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Structure-Activity Relationship of Fluoroquinolones Against *K.pneumoniae*Xiao-hong Li^{a,b*}, Rui-zhou Zhang^a, Xin-lu Cheng^b, Xiang-dong Yang^b*a.* College of Science, Henan University of Science and Technology, Luoyang 471003, China; *b.* Institute of Atom and Molecular Physics, Sichuan University, Chengdu 610065, China

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The structure-activity relationship of fluoroquinolones, which show anti-*K. pneumoniae* activity, was studied by using principal component analysis (PCA) and hierarchical cluster analysis (HCA). The PCA results showed that the lowest unoccupied molecular orbital energy, energy difference between the highest occupied and the lowest unoccupied molecular orbital, dipole moment, net atomic charge on atom I, molecular polarizability, partition coefficient and molecular refractivity of these compounds are responsible for the separation between high-activity and low-activity groups. The HCA results were similar to those obtained with PCA. By using the chemometric results, four synthetic compounds were analyzed through PCA and HCA, and three of them are proposed as active molecules against *K. pneumoniae* which is consistent with the results of clinical experiments. The methodologies of PCA and HCA provide a reliable rule for classifying new fluoroquinolones with anti-*K. pneumoniae* activity.

Key words: Structure-activity relationship, DFT, PCA, HCA

I. INTRODUCTION

Ocular infections, both superficial and deep, such as conjunctivitis, corneal ulcers and endophthalmitis, are caused by a diverse group of bacterial, viral, and fungal pathogens. Accordingly, the armamentarium of available antimicrobials used in the prevention and treatment of these infections includes antivirals, antifungals, and antibacterials. Common topical antibacterials used in the treatment of ocular infectious diseases include sulfonamides, aminoglycosides, polymyxin-based combinations and fluoroquinolones [1]. The fluoroquinolones represent an expanding class of broad-spectrum antibacterials which cover a host of Gram-negative and anaerobic species responsible for ocular infections [2,3]. Fluoroquinolones are also effective against a variety of Gram-positive organisms [4-6]. *Klebsiella pneumoniae* is a Gram-negative, rod-shaped bacteria, and clinically the most important member of the *Klebsiella* genus of Enterobacteriaceae. *K. pneumoniae* can cause bacterial pneumonia, though it is more commonly implicated in hospital-acquired urinary tract and wound infections, particularly in immunocompromised individuals. *K. pneumoniae* is an increasingly nosocomial infection as antibiotic resistant strains continue to appear. Structure-activity relationships (SAR) and quantitative SAR (QSAR) studies have been used extensively to correlate molecular structures to their biological activities [7-12]. A primary goal of QSAR/SAR methods is to find rules that can lead to reliable classifications and predictions of the biological activity for tested, untested or hypothetical compounds. The ob-

tained information can be used for the selection or design of better structures.

In order to investigate the relationship between some molecular properties and their anti-*K. pneumoniae* activity for fluoroquinolones by using quantum chemical and chemometric methods and afterwards to propose new fluoroquinolones that may be potentially active against *K. pneumoniae*, principal component analysis (PCA) and hierarchical cluster analysis (HCA), were employed in this work to analyze the data set, which are extremely useful to classify the molecules into groups that can be correlated to their anti-*K. pneumoniae* activities.

II. COMPUTATIONAL METHODS

A. Calculation of the theoretical descriptors of molecular properties

Except for the steric and hydrophobic features, all the results were obtained with the GAUSSIAN 03 series of programs [13], using the standard 3-21G basis set. Electron correlation was partially taken into account by means of density functional theory (DFT) [14] and by using GAUSSIAN 03 version of the hybrid three-parameter functional developed by Becke [15-16] and denoted as B3LYP. The steric and hydrophobic features were calculated with the HyperChem 6.0 program. All the structures were fully optimized at B3LYP/3-21G. The basic structure of fluoroquinolones can be seen in Fig.1.

Structural descriptors of compounds are usually correlated with biological activity. In this work we calculated the following structural descriptors to be correlated with the biological activity: the highest occupied molecular orbital energy (E_{HOMO}), the lowest unoccupied molecular orbital energy (E_{LOMO}), energy differ-

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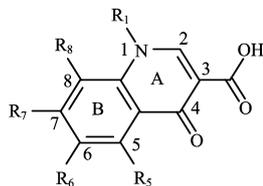


FIG. 1 The basic structure of fluoroquinolones.

ence between the highest occupied and the lowest unoccupied molecular orbital (ΔE_{HL}), dipole moment (μ), molecular softness ($1/\eta$), net atomic charge on atom I (Q_i), net charge on ring (Q_N), Mulliken electronegativity (χ), molecular polarizability (MP), partition coefficient ($\lg P$), molecular refractivity (MR), molecular volume (VOL) and surface area (A).

