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European Journal of Pharmaceutics and Biopharmaceutics 53 (2002) 317–325

European Journal of Pharmaceutics and Biopharmaceutics

www.elsevier.com/locate/ejphabio

Research paper

A novel approach to determining physicochemical and absorption properties of 6-fluoroquinolone derivatives: experimental assessment

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Received 10 May 2001; accepted in revised form 29 January 2002

Abstract

The ToSS MoDe approach is used to estimate the *n*-octanol/buffer partition coefficient, the apparent intestinal absorption rate constant and intestinal permeability from a 6-fluoroquinolone data set. Improved in silico methods for predicting a drug's ability to be transported across biological membranes and other biopharmaceutical properties is highly desirable to optimize new drug development. The physicochemical property (Log *P*) of 26 6-fluoroquinolone derivatives and the absorption properties (Log K_a and Log P_{eff}) of 21 derivatives were well described by the present approach. The models obtained confirm the important role of lipophilicity in the absorption process and its relation with the piperazinyl ring spectral moment and general local spectral moment. The normalized group contributions to each property, at the R_4 and R_5 positions of a 6-fluoroquinolone framework, were calculated. Principal factor analysis between these contributions and the Hammett and Hansch constant, molar refractivity and sterimol parameters was also carried out. Three principal factors explained 78% of the total variance and the correlation coefficients were higher than 0.98. The isocontribution zone analysis for the Log *P* and Log K_a of Sarafloxacin and Sparfloxacin, used as external corroboration compounds, was carried out. The absorption rate constants (in situ rat gut technique) for these drugs were also evaluated, and the results were compared with the values predicted by theoretical models for evaluating predictive performance. The present approach proved to be a good method for studying the oral absorption of drug candidates in drug development studies.

Keywords: ToSS MoDe approach; 6-Fluoroquinolone; Structure–property relationship; Sarafloxacin; Sparfloxacin; Physicochemical property; Absorption

1. Introduction

During the last decade a large number of novel antimicrobial agents have been developed and marketed. One of the most important groups is a class of synthetic antibacterial drugs, called fluoroquinolones [1,2]. These agents inhibit DNA gyrase, thus interfering with the supercoiling of bacterial chromosomal material, and they show a broad-spectrum bactericidal activity [3]. The fluoride atom on position 6 and the 7-piperazinyl group in the quinolone framework are essential for the bactericidal spectrum and the high potency shown by these drugs. Furthermore, both groups affect pharmacokinetics properties, secondary effects and induce frequent spontaneous bacterial resistance [4].

Taking into consideration that in clinical practice most 6-fluoroquinolones are orally administered, it is important to study their gastrointestinal absorption process extensively and to establish correlations between their absorption rate constants and physicochemical properties [5]. Some biophysical models [6–8] which include physicochemical parameters like lipophilicity have been developed in order to study the absorption process of 6-fluoroquinolones [9,10]. On the other hand, the structural features of fluoroquinolones have been used to predict antibacterial activity, pharmacokinetics and physicochemical properties [5,10–13].

In the last few years, graph-theoretical methods have become one of the most important tools for quantifying molecular structure. This theoretical approach appears to be a good alternative to molecular design methods [14] and has been very useful in elucidating quantitative structure–property (QSPR) and quantitative structure–activity (QSAR) relationships.

The aims of this study were, first, to use a topological substructural molecular design (ToSS-MoDe) approach [15–

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0939-6411/02/$ - see front matter
PII: S0939-6411(02)00013-9

18] in order to obtain predictive models for the *n*-octanol/buffer partition coefficient (Log *P*), apparent intestinal absorption rate constant (Log K_a) and intestinal permeability (Log P_{eff}) of 6-flouroquinolone derivatives; to interpret the obtained models in terms of group contributions, using principal factor analysis to determine their relationship with lipophilic, electronic and steric features; to predict the theoretical values of the apparent intestinal absorption rate constant for Sarafloxacin and Sparfloxacin and to compare them with those obtained experimentally by an in situ rat gut technique; and finally to introduce isocontribution zone analysis (IZA) to carry out a substructural quantification, influenced by chemical fragments, of the studied properties.

2. Materials and methods

2.1. The ToSS-MoDe approach

The present approach is based on calculation of the spectral moments of the hydrogen suppressed molecular graph edge-adjacency matrix, using the bond dipole moments to weigh the diagonal entries of the edge matrix [18]. These variables are molecular descriptors calculated by the ToSS-MoDe software [19]. In our study we determined the local spectral moment carrying out the trace summation only on the bonds that constitute the piperazinyl ring and the piperazinyl ring and the N_1 substituent together in the quinolone molecular base. These indexes were called respectively, the i-esimal piperazinyl ring moment ($\mu_{iL\text{-}PR}$) and general local moment (μ_{iL}). The last index can be calculated as follows: $\mu_{iL} = \mu_{iL\text{-}PR} + \mu_{iL\text{-}N1sust}$. Due to the influence of the hydrogen bond on physicochemical and absorption properties the spectral moment in the hydrogen bond (μ_{iH}) was defined as the trace summation on the hydrogen–heteroatom edge in the weighted adjacency matrix of a non-hydrogen-suppressed molecular graph. The first 15 local spectral moments for each index were determined and the selection took into account the collineality tendency when the order of the spectral moment is increased [18]. The total amount of analyzed variables was 45.

