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Research paper

A topological-substructural molecular design (TOPS-MODE) approach to determining pharmacokinetics and pharmacological properties of 6-fluoroquinolone derivatives

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Abstract

The topological-substructural molecular design approach was used to estimate the human bioavailability (F) and the minimum inhibitory concentration (MIC_{90}) against *Streptococcus pneumoniae* from a data set of 17 and 19 fluoroquinolone derivatives, respectively. Both pharmacokinetics and pharmacological properties were well described by the present approach. The total spectral moments and local spectral moments that include the different fluoroquinolone rings, polar and non-polar areas and their interactions were calculated and weighted with the standard dipole moments and the electronegative difference between the atoms that form a bond. In order to obtain a qualitative model that permits the classification of drugs with high and moderate bioavailability, a linear discriminant analysis was carried out. The percentage of correct classification was 100% for compounds of the training set. The leave-one-out cross validation procedure showed an 88.23% of correct classification. Also, a quantitative model, by the piecewise linear regression was developed. The theoretically predicted values for human bioavailability was assessed by a correlation with in vivo rat bioavailability and the regression equation was used to predict this biopharmaceutical property for two new pre-clinical 6-fluoroquinolone derivatives. On the other hand, a linear regression model that explained the 84% of variance was developed to predict the MIC_{90} values. Finally, the role of a pharmacokinetic and pharmacological relationship in the design of new fluoroquinolones was evaluated in the Sitafloxacin framework, where 13 substituents were analyzed; halogens and methoxy groups had the best contributions to both properties. The present approach proved to be a good method for studying the pharmacokinetics and the pharmacological properties of new 6-fluoroquinolone candidates in drug development studies.

Keywords: Topological-substructural molecular design approach; 6-Fluoroquinolones; Structure-property relationship; Bioavailability; Oral absorption; Pharmacokinetic

1. Introduction

The primary goal of the drug discovery and development process is to obtain a new molecule possessing good pharmacokinetic and pharmacological properties. Considering that 50% of compounds fail in pre-clinical study phases, which leaves unsuitable compounds to progress into expensive clinical testing [1], a great interest has been focused on the discovery stage assessment of pharmacokinetic properties [2] of compounds, as well as their pharmacological activity [3–8].

Important tools in the early state of drug development are the quantitative structure-activity (QSAR) and quantitative structure-property (QSPR) studies, where many kinds of molecular descriptors have been used to predict the activities and the pharmacokinetic behavior of untested drugs in the animal body [9–13]. The structural descriptors used in these studies are based on hydrophobic, electronic, and steric parameters. These descriptors also include partition coefficient, solubility, substituent constant, Hammet constant, molecular volume, quantum chemical parameters,

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alkyl side-chain length, molecular weight, reverse-phase thin-layer chromatographic Rm value and Van der Waals' dimension [14–16].

In the last few years, graph-theoretical methods have evolved as some of the most important tools for quantifying the contributions of molecular structures to kinetic-dynamic properties. This theoretical approach appears to be a good alternative to molecular design methods [17] and has been very useful in elucidating QSPR and QSAR relationships.

The topological-substructural molecular design (TOPS-MODE) approach stands out among these graph-theoretical methods as a very reliable and functional instrument for computational prediction. This novel approach, whose main advantage is that molecular descriptors can be expressed as a linear combination of structural fragments of molecule, has been successfully used to predict physical and biological properties in terms of substructural features of molecules [18–21].

In a previous paper we presented a theoretical method, based on the use of the TOPS-MODE approach, in order to obtain predictive models for the *n*-octanol/buffer partition coefficient, apparent intestinal absorption rate constant and intestinal permeability of 6-fluoroquinolones derivatives [22].

Taking into consideration the broad antibacterial spectrum of 6-fluoroquinolones and the variety of pharmacokinetic and pharmacological QSPR/QSAR studies conducted [22–31], our approach was based on the following question: are the spectral moments, as a new topological descriptor, useful in predicting the pharmacokinetics and pharmacological properties of 6-fluoroquinolones derivatives? By answering this question the aims of the present work were, from the pharmacokinetics point of view, to use the TOPS-MODE approach in the generation of a discriminant function that permits the classification of 6-fluoroquinolone molecules as a high/moderate bioavailability (F); to obtain quantitative models for this biopharmaceutical property through the piecewise linear regression (PLR); to predict the theoretical values of F for six new 6-fluoroquinolones derivatives and finally to assess the human predicted F with in vivo determination of F using rats as animal model. From a pharmacological sense, the aim was to obtain a quantitative model of minimum inhibitory concentration (MIC_{90}) against *Streptococcus pneumoniae* by a multivariable linear regression model (MLR) and select a compound with good pharmacological and pharmacokinetics properties in order to evaluate the influence of different substituent on the 6-fluoroquinolone framework.

