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RESEARCH ARTICLE

# QSAR study on fluoroquinolones as antibacterial agents active for *Pseudomonas aeruginosa*

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**Density functional theory (DFT) was used to calculate the properties of a set of molecular descriptors for 14 fluoroquinolone with anti-*Pseudomonas aeruginosa* activity. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed in order to reduce dimensionality and investigate the effectiveness of variables, i. e., which subset of variables should be more effective for classifying fluoroquinolones according to their antibacterial activities against *P. aeruginosa*. The PCA results showed that the variables $E_{LUMO}$, $\Delta E_{HL}$, $Q_5$, $Q_6$, log*P*, *MR*, and *MP* are responsible for the separation between compounds with higher and lower anti-*P. aeruginosa* activity. The HCA results were similar to those obtained using PCA. By using the chemometric results, four synthetic compounds were analyzed through the PCA and HCA. Two of them are proposed as active molecules against *P. aeruginosa*. The result is consistent with the observations of clinic experiments. The methodologies of PCA and HCA provide a reliable rule for classifying new fluoroquinolones with anti-*P. aeruginosa* activity.**

**Keywords** structure-activity relationship, density functional theory (DFT), principal component analysis (PCA), hierarchical cluster analysis (HCA)

## 1 Introduction

*Pseudomonas aeruginosa* is widely distributed. It can grow in almost any aqueous habitat, including soil, surfact waters, sewage, plant, and various foods (such as leafy vegetables and fresh fruit juice). It is a common human intestinal bacterium. The bacterium can cause serious infections in patients who are mechanically ventilated, immunocompromised, and with malignancies or HIV infection [1]. Due to the fact that this pathogen reaches millions of people each year, both in developed countries and in developing ones, many studies were carried out on the main factors responsible for diseases caused by *P. aeruginosa*. Fluoroquinolones (FQs) are the only oral therapy available for *P. aeruginosa* infections, but resistance is increasingly prevalent [2]. The FQs are also the only synthetic antibacterial agents to rival $\beta$-lactams for impact in clinical usage in the antibacterial field [3]. Schaumann et al. discussed the activities of quinolones against obligately anaerobic bacteria [4]. In recently years, there has been a considerable interest in the development of new FQ agents [5,6].

Structure-activity relationships (SAR) and quantitative SAR (QSAR) studies have been extensively used to correlate molecular structures to their biological activities [7–9]. For example, Gupta investigated zinc-containing metalloproteinase inhibitors by using QSAR [10]; Al-masri et al. discovered DPP IV inhibitors by QSAR analysis [11].

A primary goal of QSAR/SAR methods is to find rules leading to reliable classifications and predictions of the biological activity for tested, untested, and hypothetical compounds. The obtained information can be used for the selection or the design of better structure. QSAR is based on the assumption that the structure of a molecule (i.e., its geometric and electronic properties) must contain the features responsible for its physical, chemical, and biological properties and on the ability to represent the chemical by one or more numerical descriptors [12].

The aim of the present work is to investigate the relationship between some molecular properties and their anti-*P. aeruginosa* activities for fluoroquinolones by using quantum chemistry and chemometric methods. New FQs are proposed afterwards to be potentially active against *P. aeruginosa*. The calculated molecular properties were selected so that some steric, electronic, and hydrophobic characteristics of these compounds could be taken into account since each one of them can contribute to the biological activity and can give information about the interactions between the compounds and the biological receptor. The principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed in this work to analyze the data set. They are extremely useful to classify the molecules into groups that can be correlated to their anti-*P. aeruginosa* activity.

## 2 Computational methods

### 2.1 Calculation of the theoretical descriptors of molecular properties

Except for the steric and hydrophobic features of the studied compounds, all the results were obtained with the

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GAUSSIAN 03 series of programs [13] using the standard 6-31G* basis set. Electron correlation was partially taken into account by means of density functional theory (DFT) [14] developed by Lee et al. and Becke [15,16] and denoted as B3LYP. All the structures have been fully optimized at B3LYP/6-31G*. The normal mode analysis for each structure resulted in no imaginary frequencies for the remaining 3N–6 vibrational degrees of freedom, where N is the number of atoms in the system. This result indicates that the structure of each molecule corresponds to a local minimum on the potential energy surface. The steric and hydrophobic features of the studied compounds were calculated using the HyperChem 6.0 program [17]. The optimized geometries were obtained by setting the gradient in the energy hyper surface to be lower (in module) than 0.01 kcal·mol$^{-1}$ in order to assure good quality results. The basic structure of fluoroquinolones can be seen in Figure 1.