The quantitative relationship between the physicochemical and absorption properties and the spectral moments was developed using regression statistical techniques. The spectral moments to be included in the equation were selected using a variable selection strategy, such as the forward stepwise, backward stepwise and standard procedures.

In the present work 30 compounds composed the data set of 6-fluoroquinolone derivatives. Twenty-six were used in the prediction of partition coefficient and 20 for the absorption rate constant and intestinal permeability. Nine compounds were used as an external prediction set, and Sarafloxacin and Sparfloxacin were the external corroboration set of the predictive performance of the models. The molecular structures of all these compounds are outlined in Fig. 1.

The first 15 spectral moments of the hydrogen and non-hydrogen suppressed edge-weighted adjacency matrixes were calculated using bond dipole moments [20].

The best linear regression equations for the description of each property were obtained using the forward stepwise regression in the STATISTICA version 4.13 [21]. The quality of the model was determined by examining the correlation coefficient, standard deviation of regression, standard deviation of the cross-validation 'leave-one-out' procedure, Fisher ratio ($F_{exp} > F_{tab}$, $\alpha = 0.05$) and the number of variables in the equation, which was selected considering that the ratio between cases and variables number were greater or equal than 5. In the leave-one-out procedure the model was built after removing one compound and the resulting model was used to predict the property of the one removed. This was repeated to obtain a prediction for every compound.

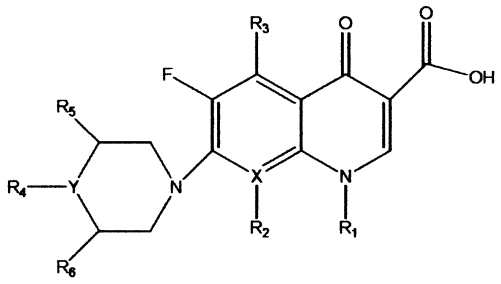
In order to study the influence of electronic and steric effects on the physicochemical and absorption properties, the normalized group contributions were analyzed at the R_4 and R_5 positions of a 6-fluoroquinolone framework. The *n*-methylciprofloxacin contributions were calculated taking the difference between the predicted property in the molecule, with the substituent in the specified positions, and the *n*-methylciprofloxacin skeleton contribution. Their relation with the Hammett electronic parameters (σ_m, σ_p), Hansch constant (π), molar refractivity (MR) and STERIMOL principal steric parameter (*L*) [22] were determined by means of principal factor analysis. These parameters were selected in order to evaluate the influence of electronic, lipophilic and steric effects on the physicochemical and absorption properties. The normalization was obtained by dividing each magnitude by its highest value.

Isocontribution zones were determined in the drugs used in the experimental study (Sarafloxacin and Sparfloxacin) by calculating the local spectral moment for each bond of selected molecules and then evaluating them in the adequate property equation. These zones, which permit us to predict the influence of different groups on the studied properties, are defined as a molecular fragment where all the bonds have the same sign on their contributions. The total contribution of an isocontribution zone was calculated by summing up the contribution of all the edges that constitute the zone.

2.2. Absorption studies

2.2.1. Test compounds

Sarafloxacin and Sparfloxacin were used as external corroboration of the predictive performance of the proposed models. Each compound was identified by its infrared spectrum. Its purity was checked by reversed phase high-performance liquid chromatography (HPLC) and was shown to be above 99.9%. The names and structures are given in Fig. 1 along with the names and structures of the 6-fluoroquinolones used in the model development. The compounds exhibited a



No	Name	X	Y	R_1	R_2	R_3	R_4	R_5	R_6
1	Norfloxacin	C	N	C_2H_5	H	H	H	H	H
2	Pefloxacin	C	N	C_2H_5	H	H	CH_3	H	H
3	N-ethylnorfloxacin	C	N	C_2H_5	H	H	C_2H_5	H	H
4	N-propylnorfloxacin	C	N	C_2H_5	H	H	C_3H_7	H	H
5	N-butylnorfloxacin	C	N	C_2H_5	H	H	C_4H_9	H	H
6	N-pentylnorfloxacin	C	N	C_2H_5	H	H	C_5H_{11}	H	H
7	N-hexylnorfloxacin	C	N	C_2H_5	H	H	C_6H_{13}	H	H
8	N-heptylnorfloxacin	C	N	C_2H_5	H	H	C_7H_{15}	H	H
9	Ciprofloxacin	C	N	Cyclopropyl	H	H	H	H	H
10	N-methylciprofloxacin	C	N	Cyclopropyl	H	H	CH_3	H	H
11	Enrofloxacin	C	N	Cyclopropyl	H	H	C_2H_5	H	H
12	N-propylciprofloxacin	C	N	Cyclopropyl	H	H	C_3H_7	H	H
13	N-butylciprofloxacin	C	N	Cyclopropyl	H	H	C_4H_9	H	H
14	N-pentylciprofloxacin	C	N	Cyclopropyl	H	H	C_5H_{11}	H	H
15	N-hexylciprofloxacin	C	N	Cyclopropyl	H	H	C_6H_{13}	H	H
16	N-heptylciprofloxacin	C	N	Cyclopropyl	H	H	C_7H_{15}	H	H
17	CNV 97100	C	N	Cyclopropyl	H	H	H	CH_3	H
18	CNV 8804	C	O	Cyclopropyl	H	H	--	H	H
19	CNV 8919	C	S	Cyclopropyl	H	H	--	H	H
20	Flumequine	C	--	$CH_3CH(CH_2)_2$-		H	--	--	--
21	Ofloxacin	C	N	*CH_3CHCH_2O-		H	CH_3	H	H
22	Enoxacin	N	N	C_2H_5	--	H	H	H	H
23	Sparfloxacin	C	N	Cyclopropyl	F	NH_2	H	CH_3	CH_3
24	Sarafloxacin	C	N	4-FPh	H	H	H	H	H
25	Lomefloxacin	C	N	C_2H_5	F	H	H	CH_3	H
26	CNV 8706	C	N	Cyclopropyl	H	H	C_2H_4OH	H	H
27	CNV 9201	C	C	Cyclopropyl	H	H	OH	H	H
28	Grepafloxacin	C	N	Cyclopropyl	H	CH_3	H	CH_3	H
29	Fleroxacin	C	N	C_2H_4F	F	H	H	CH_3	H
30	Temafloxacin	C	N	2,4-diFPh	H	H	H	CH_3	H