2. Materials and methods

2.1. The TOPS-MODE approach

The present approach is based on the calculation of the spectral moments of the topological bond matrix, which mathematical basis was described in previous reports [18–21,32–34]. In this work, once the hydrogen-suppressed molecular graphs for each drug of the data set were drawn, the standard dipole moments (SD) and the electronegative difference between atoms that form a bond (DE) were used to weight the diagonal entries of the matrix. These variables (spectral moments) are molecular descriptors calculated by the TOPS-MODE software [35]. In our study we determined the local spectral moment carrying out the trace summation only on the bonds that constitute the different rings of the quinolone molecular base (see Fig. 1). These indexes were called $\mu_{i\text{-}R1}$, $\mu_{i\text{-}R2}$ and $\mu_{i\text{-}R3}$ for first, second and third rings respectively, of 6-fluoroquinolones framework. Also, the total spectral moments (μ_i) were calculated. Due to the influence of the polar surface area as well as the hydrogen-bonding capacity [36–39] on the physicochemical and absorption properties, in our study, the local spectral moment on the polar groups ($\mu_{i\text{-}PA}$) of the molecules and the local spectral moment over non-polar area ($\mu_{i\text{-}NPA}$) were determined. The local spectral moments on the polar groups were calculated by carrying out the trace summation over the N—H and O—H bonds only, and the local spectral moment over the non-polar areas were obtained by computing the difference between the total and the polar spectral moment. 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 [21]. The total amount of analyzed variables was 96.

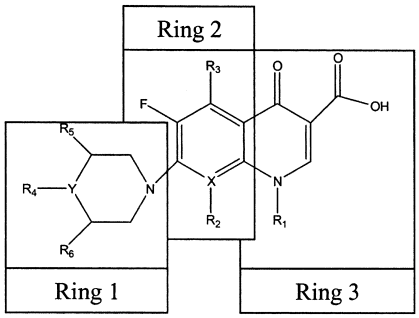
2.2. Development of the discrimination function for human bioavailability

The development of a discriminant function, through a linear discriminant analysis (LDA), that permits the classification of molecules with high and moderate F, is the first step in order to obtain a function capable of predicting this property from a data set.

In the present work, the data set of 6-fluoroquinolones derivatives was composed by 24 compounds. Seventeen compounds were used as a training set in the prediction of bioavailability (F) and seven compounds as an external prediction set (see Table 1). The molecular structures of all these compounds are outlined in Fig. 1.

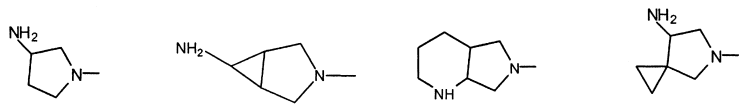
Bioavailability data of the 6-fluoroquinolones (humans and rats) were collected from the literature [40–47] and experimentally determined in vivo in rats for two of the fluoroquinolones belonging to the external prediction set. In this paper, bioavailability represents the percentage of the administered dose that reaches the systemic circulation after an oral administration, so the used values include all the pre-systemic loses as chemical degradation in gastrointestinal tract and/or intestinal or hepatic first pass effects.

The classification criterion for the discriminant function was selected, according to other authors [48], in the following way: If the compound has an oral bioavailability $\geq 90\%$,



