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QUANTITATIVE STRUCTURE – PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIP FOR FLUOROQUINOLONES

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Quantitative structure – pharmacokinetic/pharmacodynamic (PK/PD) relationship (QSPR) techniques and chemometric methods were employed to classify fluoroquinolones with respect to their activity against *Streptococcus pneumoniae*. Density functional theory (DFT) was used to calculate a set of molecular descriptors (properties) for 13 synthetic fluoroquinolones. The descriptors were further analyzed using chemometric methods including the principal component analysis (PCA), hierarchical cluster analysis (HCA), and stepwise discriminant analysis (SDA). The PCA and SDA methods were employed in order to reduce the dimensionality and select a subset of variables that would be more effective for classifying the fluoroquinolones according to their degree of antipneumococcal activity. The methods of PCA, SDA and HCA were quite efficient to classify 13 compounds in two groups (active and inactive), and the net charge on ring B (Q_B), molecular volume (VOL), and partition coefficient (log P) were found to be descriptors important for the classification. These methodologies of PCA, SDA and HCA provide a reliable rule for classifying new fluoroquinolones with respect to antipneumococcal activity. The application of SPP relationship is of considerable value for clinicians, drug developers, and regulators because PK/PD principles form the basis of modern antimicrobial chemotherapy.

1. Introduction

Four decades after the discovery of nalidixic acid – the first member of the quinolone antibacterial family – more than 7000 new analogs have been documented in the literature. Since 1977, this class of synthetic antibacterial agents has been widely used in clinics. In recent years, there has been a considerable interest in the development of new fluoroquinolone agents. Quinolones are among the most important classes of antimicrobial agents discovered in recent years and one of the most widely used classes of antimicrobial drugs in clinical medicine.

The antibacterial activity of antibiotics has traditionally been expressed as minimal inhibitory concentrations (MICs) or minimum bactericidal concentrations (MBCs). Although these parameters describe antimicrobial activity at one point in time, they do not provide any time course of the antimicrobial activity and account for fluctuations of drug concentrations within the body. Recently, pharmacokinetic/pharmacodynamic (PK/PD) parameters – that is, those describing the relationship between a drug concentration in the blood serum and the drug pharmacology and toxicology – have assumed greater importance. In vivo bactericidal efficacy can be perfectly predicted by PK/PD parameters, such as the time for which the drug concentration is above the MIC level ($T_{>\mathrm{MIC}}$), the ratio of the peak concentration to the MIC, and the ratio of the area under the 24-h concentration – time curve to the MIC (AUC_{24}/MIC). The pharmacokinetic (PK) and pharmacodynamic (PD) analyses can be used to predict bacterial eradication and applied to the assessment and choice among the existing agents for the treatment of respiratory tract infections as well as to the development of improved formulations and new antimicrobial agents. There are data to support the general conclusion that PK/PD may be able to predict the likelihood of emergence of resistance during therapy. Today, the integration of PK and PD characteristics (i.e. the PK/PD relationship for a drug) is central to our understanding of the drug dose and efficacy [1].

The PK/PD profile of quinolones as antibacterial agents has been well elucidated. Quinolones eliminate bacteria most rapidly when the drug concentration is appreciably above the MIC of the target microbe and exhibit a moderate-to-prolonged persistent killing effect. These agents are classified as concentration-dependent killing antibacterial agents [2, 3]. In order to study a correlation between the PK/PD parameters and structure of fluoroquinolones, we performed geometric optimization on the structure of drugs studied.

Traditional quantitative structure – pharmacokinetic/pharmacodynamic relationship (QSPR) studies employ empirical physicochemical (electronic, sterical, hydrophobic, and topological) parameters related to a series of compounds. However, structural descriptors (variables) obtained from molecular orbital calculations have been successfully correlated with a variety of biological data in QSPR. The paper focuses on the relationship of the structure descriptors and PK/PD parameters of fluoroquinolones against community-acquired respiratory pathogens such as *S. pneumoniae*.