Fig. 1. Names and chemical structure of substituted 6-fluoroquinolones.

pK_{a1} between 5.5 and 6.5, and a pK_{a2} between 7.5 and 8.8, and at the working pH (7.00) they were in their zwitterionic form.

2.2.2. Biological technique

The in situ rat gut technique [23], adapted as previously described [7], was performed using the whole small intestine of male Wistar rats weighing 210–295 g (six animals per compound). In order to prevent enterohepatic recycling, the bile duct was cannulated before perfusion. An isotonic saline solution was prepared and buffered to pH 7.00 by adding of 10% (v/v) Sörensen phosphate solution 0.066 M. This concentration prevents the disturbing effects that phosphates have on the intestinal membrane [24] while maintaining the ionization of the substances. Test solutions were prepared immediately before use by dissolving a fixed amount of each compound in the vehicle solution (w/v), depending on its solubility. The concentrations (120 μg/ml for Sparfloxacin and 5 μg/ml for Sarafloxacin) were low enough to avoid precipitation in the lumen during the absorption tests. After dissolving the xenobiotic, the pH of the solution was checked and readjusted when necessary. Samples of the perfusate were collected in silanized glass tubes at fixed times, after 5 min, at intervals of 5 min. All samples were analyzed immediately.

2.2.2.1. Water reabsorption studies. The reduction in the volume of the perfused solutions at the end of the experiments was significant (up to 20%), and a correction became necessary in order to calculate the absorption rate constants accurately. Water reabsorption was characterized as an apparent zero-order process [7,25]. A method based on direct measurement of the remaining volume of the test solution was employed [7]. The volume at the beginning of the experiment (V_0) for each compound was determined in groups of three animals, while the volume at the end (V_t) was measured on every animal used. The concentration in the sample C_e was corrected as follows:

$$A_t = C_e \cdot \frac{V_t}{V_0} \quad (1)$$

where A_t represents the concentration in the gut that would exist in the absence of the water reabsorption process at time t. The A_t values were used to calculate the actual absorption rate constant.

2.2.3. In situ absorption rate constants

The absorption rate constants of the compounds, Ka, were determined by non-linear regression analysis of the remaining concentrations A_t versus time, using Sigma Plot 2.0 (Jandel Scientific), as it had previously been established that the process follows first-order kinetics. In order to prevent adsorption on the intestinal mucosa and residual sample dilution effects [7,26], only the calculated values after 5 min were used for regression, as it was found that, in general, after that time adsorption equilibrium was reached. The intestinal permeability values were calculated taking into account the relationship between Ka and P_{eff}: $P_{eff} = (K_a R)/2$, where R is the radius of the perfused intestinal segment.

2.2.4. Analysis of the samples

An HPLC procedure was used to quantify the solute concentration in biological samples. The analysis was carried out on a Novapak C18 column (3.9 × 150 mm), using as mobile phase a mixture of acetonitrile and 1/15 M phosphate buffer (20:80 v/v) adjusted to pH 2.4 with orthophosphoric acid for Sarafloxacin and a mixture of acetonitrile–phosphate buffer 10 mM (adjusted to pH 3.0) with 0.012% of heptane (23:77 v/v) for Sparfloxacin. The equipment consisted of a Series 1050 Hewlett-Packard quaternary pump, a Hewlett-Packard Agilent Series 1100 autosampler injector and a Perkin Elmer LCI-100 integrator. Quantification was done by spectrophotometry at 291 nm for Sparfloxacin and by fluorimetry for Sarafloxacin with excitation and emission wavelengths of 285 and 442, respectively. Both procedures offer a high degree of selectivity and specificity. The procedures were validated for inter- and intra-day runs before use. Accuracy was estimated from the percentage error associated with measuring 5–8 standards, analyzed at least three times. Accuracy was demonstrated to be less than 15%, regardless of the analyte concentration. Precision was calculated as the coefficient of variation of five determinations over the same standards. Precision was shown to be less than 5%. Linearity was established over the range of concentrations present in the samples for every compound (correlation coefficients always over 0.999).