No	Name	X	Y	R_1	R_2	R_3	R_4	R_5	R_6
1	Balofloxacin	C	C	Cyclopropyl	OCH_3	H	H	H	$NHCH_3$
2	Ciprofloxacin	C	N	Cyclopropyl	H	H	H	H	H
3	Clinafloxacin	C	-	Cyclopropyl	Cl	H		Ring 4	
4	Difloxacin	C	N	4-FPh	H	H	CH_3	H	H
5	Enoxacin	N	N	C_2H_5	--	H	H	H	H
6	Fleroxacin	C	N	C_2H_4F	F	H	H	CH_3	H
7	Gatifloxacin	C	N	Cyclopropyl	OCH_3	H	H	CH_3	H
8	Grepafloxacin	C	N	Cyclopropyl	H	CH_3	H	CH_3	H
9	Lomefloxacin	C	N	C_2H_5	F	H	H	CH_3	H
10	Moxifloxacin	C	-	Cyclopropyl	OCH_3	H		Ring 4	
11	Norfloxacin	C	N	C_2H_5	H	H	H	H	H
12	Ofloxacin	C	N	$^{*}CH_3CHCH_2O$-		H	CH_3	H	H
13	Pefloxacin	C	N	C_2H_5	H	H	CH_3	H	H
14	Rufloxacin	C	N	-S-$(CH_2)_2$-		H	H	H	H
15	Sitafloxacin	C	-	F-cyclopropyl	Cl	H		Ring 4	
16	Sparfloxacin	C	N	Cyclopropyl	F	NH_2	H	CH_3	CH_3
17	Temafloxacin	C	N	2,4-diFPh	H	H	H	CH_3	H
18	Tosufloxacin	N	-	2,4diFPh	--	H		Ring 4	
19	Trovafloxacin	N	-	2,4diFPh	--	H		Ring 4	
20	4N-Propylnorfloxacin	C	N	C_2H_5	H	H	C_3H_7	H	H
21	4N-Propylciprofloxacin	C	N	Cyclopropyl	H	H	C_3H_7	H	H
22	CNV 97100	C	N	Cyclopropyl	H	H	H	H	CH_3
23	CNV 97102	C	N	Cyclopropyl	H	H	C_2H_5	H	CH_3
24	CNV 97104	C	N	Cyclopropyl	H	H	C_4H_9	H	CH_3

Ring 4



Tosufloxacin Trovafloxacin Moxifloxacin Sitafloxacin

Clinafloxacin

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

it is a well-absorbed drug (high F) and if a bioavailability value is between 90 and 10%, the drug is moderately absorbed. In addition, the validation of the model was carried out by a cross-validation (leave-one-out) procedure, where the model is built after removing one compound and the resulting model is used to predict the property of the one removed. This was repeated to obtain a prediction for every compound.

The discrimination function was obtained by using the stepwise LDA as implemented in STATISTICA version 5.5 [49]. The default parameters of this program were used in the development of the model. The variables to be included in the equation were selected using a forward stepwise procedure as a variable selection strategy.

The quality of the model was determined by examining the Wilks' λ statistic, which establishes a perfect discrimination for $\lambda = 0$ and no discrimination when $\lambda = 1$. It also establishes the Mahalanobis distance (D^2) that indicates the separation of the respective groups, the Fisher ratio and the number of variables in the equation.

2.3. Development of PLRs function for human bioavailability

The development of the PLR, as a second step, in order to obtain quantitative values of human bioavailability was carried out. The molecular descriptors used were the same than those described in Section 2.2. The model was obtained over the general data set.

The estimation procedure was carried out by the *Simplex* and *Quasi-Newton* method. The breakpoint was 90 because this value was the limit between 6-fluoroquinolones with high and moderate bioavailability as selected in the previous discriminant analysis.

After estimating the regression parameters, the test of the appropriateness of the overall model was developed by

Table 1
Experimental and predicted values of bioavailability (F) and minimum inhibitory concentration (MIC_{90}) of 6-fluoroquinolones by qualitative and quantitative theoretical methods