The calculated parameters were selected so that they represent the electronic (E_{HOMO} , E_{LUMO} , ΔE_{HL} , χ , μ , $1/\eta$, Q_i , Q_N , MP , MR), steric (VOL , A) and hydrophobic ($\lg P$) features of the compounds studied. These features are thought to be important for investigating the SAR of a drug [17-19].

B. Analysis method

Principal components analysis is a statistical technique that seeks to find a few mutually orthogonal linear combinations of the original variables which capture most of the variability present within the original system. In other words, a large part of the variance can usually be captured with a small number of components. In PCA, the first rows are standardized (unit variance, zero mean) to give a square matrix of moment correlations 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 the 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 independent principal components are ranked in terms of the amount of total variance each component explains.

HCA together with principal components, has become another important tool in chemometrics [20]. Hierarchical clustering methods takes as input either a similarity matrix between a set of items, or some attributes describing the items. The result of HCA 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 further the node is from the tree root, the more similar the items are under the node.

III. RESULTS AND DISCUSSION

A. Pre-processing of the molecular descriptors

Before applying the PCA technique, each of the variables was autoscaled so that they could be compared

to each other in the same scale. This method is very important because each variable is weighed equally and this provides a measure of the ability of a descriptor to discriminate classes of compounds [21].

B. Principal component analysis

In the application of PCA to the compounds listed in the Fig.2, several attempts were made to obtain a separation of the active compounds from the inactive compounds. The best separation was obtained when the 21 initial variables were reduced to 12 final variables. This suggests that the other 9 variables are not so important for classifying these compounds. Moreover, from the 12 variables selected, we obtained the correlation matrix between these variables and the respectively calculated values. This correlation matrix is presented in Table I. From Table I, we can see that the variables are correlated to each other (we considered correlated variables that possess correlation coefficients above 0.70), and according to the results shown in Table I, only 9 variables are important for the separation between active and inactive compounds. The best separation was obtained by using the following variables: E_{LUMO} , ΔE_{HL} , μ , Q_3 , Q_5 , Q_7 , Q_8 , Q_A , $\lg P$, MR , A , MP , whose values are presented in Table II.

The PCA results show that the first principal component explained 24.37% of the total variance. The first two principal components explained 48.235% of the total variance. The first three principal components explained 67.368% of the total variance. The first four and five principal components explained 80.143% and 91.644% of the total variance, respectively. The eigenvalues of the five principal components are 2.193, 2.148, 1.722, 1.15 and 1.035, respectively. According to the principle of choosing eigenvalues, the eigenvalue of each principal component is larger than 1 and the total variance is larger than 85%, so we chose the five principal components. Table III shows the loading vectors for PC1, PC2, PC3, PC4 and PC5.

According to Table III, PC1 can be expressed through the following equation:

$$\begin{aligned} \text{PC1} = & 0.359E_{LUMO} + 0.604\Delta E_{HL} + 0.5\mu \\ & + 0.094Q_3 - 0.127Q_5 + 0.623Q_A \\ & - 0.733\lg P - 0.167MR - 0.687MP \end{aligned}$$

From this equation, we can see that the more active molecules can be obtained when we have bigger values for the variables E_{LUMO} , ΔE_{HL} , μ , Q_3 and Q_A , combined with smaller values of Q_5 , $\lg P$, MR and MP . These characteristics can be useful in the design of new compounds with high anti-*K. pneumoniae* activity, as some of these variables can be related to properties such as strength of molecular association by electrostatic interaction.

The plot of the score vectors for the first two principal components (PC1×PC2) is shown in Fig.3. From