3. Results

The best predictive model obtained for the *n*-octanol/water partition coefficient (Log P) is given below:

$$\text{Log } P = 1.16(\pm 0.30) - 4.50(\pm 0.59)\cdot\mu_{1L-PR} + 1.81(\pm 0.20)\cdot\mu_{2L-PR} + 1.14(\pm 0.17)\cdot\mu_{3L-PR} - 0.50(\pm 0.06)\cdot\mu_{4L-PR} - 1.42\times 10^{-8}(\pm 1.1\times 10^{-9})\mu_{15H} \quad (2)$$

$$N = 25;\ R = 0.979;\ R^2 = 0.958;\ S = 0.31;\ S_{CV} = 0.45;\ F(5,19) = 88.75$$

where N is the number of compounds used, R is the regression coefficient, S is the standard deviation of the regression, S_{CV} is the standard deviation of the cross-validation and F is the Fisher ratio at the 95% confidence level.

This model shows that the partition coefficient of the 6-fluoroquinolones employed in the study is dependent on the local spectral moments in the piperazinyl ring and on the molecule's capacity to release a hydrogen atom, given by μ_{15H}.

Experimental and calculated Log P of 26 6-fluoroquinolone derivatives used as experimental data are summarized in Table 1 (first and second columns of data). The same predictive model was used to determine the non-reported partition coefficients of four 6-fluoroquinolone derivatives, and the results appear in Table 1 (second column of data).

The best predictive model for the Log K_a values of 20 6-fluoroquinolones is given below:

$$\text{Log } K_a = 0.45(\pm 0.08) + 0.39(\pm 0.03)\cdot\text{Log } P - 0.15(\pm 0.02)\cdot\mu_{0L-PR} + 3.20\times 10^{-5}(\pm 0.6\times 10^{-5})\mu_{9L-PR} \quad (3)$$

$$N = 20;\ R = 0.968;\ R^2 = 0.934;\ S = 0.10;\ S_{CV} = 0.11;\ F(3,16) = 80.92$$

The experimental and calculated Log K_a for all the analyzed derivatives are given in Table 1 (fourth and fifth columns of data). In this case the model obtained depends on the partition coefficient and local spectral moment in the piperazinyl ring.

The model determined for the Log P_{eff} is the following:

$$\text{Log } P_{eff} = -3.90(\pm 0.10) + 0.39(\pm 0.02)\cdot Log\ P - 0.13(\pm 0.01)\cdot\mu_{0L} + 2.30\times 10^{-3}(\pm 0.31\times 10^{-3})\mu_{5L} \quad (4)$$

Table 1
Experimental and predicted partition coefficients, absorption rate constants and in situ intestinal permeability of 6-fluoroquinolones

No.	Name	Log P_e[a]	Log P_p[f]	Res[g]	Log K_{ae}[h]	Log K_{ap}[j]	Res[k]	Log P_{effe}[l]	Log P_{effp}[m]	Res[n]
1	Norfloxacin	−1.52	−1.36	0.16	−0.38	−0.34	0.04	−4.98	−4.99	−0.01
2	Pefloxacin	0.26	0.47	0.21	0.28	0.49	0.21	−4.32	−4.16	0.16
3	*N*-Ethylnorfloxacin	0.37	0.24	−0.13	0.44	0.48	0.04	−4.16	−4.19	−0.03
4	*N*-Propylnorfloxacin	1.05	0.86	−0.19	0.61	0.61	0.00	−3.99	−4.06	−0.07
5	*N*-Butylnorfloxacin	1.48	1.48	0.00	0.75	0.63	−0.12	−3.86	−4.02	−0.16
6	*N*-Pentylnorfloxacin	2.11	2.10	−0.01	0.76	0.73	−0.03	−3.85	−3.91	−0.06
7	*N*-Hexylnorfloxacin	2.71	2.73	0.02	0.78	0.81	0.03	−3.83	−3.81	0.02
8	*N*-Heptylnorfloxacin	3.22	3.35	0.13	0.78	0.86	0.08	−3.83	−3.75	0.08
9	Ciprofloxacin	−1.10	−1.36	−0.26	−0.20	−0.17	0.03	−4.81	−4.74	0.07
10	*N*-Methylciprofloxacin	0.15	0.47	0.32	0.41	0.44	0.03	−4.19	−4.12	0.07
11	Enrofloxacin	0.53	0.24	−0.29	0.55	0.54	−0.01	−4.06	−4.05	0.01
12	*N*-Propylciprofloxacin	1.07	0.86	−0.21	0.68	0.62	−0.06	−3.92	−3.96	−0.04
13	*N*-Butylciprofloxacin	1.55	1.48	−0.07	0.76	0.66	−0.10	−3.85	−3.91	−0.06
14	*N*-Pentylciprofloxacin	2.06	2.10	0.04	0.78	0.71	−0.07	−3.82	−3.84	−0.02
15	*N*-hexylciprofloxacin	2.56	2.73	0.17	0.78	0.75	−0.03	−3.82	−3.79	0.03
16	*N*-Heptylciprofloxacin	3.02	2.90	−0.12	0.78	0.88	0.10	−3.82	−3.74	0.08
17	CNV 97100	−0.68	−0.38	0.30	0.09	0.03	−0.06	−4.51	−4.55	−0.04
18	CNV 8804	0.97	1.30	0.33	0.85	0.67	−0.18	−3.75	−3.77	−0.02
19	CNV 8919	1.74	1.03	−0.71	0.88	0.98	0.10	−3.73	−3.55	0.18
20	Flumequine	0.97	0.98	0.01	0.84	0.83	−0.01	−3.77	−3.75	0.02
21	Ofloxacin	−0.47[b]	−0.03	0.44		0.30			−4.40	
22	Enoxacin	−1.02[b]	−2.77	−1.75		0.22			−4.44	
23	Sparfloxacin	−0.31[c]	−0.72	−0.41	0.38[i]	0.37			−4.29	
24	Sarafloxacin		−1.36		−0.31[i]	−0.28			−5.11	
25	Lomefloxacin	−1.28[d]	−0.87	0.41		−0.09			−4.74	
26	CNV 8706	−0.33[e]	−0.18	0.15		0.08			−4.49	
27	CNV 9201	0.22[e]	−0.02	−0.24		0.31			−4.23	
28	Grepafloxacin		−0.38			0.15			−4.46	
29	Fleroxacin		−1.86			0.37			−4.30	
30	Temafloxacin		−0.38			0.29			−4.21	