No	Name	High F[a] (%)	Moderate F[a] (%)	F_H exp[b]	F_H pred[c]	F_R exp[d]	F'_H pred[e]	MIC_{90} exp[f]	MIC^{90} pred[g]
1	Ciprofloxacin		99.8	70	60	44.8		2	2.2
2	Clinafloxacin		99.7	80	66			0.06	0.35
3	Difloxacin	99.9		90	91			2	5.7
4	Enoxacin	52.7		90	93			16	11.1
5	Fleroxacin	98.0		96	93			8	3
6	Gatifloxacin	97.6		96[h]	95			0.39	0.28
7	Grepafloxacin		98.5	70[i]	69			0.39	0.53
8	Lomefloxacin	81.3		95	93			16	4
9	Moxifloxacin		81.7	86	87			0.25	0.22
10	Norfloxacin		82.4	40[j]	48	33.4		16	27
11	Ofloxacin	99.9		90	93			2	1.3
12	Pefloxacin	99.3		95[j]	93	79.5		8	23
13	Rufloxacin		97.7	50	53			16	6
14	Sitafloxacin		99.9	84[k]	97			0.05	0.05
15	Sparfloxacin	99.9		92[i]	94			0.5	0.6
16	Temafloxacin	99.5		92[l]	91			1	0.8
17	Trovafloxacin		96.2	80	77			0.25	0.42
		External prediction set							
18	Tosufloxacin	99.8		NA[m]	91			0.39	0.35
19	Balofloxacin	97.7		NA	95	87.5[n]		0.39	0.28
20	4N-propylciprofloxacin	99.9		NA	94	92.8			1.14
21	4N-propylnorfloxacin	95.6		NA	93	93.6			14.18
22	CNV 97100		99.8	NA	64	72	77.9		1.78
23	CNV 97102	94.1		NA	94	97[o]	100		1.37
24	CNV 97104	99.6		NA	95	83[o]	89.2		0.83

[a] Classification probabilities by Eq. (1).
[b] F, Ref. [40].
[c] Human bioavailability value (F) predicted by Eqs. (2) and (3).
[d] In vivo rat bioavailability, taken from Ref. [46].
[e] Human bioavailability predicted by Eq. (4).
[f] Minimum inhibitory concentration against *Streptococcus pneumoniae* (MIC_{90}), taken from Ref. [40].
[g] Minimum inhibitory concentration against *Streptococcus pneumoniae* (MIC_{90}), predicted by Eq. (5).
[h] F, Ref. [41].
[i] F, Ref. [42].
[j] F, Ref. [43].
[k] F, Ref. [44].
[l] F, Ref. [45].
[m] NA, data not available.
[n] In vivo rat bioavailability, taken from Ref. [47].
[o] In vivo rat bioavailability experimentally determined.

examining the correlation coefficient (R) and the determination coefficient (R^2).

Taking into consideration that a new chemical entity needs several pre-clinical studies before progressing to clinical trials, it is very important to carry out previous pharmacokinetics studies in animal models. For this reason and considering it as a validation procedure of the computational prediction, a correlation between predicted human bioavailability and the rat experimental bioavailability was developed in our study. The rationale of this approach was based on the good predictive performance for human permeability and oral fraction absorbed shown by this animal model [50,51]. A linear regression between human predicted bioavailability and in vivo rat bioavailability was obtained using six compounds (ciprofloxacin, norfloxacin, pefloxacin, 4N-propylnorfloxacin, 4N-propylciprofloxacin and balofloxacin) for which the rats in vivo values were reported [46,47]. Subsequently, the F predicted values for CNV 97100, CNV 97102 and CNV 97104 (novels pre-clinical 6-fluoroquinolones) were obtained by the above linear equation and compared with the originally predicted values 97104 (novels pre-clinical 6-fluoroquinolones) were obtained by the above linear equation and compared with the originally predicted values.

2.4. Pharmacokinetic and pharmacological relationship

In order to design a novel 6-fluoroquinolone with good pharmacokinetic and pharmacological properties, the influence of different fragments and substituents over a

6-fluoroquinolone framework should be evaluated. Firstly, the best linear equation for the description of MIC_{90} against *Streptococcus pneumoniae* was obtained over 19 compounds (see Table 1) by a MLR method employing the STATISTICA version 5.5 and using the forward stepwise regression as a strategy for variable selection [49]. The quality of the model was determined by examining the correlation coefficient, the standard deviation of regression, the standard deviation of the cross validation 'leave-one-out' procedure, the Fisher ratio ($F_{exp} > F_{tab}$, $\alpha = 0.05$) and the number of variables in the equation. The number of variables in the model was selected considering a ratio between compounds and number of variables greater or equal than 5.

Once the model was obtained and taking into consideration the variables included in the linear model, a compound was selected (Sitafloxacin, thanks to it is good pharmacokinetic and pharmacological properties). Thirteen substituents were also evaluated in two positions of the Sitafloxacin framework (see Fig. 3). The selection criterion was based on the results obtained by Eq. (1) (for bioavailability) and Eq. (5) (for MIC_{90}), previously described.