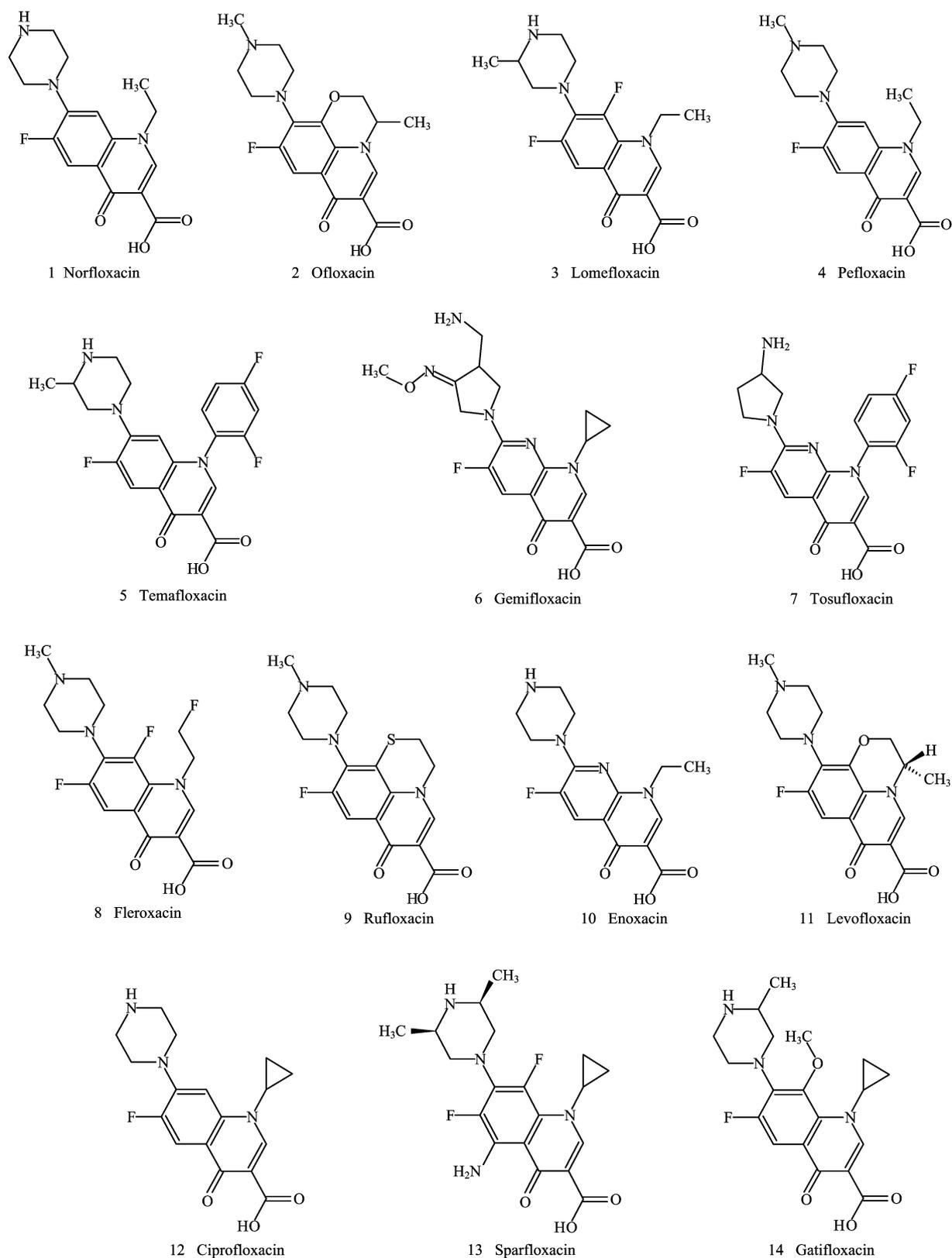


FIG. 2 Molecular structural schematic diagrams of fourteen fluoroquinolones.

TABLE I Correlation matrix between the selected variables

	E_{LUMO}	ΔE_{HL}	μ	Q_3	Q_5	Q_7	Q_8	Q_A	$\lg P$	MR	A	MP
E_{LUMO}	1.000	0.09	0.49	-0.003	0.38	-0.24	0.43	-0.21	-0.44	0.48	-0.63	-0.10
ΔE_{HL}		1.00	0.11	-0.077	0.20	0.63	-0.66	0.56	-0.18	-0.41	0.06	-0.17
μ			1.00	0.021	-0.43	0.07	-0.04	-0.06	-0.14	-0.21	-0.20	-0.28
Q_3				1.00	0.13	0.18	-0.24	0.31	-0.16	0.08	0.10	0.18
Q_5					1.00	0.02	0.14	0.04	-0.05	0.35	-0.12	0.31
Q_7						1.00	-0.77	0.89	-0.09	-0.50	0.32	0.00
Q_8							1.00	-0.78	0.03	0.57	-0.31	0.07
Q_A								1.00	-0.44	-0.26	-0.00	-0.17
$\lg P$									1.00	-0.49	0.84	0.50
MR										1.00	-0.60	0.26
A											1.00	0.56
MP												1.00

TABLE II Values obtained for the nine most important properties (descriptors) that classified the 14 compounds of the training set as active and inactive molecules^a

