k_{11} and k_{12} are equivalent to the macroscopic K_2 and K_1 values, respectively, since the k_{11} value describes the dissociation of the carboxylic acid proton. The



Scheme 1.

TABLE 1

Microscopic dissociation constants of the quinolones studied

Compound	$pk_{11}(pk_{22}) \equiv pK_2$	$pk_{12}(pk_{21}) \equiv pK_1$	$[HQ^{\pm}]/[HQ^0]$
Amifloxacin	7.35 [a]	6.32	11
	5.7 (7.3) [b]	7.2 (5.8)	1.35
Ciprofloxacin	8.74 [a]	6.09	444
Difloxacin	7.62 [a]	6.07	35
Enoxacin	8.69 [a]	6.31	238
Fleroxacin	8.10 [a]	5.46	435
Lomefloxacin	9.3 [a]	5.82	3018
	5.7 (8.6) [b]	8.3 (6.0)	2.24
Norfloxacin	8.38 [a]	6.30	118
	6.3 (8.5) [b]	7.6 (7.2)	7.64
Ofloxacin	8.22 [a]	6.05	146
Temafloxacin	8.75 [a]	5.61	1378

[a] This work.

[b] Values reported by Takacs-Novak and co-workers (1990). Note that these authors have different values for pk_{11} and pk_{22} (in parentheses) and for pk_{12} and pK_{21} (in parentheses).

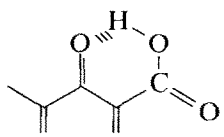
fraction of zwitterionic species and neutral species can then be calculated from the following relationships:

$$f_{HQ^0} = \frac{k_{11}[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (5)$$

$$f_{HQ^\pm} = \frac{k_{12}[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (6)$$

The values estimated for k_{11} (and k_{22}) and k_{12} (and k_{21}) and the ratio of zwitterion/neutral molecule ($f_{HQ^\pm}/f_{HQ^0} = k_{12}/k_{11}$) for each of the quinolones studied are listed in Table 1 along with microscopic dissociation constant values determined by Takacs-Novak et al. (1990) for comparison. It should be noted that in order to obtain these values, Takacs-Novak et al. (1990) assumed that there was no spectral shift associated with the protonation of the piperazinyl function. They were actually determining the macroscopic dissociation constant associated with the piperazinyl function when they were attempting to determine the microscopic constant associated with the neutral species at equilibrium with the anion. It should be noted that the microscopic constants determined by Takacs-Novak et al. (1990) are similar to the values of the macroscopic constants associated with the other ionizable function. For the purposes of this paper, the macroscopic dissociation constants will be used to estimate the fractions of the zwitterionic and neutral species.

The apparent pK_a values associated with the carboxylic acid function for the compounds studied here ranged from 5.46 to 6.31. The values for pK_{a_1} were higher than what is generally observed with aromatic carboxylic acids such as benzoic acid which has a pK_a of 4.2. This decrease in acidity can be attributed to an intramolecular H-bond formation with the neighboring keto function resulting in stabilization of the protonated species:



The dissociation constants may also be affected by electron-donating or electron-withdrawing groups which stabilize or destabilize the charged species. The fluoroquinolones have one or more fluorines which are conjugated to the carboxylic acid functional group. Although the diversity in the pK_{a_1} values of the fluoroquinolones studied here was only 1 pH unit, it was very likely that the dissociation of the carboxylic acid function was affected by the presence of fluorine atoms. Those fluoroquinolones with only one fluorine substituent at the 6-position had pK_{a_1} values ranging from 6.05 to 6.31. Ofloxacin had the lowest pK_{a_1} of this group (6.05) due to the oxygen attached to C8, which exerts an inductive electron-withdrawing effect. Those compounds containing a second fluorine atom had lower pK_{a_1} values with the exception of difloxacin. The inductive effect of the additional fluorine was electron-withdrawing and therefore stabilized the carboxylic acid anion resulting in a decrease in the pK_{a_1}. The second fluorine of difloxacin and the third fluorine of temafloxacin did not seem to have much effect on

the carboxylic acid ionization probably because the electron-withdrawing effect was apparently attenuated by the distance from the ionization site. The pK_{a_1} values of lomefloxacin, fleroxacin, and temafloxacin were from 0.5 to 0.8 pH units lower those of enoxacin, amifloxacin, or norfloxacin due to the negative inductive effect of the second fluorine.