2.5. Absorption studies: in vivo bioavailability studies

2.5.1. Design of the studies

Male Wistar rats weighing 270–315 g, aged 3 months were used. Twenty-four hours before the experiment all the animals were permanently cannulated in the jugular vein with the aid of a previously validated technique [52] in order to facilitate blood sampling and intravenous administration while maintaining the animals non-anaesthetized. The rats were randomly distributed in two groups, each of eight to 12 animals. The first group received an intravenous bolus/perfusion of 4 or 8 mg, depending on the quinolone, and the second an oral administration by gastric sounding of 4 or 8 mg, both dissolved in a mixture of propyleneglycol and saline solution (50/50 V/V) in order to prevent precipitation.

Blood samples (0.4–0.5 ml) were drawn with heparinized syringes, and replaced by heparinized saline solution (10 U.I./ml) at previously established sampling times. The plasma was immediately separated by centrifugation (8000 rpm for 10 min) and frozen at −20°C until analyzed.

2.5.2. Analysis of the samples

The plasma was deproteinized with methanol to which the internal standard was added, (3,4,5-trimethoxybenzaldehide for CNV97 102 and N′Hexyl-cyprofloxacin for CNV97 104), and centrifuged at 8000 rpm for 10 min. An original HPLC procedure was used to quantify the quinolone concentration in the supernatant. The equipment used was a Hewlett-Packard 1045 system. A Novapak C 18 column was used as the stationary phase, while the mobile phase was adjusted for each test compound in order to obtain the best chromatographic resolution. The mobile phase consisted of mixtures of methanol/acetonitrile/phosphate buffer in 5/20/75 volumetric proportions for CNV 97102 and 5/30/65 for CNV97104. The optimized excitation and emission wavelengths were 285 and 442 nm, respectively. Validation of the procedure was carried out as usual [27,53,54].

2.5.3. Data analysis

The pharmacokinetic analysis was carried out for individual and mean experimental data on every compound employing Winnonlin 1.0. As similar results were obtained, only those of mean experimental data are reported. Bioavailability (F) was calculated as the ratio of the area under the plasma concentration versus the time curve for the oral and intravenous administrations (AUCoral and AUCiv, respectively), which was normalized by the corresponding dose. AUC was calculated by the trapezoidal rule [55] for the data up to the last sampling time point. The total area, AUC_0^{∞}, was obtained by adding the value of Cp/k to AUG_0^t where Cp is the last experimental drug concentration, and k represents the terminal monoexponential rate constant calculated for the mean plasma levels of the test compound.

3. Results

The best discriminant function obtained for the human bioavailability in the training set is given below:

Bioavailability

$$= -0.956 - 0.119\mu_{1\text{-DE}} + 1.559 \cdot 10^{-10}\mu_{15\text{-DE}} + 7.916 \cdot 10^{-10}\mu_{15\text{-PA-DE}} \quad (1)$$

$$N = 17 \qquad \lambda = 0.268 \qquad D^2 = 9.673 \quad F(3, 13) = 11.85$$

Where λ is the Wilks statistic, D^2 is the squared Mahalanobis distance and F is the Fisher ratio. There was not any compound detected as a statistical outlier in the training set. The percentage of probabilities obtained in the classification of compounds of the training and the external prediction set (first and second columns of data) is illustrated in Table 1. This model shows that the F values employed in the study are mainly dependent on the total spectral moment (μ_i) and the local spectral moment for the polar area ($\mu_{i\text{-PA}}$) of the molecules.

The percentage of overall accuracy of the model was 100% for the training set. If we take into account the cross-validation by the leave-one-out procedure, the model showed an 88.23% of correct classification (15/17). The results when the discrimination function is applied to the external prediction set of seven compounds appear in Table 1 (first and second columns of data).

Once the human bioavailability was classified by a discriminant function, a predictive PLR model for drugs with high and moderate F values was carried out. The feasibility of this model is justified due to drugs with high F

values only, which would have interest for oral administration. If the drug is classified as a moderate F, the wide range of values for this biopharmaceutical parameter in this classification group (between 90 and 10) will show a high variability hence a quantitative value will be necessary.