Compound	E_{LUMO}	ΔE_{HL}	μ	Q_3	Q_5	Q_A	$\lg P$	MR	MP	MIC ₉₀ /(mg/L)[22-32]
1	-0.0416	0.1583	7.5264	-0.2062	-0.1966	-0.1248	4.76	47.98	31.81	0.39
2	-0.0400	0.1354	7.7856	-0.2045	-0.2051	-0.1433	4.77	57.40	35.35	0.12
3	-0.0445	0.1476	6.1790	-0.2845	-0.1982	-0.2156	5.42	52.30	33.56	0.25
4	-0.0393	0.1470	8.0862	-0.2059	-0.1970	-0.1293	5.12	53.27	33.65	2.00
5	-0.0507	0.1403	6.7175	-0.2092	-0.1981	-0.2471	7.08	46.23	39.46	0.25
6	-0.0437	0.1609	6.9291	-0.2052	-0.1501	0.1448	4.25	6.64	37.41	0.12
7	-0.0505	0.1551	6.3508	-0.2071	-0.1478	0.0495	6.05	40.68	36.91	0.20
8	-0.0506	0.1386	4.9416	-0.2022	-0.1975	-0.1493	5.19	53.03	33.47	0.39
9	-0.0521	0.1394	4.2886	-0.2074	-0.2043	-0.0954	4.80	59.15	35.88	0.19
10	-0.0478	0.1494	6.8119	-0.2041	-0.1450	0.1795	4.43	47.08	31.11	1.56
11	-0.0401	0.1361	7.7210	-0.2044	-0.2050	-0.1449	4.77	57.40	35.35	0.06
12	-0.0406	0.1498	9.6695	-0.2187	-0.1983	-0.1553	4.81	50.44	32.88	0.10
13	-0.0368	0.1489	4.6780	-0.2045	0.3471	-0.1430	5.02	62.51	37.81	0.06
14	-0.0422	0.1386	7.5802	-0.2077	-0.2055	-0.1785	4.99	61.28	37.18	0.10

^a The units of energy and charge are all atomic unit.

TABLE III The loading vectors for PC1, PC2, PC3, PC4 and PC5

	PC1	PC2	PC3	PC4	PC5
E_{LUMO}	0.359	0.747	-0.326	0.339	0.260
ΔE_{HL}	0.604	-0.240	0.430	0.538	0.103
μ	0.500	-0.049	-0.643	0.048	0.544
Q_3	0.094	0.182	0.404	-0.601	0.585
Q_5	-0.127	0.579	0.567	0.458	0.034
Q_A	0.623	-0.221	0.645	-0.194	0.0015
$\lg P$	-0.733	-0.510	-0.049	0.294	0.306
MR	-0.167	0.904	-0.034	-0.205	-0.212
MP	-0.687	0.185	0.335	0.079	0.423

Fig.3, it can be seen that fluoroquinolones studied are separated into two groups, inactive group and active group against *K. pneumoniae*, when we used

the variables E_{LUMO} , ΔE_{HL} , μ , Q_3 , Q_5 , Q_7 , Q_8 , Q_A ,

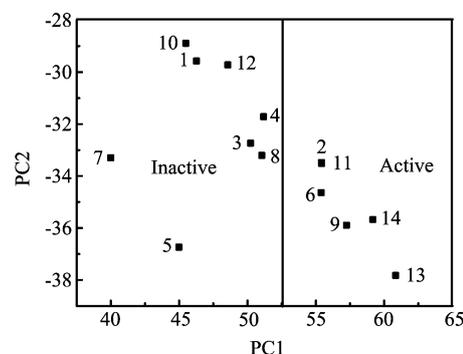


FIG. 3 PCA score for the 14 fluoroquinolones with anti-*K. pneumoniae* activity. The PC analysis leads to a separation into two groups: inactive group and active group.

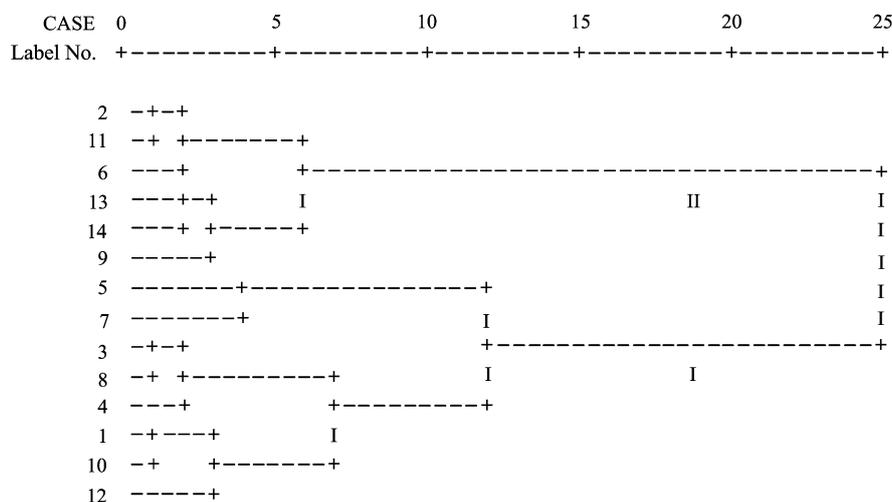


FIG. 4 Dendrogram of the training set, which are separated into two groups: I (inactive) and II (active).