2. Computation methods

2.1. Calculation of Theoretical Descriptors of Molecular Properties

Except the sterical and hydrophobic features of the compounds studied, all the results were obtained with the GAUSSIAN 03 program package [4], using the standard 3 – 21G basis set. Electron correlation was partially taken into account by means of density functional theory (DFT)

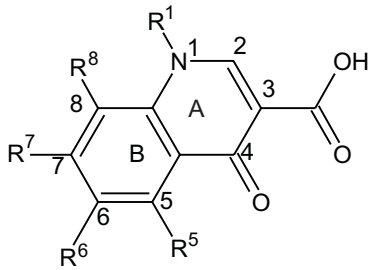

Fig. 1. The basic structure of fluoroquinolones.

[5] using the GAUSSIAN 03 version of the hybrid three-parameter functional developed by Becke [6 – 8] and denoted as B3LYP. The sterical and hydrophobic features of the compounds studied were calculated with the HyperChem 6.0 program. All structures have been fully optimized on the B3LYP/3 – 21G level. The PK/PC parameters of fluoroquinolones against *S. pneumoniae* are shown in Table 1.

Because fluoroquinolones are classified as concentration-dependent killing antibacterial agents, the AUC_{24}/MIC ratio is currently considered and commonly accepted as a pharmacodynamic correlation of fluoroquinolone efficacy [22 – 23]. The greater the AUC_{24}/MIC value, the more pronounced the bactericidal effect. It can be seen from Table 1 that all new fluoroquinolones (gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin) achieve free AUC_{24}/MIC that is higher than 30 against *S. pneumoniae* [24]; at the same time, the other (old) fluoroquinolones such as ciprofloxacin achieve free AUC_{24}/MIC that is smaller than 30. This implies that the newly synthesized fluoroquinolones have greater bactericidal activity than the older ones. The basic structure of fluoroquinolones is depicted in Fig. 1.

Structural descriptors of compounds are usually correlated with biological activity. In this work we calculated using B3LYP/3 – 21G the following structural descriptors to be correlated with the biological activity: highest occupied molecular orbital energy (E_{HOMO}); lowest unoccupied molecular orbital energy (E_{LUMO}); energy difference between the highest occupied and the lowest unoccupied molecular orbital (ΔE_{HL}); dipole moment (μ); molecular hardness (η); molecular softness ($1/\eta$); hydration energy (*HE*); net atomic charge on *i* th atom (Q_i); net charge on *n* th ring (Q_n); Mulliken's electronegativity (χ); molecular volume (VOL); molecular polarizability (*Pol*); partition coefficient (log *P*); molecular refractivity (*MR*); molecular mass (*M*); and surface area (*A*).

During the investigation of the relationship between PK/PD parameters and structural descriptors of fluoroquinolines, we have found that the PK/PD parameters of fluoroquinolones are strongly correlated with the Q_B, log *P*, VOL and Q_5. A correlation between the molecular properties and PK/PD parameters was established using the pattern recognition methods built in the SPSS program.

2.2 Principal component analysis

Principal component analysis (PCA) is a statistical technique that seeks to find a few mutually orthogonal linear combinations of the original variables, which account for most of the variability present within an original system. In other words, a large part of the variance can usually be related to a small number of components.

In the PCA, the first rows are standardized (unit variance, zero mean) to give a square matrix of the moment correlation coefficients between pairs of rows. Computing the principal components of this matrix involves the computation of its eigenvalues and eigenvectors. The importance of these vectors is that they are orthogonal. In other words, a large proportion of the dispersion through *n* rows over *m* columns may be accounted for by *p* dimensions. The *p*-normalized vectors give the directions of a set of *p*-orthogonal axes in *p*-dimensional space. The linearly inde-

Table 1

Pharmacokinetic and Pharmacodynamic Parameters Relevant to Antipneumococcal Activity of Fluoroquinolones