In the estimation of the microscopic dissociation constants, the assumption was made that the spatial separation of the ionizable groups on the fluoroquinolones resulted in the charge on one functional group not affecting the dissociation constant of the other ionizable function. This assumption appeared to be validated by the fact that nalidixic acid, which did not contain a piperazinyl moiety, had a pK_{a_1} value of 5.95 which was very comparable to the pK_{a_1} values determined for the fluoroquinolones. To further validate the assumption that the pK_{a_1} was not affected by the pK_{a_2} and to better understand the effect of structure on pK_{a_1}, multiple-regression analysis was performed. The general regression equation resulting from an analysis which correlated the effects of the number of fluorines (f) and the pK_{a_2} on the pK_{a_1} value was:

$$pK_{a_1} = -0.071(\pm 0.076)pK_{a_2} - 0.32(\pm 0.052)f + 7.1(\pm 0.63) \quad (7)$$

$$R^2 = 0.87, \quad p = 0.0021$$

where the numbers in parentheses represent the standard deviations of the coefficients, and the R^2 value is the square of the correlation coefficient, indicating the fraction of the variance of the dependent variable that is predicted by the regression model. In this case, the multiple-regression model (Eqn 7) accounted for 87% of the variance associated with the pK_{a_1} of the fluoroquinolones. The value of p (0.0021) indicated the probability that correlation would occur by chance sampling fluctuation. The smaller the value of p, the greater the probability that the correlation was due to the influence of the independent variable(s) on the dependent variable. A p value of less than 0.05 was considered significant in this

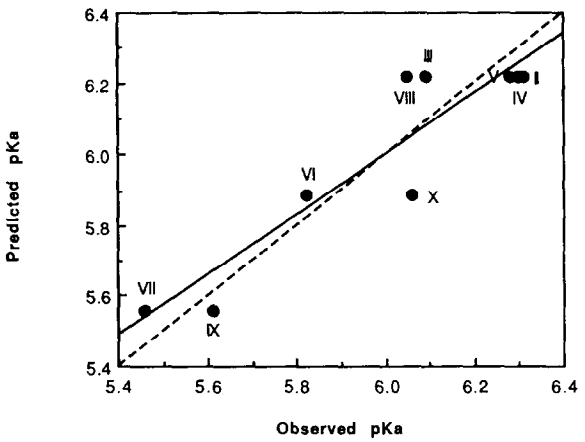


Fig. 1. Results of a simple regression analysis using the number of fluorines (f) to predict the carboxylic acid pK_a. The predicted pK_a was calculated from the regression equation: $pK_{a_1} = -0.33(f) + 6.5$. The regression line is shown by the solid line and fits the equation: $y = 0.86x + 0.87$. The coefficient of correlation (r) is 0.92. The dashed line represents the theoretical line of unit slope and zero intercept. Compounds: II, enoxacin; III, ciprofloxacin; IV, norfloxacin; V, amifloxacin; VI, lomefloxacin; VII, fleroxacin; VIII, ofloxacin; IX, temafloxacin; X, difloxacin.

study. Only the number of fluorines made a significant contribution to the regression model ($p = 0.0008$) which indicated that the pK_{a_2} ($p = 0.3865$) did not influence the variation in the pK_{a_1} in any predictable manner. This finding affirmed the validity of the assumption that the dissociation of the two ionizable functions are independent of one another. Because the pK_{a_2} value did not contribute significantly to the multiple regression model, a linear regression was performed using only the number of fluorines (f):

$$pK_{a_1} = -0.33(\pm 0.051)f + 6.5(\pm 0.095) \quad (8)$$

$$r^2 = 0.85, \quad p = 0.0004$$

Fig. 1 is the graphical representation of the observed pK_{a_1} vs the pK_{a_1} predicted using Eqn 8. The coefficient of correlation (r) was 0.924, which was very good since no consideration was given to the distance of the fluorines from the ionization site or to the oxygen at the 8-position of ofloxacin which also can lower the pK_a value through its electron-withdrawing effect. The fluo-

rine of difloxacin and temafloxacin which was in the *p*-position of the phenyl ring appeared to have its effect greatly attenuated by the large distance (nine bonds) from the ionization site. This accounted for the observed pK_{a_1} values for difloxacin and temafloxacin being higher than predicted by Eqn 8. The 8-position oxygen of ofloxacin would be expected to decrease the pK_a which accounted for the lower observed pK_a than predicted by the model.