[a] Partition coefficients in *n*-octanol (pH 7.0) buffered solution, taken from Ref. [10].
[b] Ref. [28].
[c] Ref. [29].
[d] Ref. [30].
[e] Ref. [5].
[f] Partition coefficients predicted by Eq. (2).
[g] Observed−calculated.
[h] Absorption rate constants taken from Ref. [10].
[i] Absorption rate constant determined experimentally.
[j] Absorption rate constants predicted by Eq. (3).
[k] Observed−calculated.
[l] In situ intestinal permeability adapted from Ref. [10].
[m] In situ intestinal permeability predicted by Eq. (4).
[n] Observed−calculated.

$N = 19;\ \ R = 0.979; R^2 = 0.959;\ \ S = 0.08;$

$S_{CV} = 0.08;\ \ F(3, 15) = 118.42$

The in situ intestinal permeability of 6-fluoroquinolones as well as the intestinal absorption rate constant are influenced by the partition coefficient, but in the former case the general local spectral moments appear.

The total studied groups and their normalized contributions to the physicochemical and absorption properties are depicted in Table 2.

Principal factor analysis showed that three factors explained 77.81% of the total variance. The communalities were between 95.3% and 83.7% for the selected properties, (see Eqs. (5)–(8)). Moreover, the correlation coefficients were higher than 0.984, which means that the statistical quality in the factor analysis was good. These factors can be identified through the loading coefficients as lipophilicity-absorption (*F*1, high correlation with absorption properties and Log *P*), electronic (*F*2, due to good correlation with Hammett constant) and steric (*F*3, correlation with STERIMOL, molar refractivity and Hansch constant) features.

The main equations that express the relationship between factors at positions R_4 and R_5 of the 6-flouroquinolone framework are the following:

Table 2
Normalized group contributions to physicochemical and absorption properties in R_4 and R_5 positions of the *n*-methylciprofloxacin framework