Fluoroquinolone	Dose* (mg)	PB%	C_{Pmax} free (μg/ml)	AUC_{24} free(μg/h/ml)	MIC_{90} (μg/ml)	C_{Pmax} free/MIC	AUC_{24} free/MIC
Ciprofloxacin	500 (BID)	30	1.6	14.0	2.00	0.80	7.00
Ciprofloxacin	7500 (BID)	30	2.1	20.0	2.00	1.05	10.00
Levofloxacin	400 (OD)	30	3.6	35.0	1.00	3.60	35.00
Gatifloxacin	400 (OD)	20	3.1	24.0	0.50	6.20	48.00
Moxifloxacin	400 OD	50	1.7	15.0	0.25	6.80	60.00
Gemifloxacin	320 (OD)	60	0.5	4.0	0.03	16.67	133.00
Sparfloxacin	400 (OD)	40	0.8	10.6	0.50	1.60	21.20
Trovafloxacin	400 (OD)	75	0.6	7.8	0.25	2.40	31.20
Norfloxacin	400 (OD)	15	1.3	11.6	12.50	0.10	0.93
Lomefloxacin	400 (OD)	20	3.5	27.4	8.00	0.43	3.43
Fleroxacin	400 (OD)	32	6.5	70.0	12.50	0.52	5.60
Ofloxacin	400 (OD)	31	5.6	35.0	3.00	1.87	11.67
Pefloxacin	400 (OD)	25	3.8	63.0	8.00	0.48	7.88
Enoxacin	400 (OD)	35	3.7	33.0	16.00	0.23	2.06

* Single doses simulated: OD, single daily dose; BID, double daily adminstration; AUC_{24}, area under the concentration – time curve; C_{Pmax}, maximum drug concentration in plasma; MIC_{90}, minimum inhibitory concentration (MIC) of drug necessary to inhibit 90% of strains; PB, protein binding; data summarized from [9 – 21].