Since the piperazinyl nitrogen was not in close proximity to any of the fluorines (at least seven-bond separation) and was not connected by a conjugated system, the fluorines would not be expected to have any effect on the pK_{a_2}. The only structural differences seen with this group of fluoroquinolones that would be expected to affect the pK_{a_2} was the presence of a methyl substituent on the piperazinyl nitrogen. The pK_{a_2} values of the tertiary amines (amifloxacin, difloxacin, fleroxacin, and ofloxacin) ranged from 7.39 to 8.22 (mean = 7.84), while the pK_{a_2} values of the secondary amines (ciprofloxacin, enoxacin, lomefloxacin, norfloxacin, and temafloxacin) ranged from 8.38 to 9.30 (mean = 8.77). These findings were consistent with reports in the literature for similar secondary and tertiary amines: piperazine ($pK_a = 9.73$) (Hetzer et al., 1968) and *N*-methylpiperazine ($pK_a = 9.09$) ($\Delta pK_a = 0.64$) (Enea et al., 1972); and piperidine ($pK_a = 11.12$) and *N*-methylpiperidine ($pK_a = 10.08$) ($\Delta pK_a = 1.04$) (Enea et al., 1972). The mean ΔpK_a of the fluoroquinolones for comparison of the secondary and tertiary amines was 0.93. This compares with a value of 0.64 for piperazine/*N*-methylpiperazine and 1.04 for piperidine/*N*-methylpiperidine. Nagy (1989) determined through theoretical calculations for a series of simple amines that the lower pK_a value for tertiary amines compared with secondary amines was due to different hydration states of the protonated secondary and tertiary amines. The protonated form of a secondary amine was stabilized by the greater number of water molecules involved in its hydration sphere compared with the corresponding tertiary amine.

To validate further the assumption that one ionizable group did not affect the dissociation of the other and to better understand the effect of structure on pK_{a_2}, multiple-regression analysis was performed. The general regression equation resulting from an analysis which correlated the presence (1) or absence (0) of an *N*-methyl substituent (m) and the pK_{a_1} on the pK_{a_2} value was:

$$pK_{a_2} = -0.98(\pm 0.21)m - 0.66(\pm 0.36)pK_{a_1} + 12.8(\pm 2.2) \quad (9)$$

$R^2 = 0.80, \; p = 0.0081$

Only the *N*-methyl substituent made a significant contribution to the regression model ($p = 0.0033$), indicating that the pK_{a_1} ($p = 0.11$) did not substantially influence the variation in the pK_{a_2} in any predictable manner. This finding reaffirmed the validity of the assumption that the dissociation of the two ionizable functions was independent of one another. Because the pK_{a_1} did not contribute significantly to the multiple-regression model, a simple regression was performed using only the presence (1) or absence (0) of the *N*-methyl substituent (m). The general regression equation resulting from the simple regression was:

$$pK_{a_2} = -0.94(\pm 0.24)m + 8.8(\pm 0.16) \quad (10)$$

$r^2 = 0.69, \; p = 0.0059$

Attempts to improve the model by inclusion of various other structural features including the presence or absence of a 3′-methylpiperazinyl substituent and the number of fluorines failed to reveal any other structural feature which contributed significantly to the variation in the pK_{a_2}.

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References

Enea, O., Houngbossa, K. and Berthon, G., Chaleurs de protonation de la pipérazine et de quelques-uns de ses dérivés. *Electrochim. Acta,* 17 (1972) 1585–1594.

Hetzer, H., Robinson, R. and Bates, R., Dissociation constants of piperazinium ion and related thermodynamic quantities from 0 to 50°. *J. Phys. Chem.,* 72 (1968) 2081–2086.

Nagy, P., Theoretical calculations on the basicity of amines. *J. Mol. Struct.,* 201 (1989) 271–276.

Ross, D. and Riley, C., Aqueous solubilities of some variously substituted quinolone antimicrobials. *Int. J. Pharm.,* 63 (1990) 237–250.

Takacs-Novak, K., Noszal, B., Hermeca, I., Kereszturi, G., Podany, B. and Szasz, G., Protonation equilibria of quinolone antibacterials. *J. Pharm. Sci.,* 79 (1990) 1023–1028.

Weast, R., *Handbook of Chemistry and Physics,* CRC Press Inc., Boca Raton, FL, 66th Edn, 1986, pp. D-159–D160.