Group	Log *P* R_4	Log K_a R_4	Log P_{eff} R_4	Log *P* R_5	Log K_a R_5	Log P_{eff} R_5	π	MR	σ_m	σ_p	*L*
Br	−0.40	−0.19	−0.38	−0.90	−0.74	−0.75	0.32	0.27	0.63	0.25	0.43
Cl	−0.43	−0.20	−0.40	−0.98	−0.82	−0.82	0.27	0.18	0.60	0.25	0.40
F	−0.41	−0.19	−0.38	−0.93	−0.77	−0.78	0.05	0.03	0.55	0.07	0.30
I	−0.35	−0.16	−0.34	−0.73	−0.57	−0.60	0.42	0.42	0.56	0.20	0.48
OH	−0.32	−0.17	−0.34	−0.43	−0.28	−0.35	−0.25	0.09	0.19	−0.41	0.31
SH	−0.31	−0.15	−0.32	−0.45	−0.29	−0.36	0.15	0.28	0.40	0.16	0.39
$NHNH_2$	−0.26	−0.18	−0.35	−0.58	−0.57	−0.54	−0.33	0.25	−0.03	−0.60	0.38
OCH_2CH_3	0.30	0.32	0.24	−0.27	−0.06	−0.23	0.14	0.38	0.16	−0.26	0.55
CH_2OCH_3	0.13	0.13	0.03	−0.20	−0.10	−0.20	−0.29	0.36	0.03	0.03	0.55
SCH_2CH_3	0.22	0.26	0.17	−0.36	−0.15	−0.30	0.40	0.55	0.29	0.03	0.59
$NHCH_2CH_3$	−0.14	−0.13	−0.27	−0.51	−0.50	−0.49	0.03	0.45	−0.39	−0.67	0.56
CycloPROPYL	0.61	0.63	0.56	−0.16	0.09	−0.14	0.43	0.41	−0.11	−0.23	0.47
C_3H_7-n	0.28	0.25	0.15	−0.12	0.00	−0.14	0.58	0.45	−0.11	−0.14	0.57
OC_3H_7	0.42	0.37	0.31	−0.20	−0.01	−0.19	0.32	0.51	0.16	−0.27	0.68
NHC_3H_7	−0.02	−0.09	−0.22	−0.44	−0.46	−0.46	0.23	0.59	−0.39	−0.67	0.68
$CHCHCOCH_3$	0.41	0.29	0.26	−0.20	−0.09	−0.21	−0.02	0.64	0.34	−0.01	0.65
C_4H_9	0.41	0.30	0.21	−0.04	0.04	−0.11	0.80	0.59	−0.13	−0.18	0.69
C_4H_9-ter	0.27	0.68	0.43	−0.60	−0.01	−0.44	0.74	0.59	−0.16	−0.22	0.46
OC_4H_9	0.55	0.42	0.37	−0.12	0.03	−0.16	0.58	0.65	0.16	−0.35	0.79
NHC_4H_9	0.10	−0.04	−0.15	−0.37	−0.42	−0.43	0.43	0.73	−0.55	−0.56	0.79
C_5H_11	0.54	0.35	0.27	0.03	0.08	−0.07	1.00	0.73	−0.13	−0.18	0.80
C_6H_5	0.18	0.03	−0.10	−0.57	−0.53	−0.63	0.73	0.76	0.10	−0.01	0.71
CH_2Br	0.00	0.08	−0.02	−0.28	−0.13	−0.23	0.30	0.40	0.19	0.15	0.46
CH_2Cl	−0.01	0.07	−0.03	−0.29	−0.13	−0.23	0.06	0.32	0.18	0.13	0.44
CH_2I	0.02	0.09	−0.02	0.01	0.08	0.05	0.56	0.57	0.16	0.12	0.49
CH=NOH	−0.27	−0.17	−0.30	−0.59	−0.45	−0.56	−0.14	0.31	0.35	0.11	0.55
CH_3	0.02	0.15	0.02	−0.03	0.24	0.02	0.21	0.17	−0.11	−0.19	0.34
OCH_3	−0.13	−0.03	−0.17	−0.52	−0.38	−0.45	−0.01	0.24	0.19	−0.30	0.45
$CHCH_2$	0.20	0.28	0.17	−0.32	−0.12	−0.26	0.31	0.33	0.08	−0.02	0.48
CH_2CH_3	0.15	0.20	0.09	−0.19	−0.05	−0.18	0.38	0.31	−0.11	−0.16	0.46
OC_6H_5	0.27	0.05	−0.05	−0.29	−0.28	−0.38	0.78	0.83	0.40	−0.03	0.51
NHC_6H_5	0.22	−0.06	−0.15	−0.29	−0.42	−0.43	0.51	0.90	−0.19	−0.44	0.51
CycloHEXYL	0.41	0.15	0.06	−0.29	−0.37	−0.42	0.94	0.80	−0.24	−0.24	0.69
$CH=NC_6H_5$	0.11	−0.10	−0.21	−0.01	−0.03	−0.10	−0.11	0.99	0.56	0.46	0.96
$CH_2C_6H_5$	0.44	0.15	0.09	−0.02	−0.07	−0.17	0.75	0.90	−0.13	−0.10	0.41
$CH_2OC_6H_5$	0.53	0.20	0.15	0.03	−0.03	−0.14	0.62	0.97	0.05	0.04	0.92

$$\text{Log } K_{a4} = 0.868 \cdot F1 - 0.004 \cdot F2 + 0.328 \cdot F3 + 0.860 \quad (R = 0.995) \quad (5)$$

$$\text{Log } K_{a5} = 0.968 \cdot F1 + 0.105 \cdot F2 + 0.069 \cdot F3 + 0.953 \quad (R = 0.987) \quad (6)$$

$$\text{Log } P_{eff4} = 0.877 \cdot F1 + 0.044 \cdot F2 + 0.373 \cdot F3 + 0.911 \quad (R = 0.994) \quad (7)$$

$$\text{Log } P_{eff5} = 0.896 \cdot F1 + 0.183 \cdot F2 + 0.035 \cdot F3 + 0.837 \quad (R = 0.984) \quad (8)$$

The independent term is the specific variance for each property (communalities).

The isocontribution zones of the two molecules analyzed are graphically outlined in Fig. 2.

The absorption rate constants for Sarafloxacin and Sparfloxacin determined experimentally in vivo are summarized in Table 1 (fourth column of data). As can be seen, the theoretical model is a good tool for predicting Log K_a.

4. Discussion

The statistical parameters of the model for predicting the Log *P* for 6-flouroquinolone derivatives suggest its high quality, and the low standard deviation of the cross validation procedure (0.45) evidenced its good predictive power. The experimental data revealed that Enoxacin was an outlier. The heteroatomic substitution at C_8 (C by N) produces a transformation from the quinolone to naphthyridone structure, thus increasing the electronic density around position 8 and the possibility of hydrogen bond formation using the unbound nitrogen electrons, with the consequent change in the physicochemical and biological properties.

The model offers realistic values for the four compounds predicted. Sarafloxacin has a chemical structure similar to that of Norfloxacin and Ciprofloxacin, which produces logic predicted partition coefficients; Grepafloxacin and Temafloxacin have some structural relationship with CNV 97100, and the results are consistent with that. Finally, Fleroxacin has practically the same chemical structure as Lomefloxacin, and the sight effect of the fluoride atom in the N_1 ethyl substituent explains to a large extent our findings. The

higher residual values are shown in compounds where the nitrogen atom of the piperazinyl ring has been replaced by a different heteroatom. In the case of Ofloxacin, the residual value is also high because the oxy-methylene bridge (-O–CH_2-) converts it into heterologue. Moreover, the contribution of the first methyl to the partition coefficient when it replaces the hydrogen atom could produce a variation between the experimental and predicted values. According to the Log *P* model, replacing the *N*-ethyl with *N*-cyclopropyl or an other substituent in R_1 does not produce any change in the partition coefficient. These results are consistent with those obtained by other authors [10]. At the same time the theoretical model depends, in a negative way, on the capacity of the molecule to release a hydrogen proton, thus decreasing the Log *P* value. This result permits us to validate the use of μ_{iH} (introduced in this work) in modeling phenomenon mediated by a hydrogen bond.