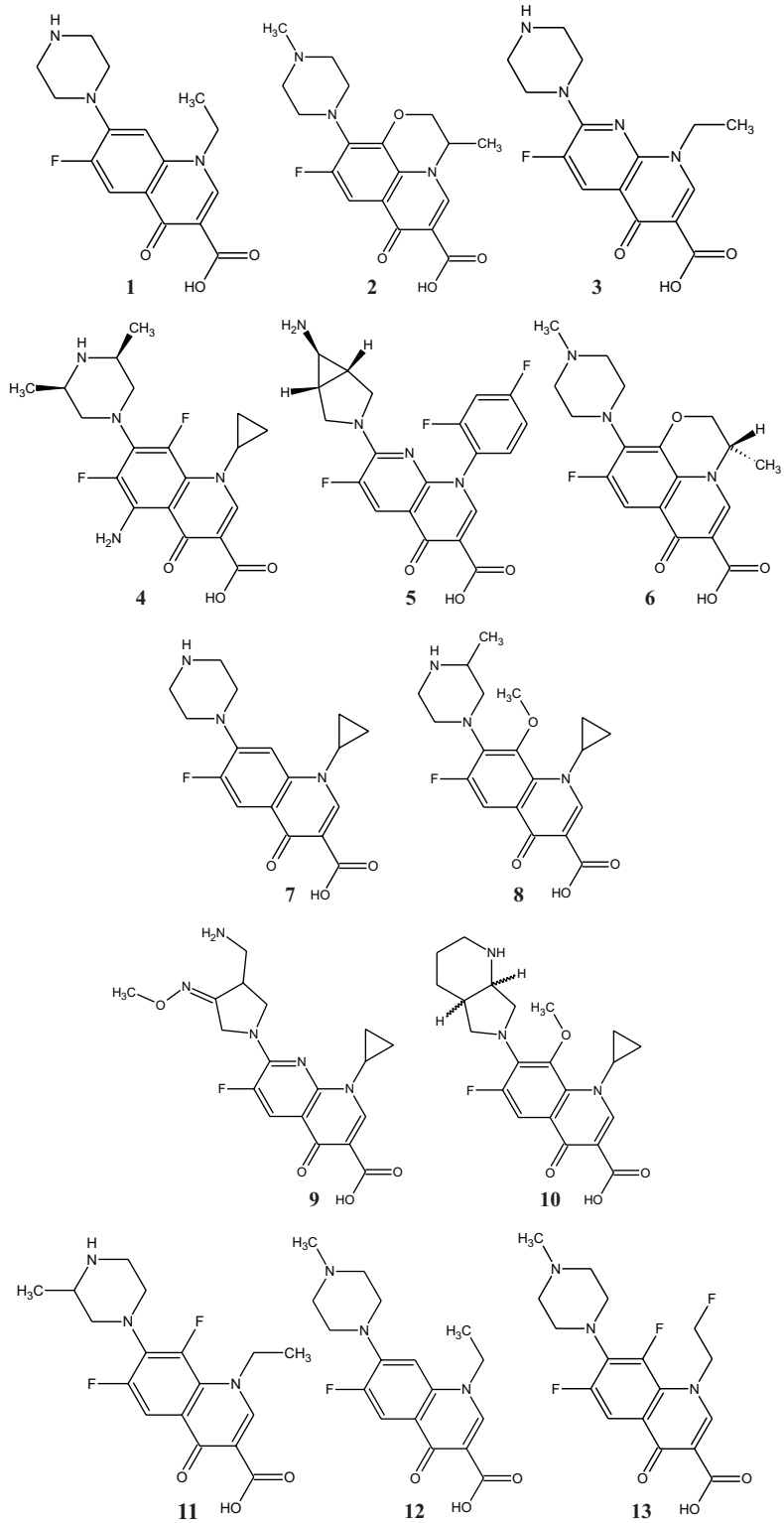

Fig. 2. Computer generated drawings of the structures of fluoroquinolones studied: (**1**) norfloxacin; (**2**) ofloxacin; (**3**) enoxacin; (**4**) sparfloxacin; (**5**) trovafloxacin; (**6**) levofloxacin; (**7**) ciprofloxacin; (**8**) gatifloxacin; (**9**) gemifloxacin; (**10**) moxifloxacin; (**11**) lomefloxacin; (**12**) pefloxacin; (**13**) fleroxacin.

pendent principal components are ranked in terms of the amount of total variance that each component explains.

2.3 Hierarchical Cluster Analysis

Hierarchical cluster analysis (HCA) has become, together with PCA, another important tool in chemometrics [25] Hierarchical clustering techniques take as input either a similarity matrix between a set of items, or some attributes describing the items. The result of such a process is a binary tree where items form the leaves of the tree and each node of the tree represents a cluster of similar items. The farther the node is from the tree root, the more similar items are under the node.

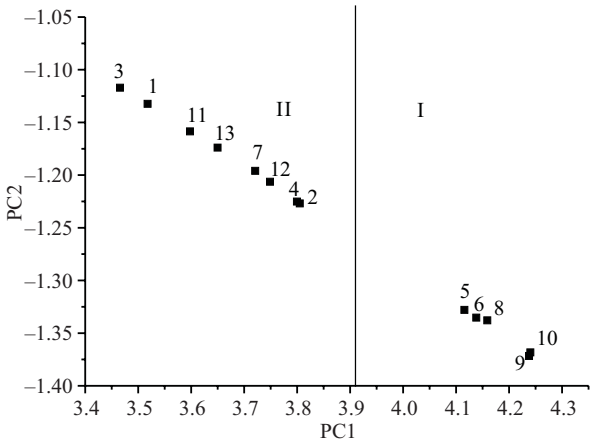

Fig. 3. Separation of the training set into two groups: I (active) and II (inactive) compounds. Notice that the first principal component (PC1) is responsible for the separation.