$\log P$, MR , A and MP to obtain the separation.

C. Hierarchical cluster analysis

In this study, we chose Ward's method [21] to separate the compounds. The method provided an informative dendrogram shown in Fig.4. The HCA separated the compounds in a way similar to that of PCA. We can see that the two groups I and II of Fig.4 are the same groups: the inactive and the active group of Fig.3 in the PCA. That is to say, HCA and PCA classified the 14 fluoroquinolones under study in the same way.

Starting from the separation of the 14 compounds (training set), we propose four synthetic compounds for analysis (test set). With the application of PCA and HCA methods of pattern recognition, we obtained a classification of 18 compounds in two groups. According to the method of PCA, the active compounds are expected to be 15, 16 and 18, and the inactive ones 17. Figure 5 gives the separation of the test set into two groups by using PCA.

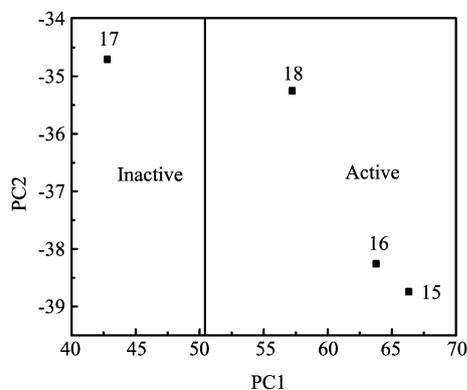
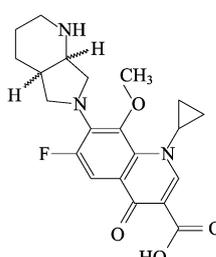
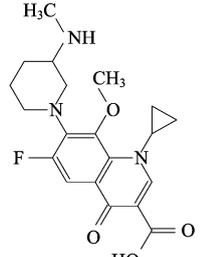
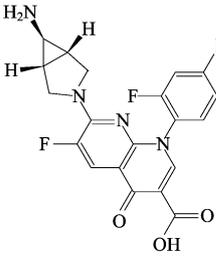
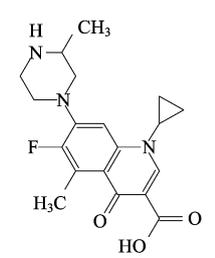


FIG. 5 PCA score for the 4 fluoroquinolones (test set) with anti-*K. pneumoniae*.

With the application of the HCA method, the result is the same as with PCA. Three compounds were classified as active by the two methods. The results of this classification are summarized in Table IV. From the results of clinical experiments, we know that the activities [33-36] in anti-*K. pneumoniae* for the four compounds are about 8.3 L/mg (Moxifloxacin), 5.0 L/mg (Balofloxacin), 4.0 L/mg (Trovafoxacin), and 10.0 L/mg (Grepafloxacin), respectively, which is nearly consistent with our classification result.

TABLE IV The results of two pattern recognitions for four synthetic compounds: active compound (+) and inactive compound (-)

Compound	PCA	HCA	Compound	PCA	HCA
	+	+		+	+
15 Moxifloxacin			16 Balofloxacin		
	-	-		+	+
17 Trovafoxacin			18 Grepafloxacin		

IV. CONCLUSION

The application of PCA and HCA in fluoroquinolones with activity in anti-*K. pneumoniae* showed that the compounds studied in this work can be correctly classified into two groups: active and inactive molecules. The PCA results showed that the variables E_{LUMO} , ΔE_{HL} , μ , Q_3 , Q_5 , Q_A , $\log P$, MR and MP are responsible for the separation of active and inactive compounds. The HCA results were similar to those obtained with PCA, i.e. both methods classified the 14 fluoroquinolones the same way. With the application of the PCA and HCA to other 4 synthetic compounds, the results of PCA and HCA are the same, compound 15, 16 and 18 are active and 17 are inactive, which is consistent with the results of clinical experiments. So the methodologies of PCA and HCA provide a reliable rule for classifying new fluoroquinolones with anti-*K. pneumoniae* activity. This also provides a theoretical basis for reasonable pharmaceutical molecular design.

V. ACKNOWLEDGMENT

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