The model for predicting the absorption rate constant for the 20 6-fluoroquinolones studied showed good statistical results. As can be observed, the Log K_a depends on the partition coefficient (Log *P*) and the local spectral moment in the piperazinyl ring. The highest residual values correspond to Pefloxacin (0.21) and CNV 8804 (−0.18). The effect of the first element could have some influence on the results, and in the case of CNV 8804 the presence of a heteroatom produces a change in the chemical structure and in the electronic parameters similar to that induced in the partition coefficient, thus enhancing the absorption process.

The sequential introduction of methylene groups at the R_4 position of the piperazinyl ring produced an increase in the absorption rate constant, and the replacement of the hydrogen atom in the aliphatic chain by the hydroxyl group (CNV 8706) caused a significant decrease in Log K_a. Obviously, the increased affinity of the hydroxyl substituent for water reduces both lipophilicity and the absorption properties.

For the predicted 6-fluoroquinolone derivatives, Sarafloxacin and Lomefloxacin have the lowest absorption rate constant. Nevertheless, the lowest Log *P* belonged to Fleroxacin. This result is influenced by the negative contribution of $\mu_{0L\text{-}PR}$, which quantifies the number of atoms at position R_5 of the piperazinyl ring. Additionally, the introduction of a methylene group at positions R_5 and R_6 (CNV 97100 and Grepafloxacin) has no influence on lipophilicity, according to our theoretical approach, while it increases absorption slightly. Overall, these results agreed with other data from the literature [10]. Finally, the predictive model suggests that absorption is an increasing function of lipophilicity [5,10].

The model for intestinal permeability (Log P_{eff}) depends positively on the partition coefficient. For this reason an

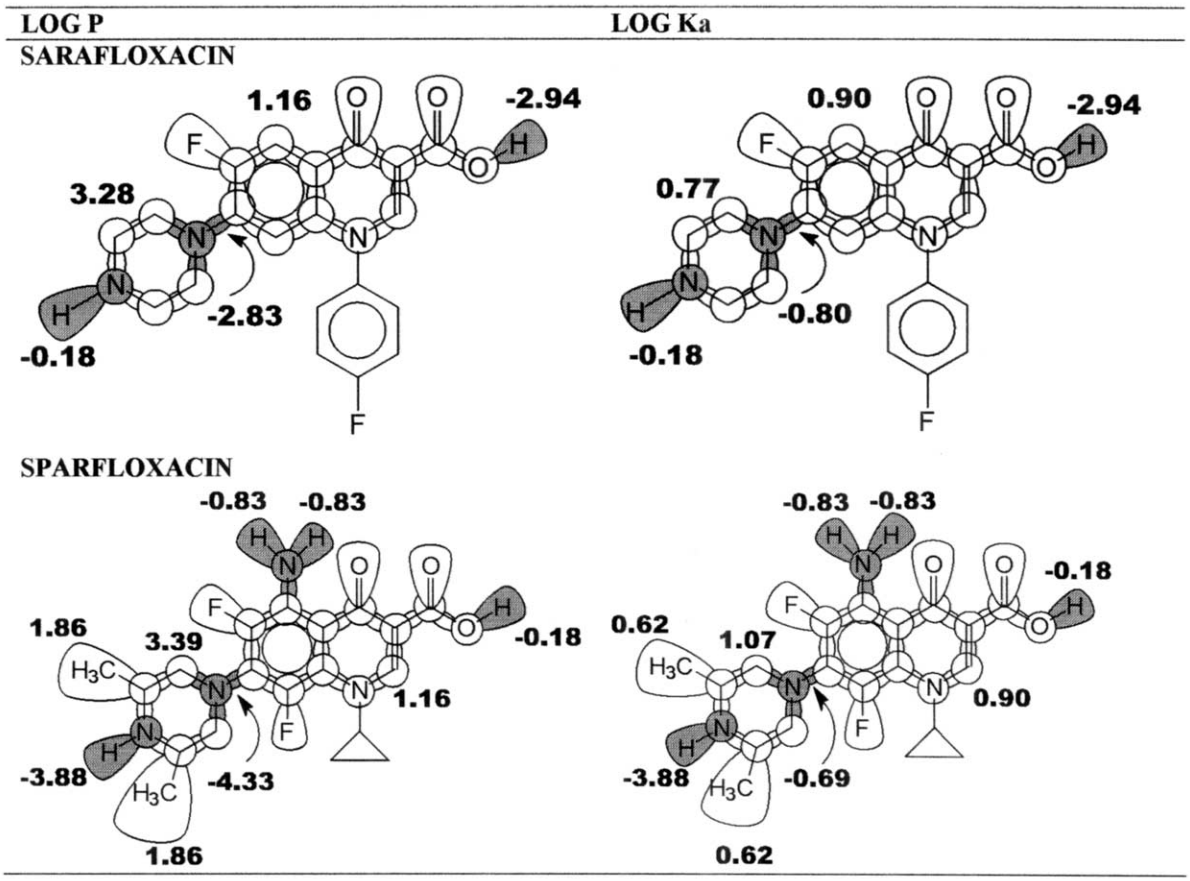


Fig. 2. Isocontribution zones for Sarafloxacin and Sparfloxacin.