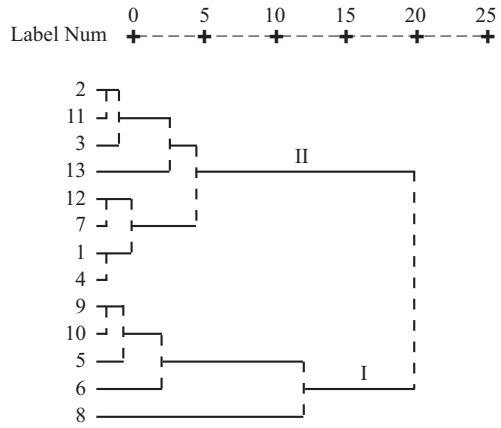

Fig. 4. Dendrogram of the training set as separated into two groups: I (active) and II (inactive).

2.4. Stepwise Discriminant Analysis

Stepwise discriminant analysis (SDA) is a multivariate technique that has two principal objectives:

(1) To separate objects from distinct populations;

(2) To allocate new objects into populations previously defined [26, 27].

The SDA is a linear discrimination method based on the *F*-test for the significance of variables. In each step, one variable will be selected on the basis of its significance.

3. Results and discussion

3.1. PCA of Variables

Before applying the PCA technique, each of the variables was autoscaled so that they could be compared to each other on the same scale. In application of the PCA to a set of compounds listed in Fig. 2, several attempts were made to ensure reliable separation of the active compounds from the inactive ones. The best separation was obtained when 21 initial variables were reduced to three final variables (see Table 2). This suggests that the other 18 variables are not as important in separating these compounds.

Table 2

Values of Three Most Important Properties (Variables) That Classify Fluoroquinolones Studied

Compound	Q_B	log *P*	VOL
1	0.4728	4.76	875.69
2	0.9609	4.77	947.01
3	0.5661	4.43	862.84
4	0.9579	4.77	945.68
5	0.4842	5.12	908.44
6	0.9277	5.42	925.58
7	0.9704	4.99	1030.12
8	0.9288	5.19	932.73
9	0.5768	4.25	1055.95
10	0.9386	5.11	1055.62
11	0.4790	4.81	895.69
12	0.5895	5.81	1035.17
13	1.4280	5.02	1024.06

The PCA results show that the first component (PC1) is responsible for 52.14% of the data variance. Upon considering the first (PC1) and second (PC2) components, the accumulated variance increases to 81.02%. The loading vectors for PC1 and PC2 are shown in Table 3. According to Table 3, PC1 can be expressed through the following equation:

$$PC1 = 0.79Q_B + 0.548\log P + 0.8\ VOZ$$

From this equation, we can see that more active compounds can be obtained when we have higher values for the variables Q_B, log *P*, *VOL*.

As can be seen from Fig. 3 showing the separation of the training set of molecules into two groups – I (active) and II (inactive) molecules against *S. pneumoniae* – the active compounds present very big values for PC1, while the inactive compounds present a progressive decrease in the PC1 values.

3.2. SDA of Compounds

The SDA is a linear discrimination method based on th3 *F*-test for the significance of variables. In each stage, one variable will be selected on the basis of its significance. Two significance variables were extracted from the 21 initial variables investigated, namely: Q_5 and VOL. For groups I and II, the discrimination functions are as follows:

$$\text{Group I: } -437.171 - 182.126Q_5 + 0.820\text{VOL};$$

$$\text{Group II: } -347.539 - 160.825Q_5 + 0.731\text{VOL}.$$

From the two discrimination functions obtained using the SDA study, one can see that the variable Q_5 has the higher weights in this classification methodology. Compa-

Table 3

PCA Loadings Obtained for Selected Variables with Two Principal Components

Variable	Q_B	log *P*	VOL
PC1	0.790	0.548	0.800
PC2	–0. 313	0.836	–0.263

Table 4

Classification Matrix

Group	True group	
	Inactive group	Active group
Inactive group	8	0
Active group	1	4
Total	9	4
Percentage	88.9%	100%

Table 5

Cross-Validation Matrix

Group	True group	
	Inactive group	Active group
Inactive group	7	1
Active group	1	4
Total	8	5
Percentage	87.5%	80%

ring the results obtained using the PCA and SDA methods, we can see also that VOL is a key property for explaining the antipneumococcal activity of fluoroquinolones studied; however, the values of Q_B, log P and Q_5 are also important when one is trying to design fluoroquinolones possessing the antipneumococcal properties.