The best predictive models obtained for F is given below:

$$\text{Log } F_M = 1.275 + 4.346 \cdot 10^{-7}\ \mu_{8\text{-DE}} + 3.840 \cdot 10^{-3}\ \mu_{2\text{-NPA}} \quad (2)$$

$$Log\ F_H = 1.963 + 5.858 \cdot 10^{-8}\mu_{8\text{-DE}} - 2.320 \cdot 10^{-4}\mu_{2\text{-NPA}} \quad (3)$$

$$N = 17 \qquad R = 0.926 \qquad R^2 = 0.858$$

This model shows that the human F values employed in the study are mainly dependent on the total and the local spectral moment of the non-polar area of the molecules.

The bioavailability values obtained by Eqs. (2) and (3) for the moderately and highly available compounds are shown in Table 1 (forth column of data). As can be observed in this table, seven compounds were used as an external prediction set. The correlation between predicted and experimental human bioavailability (F) is represented in Fig. 2.

For pre-clinical drugs, it is very difficult to obtain human experimental values of bioavailability. In order to use the theoretical model as a predictive tool for human bioavailability of novel fluoroquinolones and, at the same time to validate this procedure, a correlation between the predicted F in humans and the experimental F in rats was established with six compounds [46,47]. Only three drugs (CNV 97100, CNV 97102 and CNV 97104) were used to corroborate the final results. This procedure gave us a validation of the obtained results. The correlation equation was the following:

$$F_{H\text{-pred}} = 21.192 + 0.783 \cdot F_{R\text{-exp}} \quad (4)$$

$$N = 6 \qquad R = 0.980 \qquad R^2 = 0.962 \qquad F(1,4) = 101.71$$

$$P < 0.0005 \qquad SE = 4.54$$

The predicted results are summarized in Table 1 (sixth column of data).

The best predictive model obtained for the minimum inhibitory concentration (MIC_{90}) against *Streptococcus pneumoniae* (SP) is given below:

$$\text{Log}(1/MIC_{90})_{SP} = -10.35 - 3.98 \cdot 10^{-9}\mu_{15\text{-R1}} + 5.43 \cdot 10^{-8}\mu_{14\text{-R3}} + 2.69 \cdot 10^{-6}\mu_{8\text{-DE}} \quad (5)$$

$$N = 19; \qquad R = 0.915; \qquad R^2 = 0.837; \qquad S = 0.363;$$

$$S_{CV} = 0.379; \qquad F(3,15) = 25.85$$

where, N is the number of compounds used, R is the regression coefficient, S is the standard deviation of the regression, R^2 is the determination coefficient, 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 MIC_{90} of 6-fluoroquinolone derivatives employed in the study is dependent on the local spectral moments in the rings 1 and 3, and also of the total spectral moments weighted with electronegative difference.

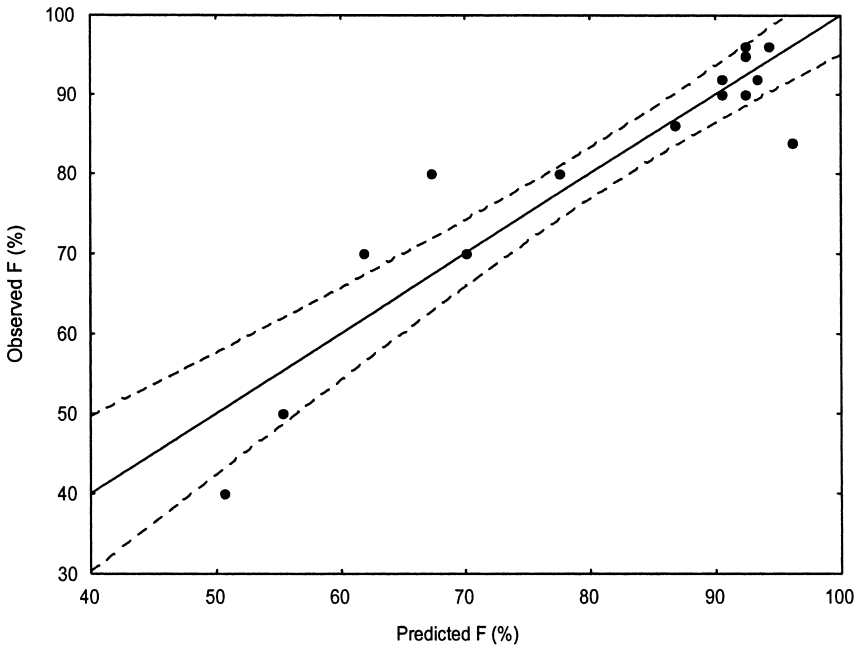


Fig. 2. Plot of observed (F_{exp}) versus calculated bioavailability (F_{pred}).