increase in this lipophilic property facilitates the diffusion of compound through the intestinal membrane, which is concordant with the findings reported by other authors [27]. Moreover, the general local spectral moments have a strong influence on intestinal permeability. The negative contribution of μ_{0L} which take into consideration the number of atoms of substituent at positions N_1 and 7 is especially noteworthy. As can be seen in Table 1, permeability increases with lipophilicity up to a certain limit, and then, depending on the substituent number, the intestinal permeability value could decrease. In this model CNV 8919 was detected as an outlier. This could be explained by the electronic changes that the thiomorpholinic substitution introduces into the molecule, which probably modifies its interactions with the absorptive membrane. For the 6-fluoroquinolone derivatives, whose Log P_{eff}s were predicted by means of a theoretical model (Eq. (4)), the results are consistent with those obtained for the experimental data set.

In order to study the group contributions to physicochemical and absorption properties and considering the strong influence of the piperazinyl ring spectral moments on the theoretical models, we decided to use *n*-methylciprofloxacin to study the steric and electronic effects of different substituents on the quinolone skeleton.

Factor analysis demonstrated that absorption is determined by an absorption-lipophilicity factor characteristic of the quinolonic framework ($F1$). This result is corroborated by the high coefficients of $F1$ in factorial equations, and is in harmony with the fact that almost all the quinolones commercially available have good absorption in spite of their different substitutions at the quinolone skeleton [10,27]. The factorial analysis showed that the electronic and steric effects produce a slight alteration in the absorption properties, which is evidenced by the lower coefficients (<0.4) of $F2$ and $F3$ in the factorial equations. This is consistent with results reported by other authors [10], who suggest that absorption is an increasing function of lipophilicity which is modulated by electronic factors.

Position R_4 is more susceptible to steric change (0.37 to 0.33 coefficient in the factorial equations) than electronic (0.044 to -0.004). In contrast, position R_5 is more susceptible to electronic change (0.18 to 0.11) than steric (0.07 to -0.04). These results could be explained by the fact that at position R_4 there is a methylene group (-CH_2- from *n*-methylciprofloxacin) between the substituent and the piperazinyl ring which decreases the electronic influence more than the R_5 position, where the substituent is directly attached to the ring.

For the IZA we used the partition coefficients (Log P) because of their direct influence on the absorption process and the absorption rate constant (Log K_a), which is the experimental property measured. This analysis permitted us to predict of the influence of different groups on intestinal absorption. As can be observed, five isocontribution zones, which can be divided into positive and negative, were detected for both properties. The positive zones were the quinolone skeletons with a constant contribution of 1.16 to the Log P and 0.9 to Log K_a in the two molecules. This result could be due to the highly lipophilic character of the benzene ring and the electronic delocalization that exists in the quinolone framework which decrease the nitrogen capacity to form hydrogen bond, especially by the withdrawing electronic effect of the fluoride and carbonyl groups. Another positive isocontribution zone is the carbonate part of the piperazinyl ring, which has variable influence on both properties that could be explained by the lipophilic character of the methylene groups in the piperazinyl ring. The methyl groups increase the positive contribution of these zones in R_5 position of the Sparfloxacin, thus constituting a positive modulating effect, as was evidenced by factor analysis. The negative isocontribution zones detected were the hydrogen proton in the piperazinyl ring, the carboxylic proton and tertiary amine groups of Sparfloxacin and Sarafloxacin, and the amine group in Sparfloxacin. This result can be explained by the possibility of these groups forming hydrogen bonds. This validates the use of IZA for the structural interpretation for absorption process.

On the other hand, the absorption rate constants experimentally determined for Sarafloxacin and Sparfloxacin were close to the predicted values. The absorption constants showed realistic results. The low Log P for Sarafloxacin, determined by the theoretical approach (-1.36), implies a low Log K_a. This result is in agreement with the absorption–partition relationship previously established [10]. Moreover, the bioavailability range of this drug (11–34%) corresponds to its low absorption rate and is consistent with the experimental result obtained by the same authors [10] (with compounds of similar lipophilicity) [27]. In the case of Sparfloxacin the absorption rate constant is also very well predicted but is not consistent with the low lipophilicity of the compound. To explain this fact it would be necessary to run further studies to determine whether a carrier-mediated mechanism is involved in the absorption of this compound.

5. Concluding remarks

A novel approach was used to predict physicochemical and absorption properties in 6-fluoroquinolone derivatives. The theoretical models revealed that lipophilicity is a very important factor in the quinolone intestinal absorption. Moreover, principal factor analysis suggests that electronic and steric factors alter slightly the absorption process. In addition, the introduction of IZA makes it possible to reach a good structural interpretation of intestinal absorption. This approach, joined to the experimental results, demonstrated that the strategy used in this work is a powerful tool in the study of influence that physicochemical and biological properties have on the intestinal absorption of drugs.

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