One way to judge on the performance of a classification rule (see the discrimination functions for inactive group and active group above) obtained with the SDA is to calculate the classification matrix or the cross-validation matrix, because they both show the actual membership for the predicted group. The difference between them is that the procedure to calculate the classification matrix considers all information necessary to develop the classification function and to classify the objects, while a procedure for the cross-validation matrix omits the first compound and develops a classification function using the remaining ones and finally classifies the omitted compound. In a second step, the first compound is included and the second one is removed, and so on, and the procedure goes on until the last compound is removed.

For the coefficients entering into the discrimination functions, the classification and cross-validation matrixes are given in Tables 4 and 5, respectively. The error obtained with the classification and the cross-validation matrices were relatively small: 7.6% and 15.2%, respectively. The separation of the two groups is quite good and, using the SDA results, we come to the following conclusion: in attempting to separate new fluoroquinolones, the first step is to calculate the value of two variables obtained here with the SDA method; then substitute these values in the two discrimination functions obtained in this work; and the last step is to check which discrimination function gives the higher value. If the higher value belongs to the discrimination function of the active group, the new fluoroquinolone is active, and the same for the inactive group.

3.3. HCA of compounds

In this study, we chose Ward's method [25] to classify a set of 13 compounds. The method provided an informative dendrogram depicted in Fig. 4. The HCA and PCA methods are complementary. Indeed, and from Figure 4, we can notice that the HCA and PCA results were quite similar for 13 studied fluoroquinolones: HCA and PCA classified 13 fluoroquinolones under study exactly in the same way.

4. Conclusions

The methods of PCA, HCA and SDA show that the PK/PD characteristics of fluoroquinolones have great relationships with the Q_B, log P, Q_5 and VOL parameters. Since the antibacterial efficacy *in vivo* can be predicted using the PK/PD characteristics, these parameters are also related to the biological activity of fluoroquinolones. The PCA method shows that 13 fluoroquinolones studied can be classified into two groups (I and II) according to their degree of antipneumococcal activity. The variables Q_B, Q_5 and VOL are responsible for the separation between the molecules with higher (Group I) and lower (Group II) antipneumococcal activity. Two significant variables extracted using the SDA method are VOL and Q_5. The low error of the SDA shows that Groups I and II were well separated, and that this method provides a reliable rule for classifying new fluoroquinolones with respect to the antipneumococcal activity.

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КОЛИЧЕСТВЕННАЯ СВЯЗЬ МЕЖДУ МОЛЕКУЛЯРНОЙ СТРУКТУРОЙ И ХАРАКТЕРСТИКАМИ ФАРМАКОКИНЕТИКИ/ФАРМАКОДИНАМИКИ ДЛЯ ФТОРОХИНОЛОНОВ

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Методы установления количественной связи между молекулярной структурой и характеристиками фармакокинетики/фармакодинамики химических соединений в сочетании с информационным хемометрическим подходом использованы для классификации фторохинолонов (ФХ) в отношении их противопневмококковой активности (ППА). Набор молекулярных дескрипторов для 13 ФХ был рассчитан с помощью теории функциональной плотности и проанализирован применительно к ППА данных соединений методами анализа главных компонент (АГК), иерархического кластерного анализа (ИКА) и пошагового дискриминантного анализа (ПДА). АГК и ПДА использованы для уменьшения размерности пространства признаков и нахождения минимального эффективного набора дескрипторов для оценки ППА. Показано, что надежное разделение 13 ФХ на активные и неактивные может быть обеспечено на основе определения заряда кольца B (Q_B), молекулярного объема (VOL), и коэффициента распределения (Log *P*).