The experimental and predicted MIC_{90} values are summarized in Table 1 (seventh and eighth column of data).

Due to the good pharmacokinetic and pharmacological properties of Sitafloxacin, this compound was chosen to analyze the influence of 13 different substituents on positions X and Y of the Sitafloxacin framework (see Fig. 3). The results of the evaluation of substituents by the Eq. (1) (contribution to bioavailability value) and Eq. (5) (contribution to MIC_{90} value) are depicted in Table 2. The best results for both properties (lower MIC_{90} and higher bioavailability contributions) were obtained when the substituents on ring 3 were halogens and methoxy groups.

4. Discussion

The statistical parameters of the discriminant model for predicting the oral bioavailability for 6-fluoroquinolone derivatives suggest its high quality; the correct classification of the cross validation procedure (88.23%) evidenced its predictive power. Almost all compounds had a probability of classification higher than 80%. Only Enoxacin, regardless of being well absorbed, had a lower classification probability. The heteroatomic substitution at C_8 (C by N), for this compound, produces a transformation from the quinolone to the naphthyridone structure, thus increasing the electronegative difference between the atoms at this position and the value of one variable of the model ($\mu_{1\text{-DE}}$) with a negative contribution to the bioavailability [22]. This fact made this structure different to the rest of the 6-fluoroquinolones analyzed, offering certain similarity to compounds with moderate bioavailability.

The three main descriptors in the Eq. (1) contain information about the size (total spectral moment) and the area of the polar groups (local spectral moment) of the molecules. The descriptors of high magnitude (μ_{15}), in both kinds of spectral moment, have a positive contribution to the bioavailability, which is in correspondence with the influence that molecular weight and the hydrogen bond capacity have on this biopharmaceutical property [39,56]. Nevertheless, it should be considered that successful drug candidates have been characterized by an optimal range of values for hydrogen bonding, lipophilicity and size [56]. For

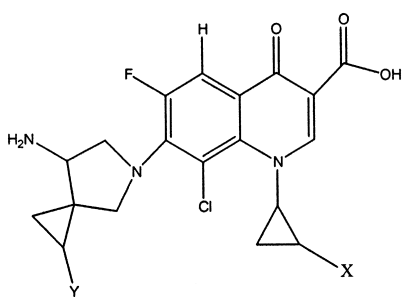


Fig. 3. Sitafloxacin structure with X and Y position for different substituents.

Table 2
Contribution of different substituents to the X and Y position of the Sitafloxacin framework for bioavailability (F) and minimum inhibitory concentration (MIC)

Substituent	MIC_{90}		F	
	X	Y	X	Y
F	0.046	1.656	0	−0.353
Cl	0.048	2.376	0.505	0.096
Br	0.065	1.976	0.544	0.146
I	0.114	1.330	0.587	0.204
OH	0.236	0.420	−0.709	−1.268
NH_2	0.302	0.325	−0.731	−1.265
CH_3	0.381	0.211	0.207	−0.213
OCH_3	0.112	0.513	0.338	−0.114
CH_2F	0.217	0.252	0.123	−0.301
CH_2Cl	0.249	0.296	0.206	−0.2 14
CH_2Br	0.260	0.298	0.207	−0.213
CH_2I	0.278	0.296	0.202	−0.217
CH_2OH	0.257	0.234	−0.165	−0.592

this reason, compounds with extreme positive values for these properties could have a marked negative effect on bioavailability. These results coincide with those reported by other authors [57].

The model offers good values for the seven predicted compounds. The CNV 97100 has a chemical structure similar to Grepafloxacin, for this reason, it seems logical that the bioavailability value is in the same range (see Table 1). The sequential introduction of methylene groups at the R_4 position of the piperazinyl ring (CNV 97102, CNV 97104) produced an increase in the lipophilicity of the molecule as well as in the absorption rate constant [22], increasing the membrane permeability and with it, the bioavailability. Tosufloxacin and Balofloxacin have some structural relationships with Trovafloxacin and Grepafloxacin respectively and the results are consistent with these. Finally, 4N-propylciprofloxacin and 4N-propylnorfloxacin have the same aliphatic effect over the R_4 position as that over the CNV family. Also, the cyclopropyl ring at R_1 position of the 6-fluoroquinolone skeleton produced higher bioavailability values than the ethyl group in the same position (see Table 1).

Due to the impossibility to obtain a multivariate linear regression model for prediction of the bioavailability property, mainly when heterogeneous structures are present in the database of analysis [37], a PLR model was carried out. The model for prediction of the human F values for seventeen 6-fluoroquinolones studied revealed good statistical results, as it is shown in Eqs. (2) and (3) and it is graphically outlined in Fig. 2.

The model for Log F depends on the total spectral moments and the local spectral moments in the non-polar area of the molecules. If we analyze Eq. (3) for example, the non-polar area is fairly high, and its negative contribution reduces the F value. This is a logical result, which coincides with the findings reported by other authors, where a balance

between the polar and non-polar areas is important in order to obtain a good absorption profile [39].

Moreover, in order to validate the computational prediction, a correlation between the 'in silico' predicted human bioavailability and the 'in vivo' rat bioavailability was developed. As can be appreciated in Eq. (4), the statistical parameters showed the good predictive power of the equation. These considerations are valid if the similarity between intestinal absorption processes in rats and in humans are considered and thus, the rat bioavailability value is used as a surrogate of the human one [50–52,58].

Eq. (4) was used to predict the human bioavailability of novel 6-fluoroquinolones (CNV family), but for this purpose an experimental determination of F, in rat model, was needed. The reached results revealed a strong relationship between the F values obtained for both methods (four and six columns of data).

One of the most interesting features of the TOPS-MODE approach to molecular design is the possibility to obtain the quantitative contribution of any kind of sub-structure to the property studied. The number of structural fragments that can be evaluated to determine their contributions to the pharmacokinetic and pharmacological properties is, of course, very large.

In order to establish a pharmacokinetic – pharmacological relationship under a structural base, as well as to design novel lead compounds, a linear regression model for Minimum Inhibitory Concentration (MIC_{90}) against Streptococcus pneumoniae was developed.

The statistical parameters obtained for the theoretical model of Log ($1/MIC_{90}$) showed good results. In Eq. (5) we can appreciate the negative contribution of the total spectral moments in ring 1 and the positive contribution in ring 3. Taking into consideration Eq. (5) (a pharmacological equation), and using Eq. (1) (a pharmacokinetics equation) in the analysis, 13 different substituents were evaluated in position X and Y (see Fig. 3) of the Sitafloxacin skeleton.

The results depicted in Table 2 showed that the best contributions for both properties (the lowest for MIC_{90} and the highest for F) mainly on ring 3, were on account of halogens and methoxy groups.

As has been widely discussed by other authors [59,60] the halogens (F or Cl) and methoxy groups at the 8-position of the fluoroquinolone framework improve the oral absorption and activity against Gram-positive bacteria. Also, the cyclopropyl and the amino-pyrrolidine groups at N_1 and C_7 of the fluoroquinolone skeleton, respectively, enhance the antimicrobial effectiveness.

In the case of Sitafloxacin, there is a cyclopropyl group at N_1 position and a 7-(7-Amino-5-aza-spiro[2.4]hept-5-yl) group at the C_7 position of the fluoroquinolone skeleton. Taking into consideration both groups, any substituent at the X position (see Fig. 3) will have better mobility and better interactions with the biological target due to the free rotation of the cyclopropyl group around the single bond C_1—N_1.

The halogens and methoxy groups attached to the X position, as bulky groups, will increase the lipophilicity and absorption properties and decrease the MIC value. On the contrary, the polar groups such as OH and NH_2 will reduce the bioavailability contribution due to the possibility to form hydrogen bond.

As can be observed during the study the proposed methodology will be of great importance in order to design new drug candidates with good pharmacological and pharmacokinetic properties. This methodology should be evaluated in a large data set with high structural variability.

5. Concluding remarks

A novel approach was used to predict the pharmacological and pharmacokinetic properties of 6-Fluoroquinolones. The combination of qualitative and quantitative values showed the influence that polar and non-polar groups had over the bioavailability predictions. Moreover, the local spectral moments had a direct influence over the pharmacological property. This computational approach, along with the experimental assessment used in this work, is a powerful tool in the design of new drug candidates. The use of this methodology on large and highly variable dataset will be the aims of future papers.

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