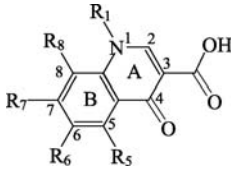



**Figure 1** The basic structure of fluoroquinolones.

Structural descriptors of compounds are usually correlated with biological activity. In this work, we calculated the following structural descriptors related to the biological activity under studies by using B3LYP/6-31G*: the highest occupied molecular orbital energy ($E_{HOMO}$); the lowest unoccupied molecular orbital energy ($E_{LUMO}$); energy difference between the highest occupied and the lowest unoccupied molecular orbital ($\Delta E_{HL}$); dipole moment ($\mu$); molecular hardness ($\eta$); net atomic charge on atom I ($Q_i$); net charge on ring ($Q_N$); Mulliken electronegativity ($\chi$); molecular polarizability (*MP*); partition coefficient (log*P*); molecular refractivity (*MR*); molecular volume (*VOL*); and surface area (*A*).

The calculated parameters were selected so that they represent the electronic ($E_{HOMO}$, $E_{LUMO}$, $\Delta E_{HL}$, $\chi$, $\mu$, $\eta$, $Q_i$, $Q_N$, *MR*, and *MP*), steric (*A*,*VOL*), and hydrophobic (log*P*) features of the studied compounds. These features are supposed to be important for investigating the SAR of drug [18–20].

### 2.2 Analysis method

PCA is a statistical technique that seeks to find a few mutually orthogonal linear combinations of original variables which capture most of the present variability within the original system. In other words, a large part of the variance can usually be captured using a small number of components [21]. In PCA, the first rows are standardized (unit variance, zero mean) to yield a square matrix of moment correlation coefficients between pairs of rows. The computation of the principal components of this matrix involves the calculation 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 independent principal components are ranked in terms of the amount of total variance explained by each component [22].

The Hierarchical cluster analysis (HCA) technique examines the distances between the samples in a dataset and represents this information as a two-dimensional plot named dendrogram. HCA has become another important tool in chemometrics together with principal components [23]. It is informative to examine the dendrogram in conjunction with PCA as they generate similar information in different forms. Hierarchical clustering methods 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 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.

## 3 Results and discussion

### 3.1 Preprocessing of the molecular descriptors

Before the application of the PCA technique to the 14 compounds under study, the first step for exploratory analysis of the dataset is the autoscaling of the variables, i.e., each variable is autoscaled so that they can be compared to each other on the same scale. Each variable is hence weighted equally. This important method provides a measure of a descriptor's ablity to discriminate classes of compounds [24].

### 3.2 Principal component analysis

In the application of PCA to the compounds listed in the Figure 2, several attempts were made to separate the active compounds from the inactive ones. The best separation was obtained when the 21 initial variables were reduced to 10 final variables. This suggests that the other 11 variables are not so important for classifying these compounds according to their anti-*P. aeruginosa* activity. Moreover, from the 10 selected

variables, we obtained the correlation matrix between these variables and the respective calculated values as presented in Table 1. The variables are not correlated to each other (we considered correlated variables those that possess correlation coefficients above 0.70). The best separation was obtained by using the following variables:$E_{LUMO}$, $\Delta E_{HL}$,$Q_5$, $Q_6$, log$P$,$MR$, and $MP$, whose values are presented in Table 2. Theese seven variables can be considered important for the separation between active and inactive compounds.

The PCA results show that the first principal component explained 35.55% of the total variance. The percentages for the first two, three and four principal components are 62.21%, 79.58%, and 88.93%, respectively. The eigenvalues of the four principal components are 2.489, 1.866, 1.216, and 1.035, respectively. According to the principle of choosing eigenvalues, the eigenvalues of each principal component is larger than 1 and the total variance is larger than 85%, hence we chose the first four principal components. Table 3 shows the loading vectors for PC1, PC2, PC3, and PC4.

According to Table 3, PC1 can be expressed through the following equation:

$$\begin{aligned}\mathrm{PC1} = {} & 0.640[E_{\mathrm{LUMO}}] - 0.591[\Delta E_{\mathrm{HL}}] + 0.141[Q_5] \\ & + 0.741[Q_6] - 0.549[\log P] + 0.91[MR] + 0.18[MP]\end{aligned}$$

From this equation, we can see that we could obtain more active molecules when we have bigger values for the variables $E_{LUMO}$, $MR$, $MP$, $Q_5$, and $Q_6$, combined with smaller values of $\Delta E_{HL}$ and log$P$. These characteristics can be useful in the design of new compounds with high anti-*P. aeruginosa* activity, as some of these seven variables can be related to properties such as strength of molecular association by electrostatic interaction ($E_{LUMO}$,$\Delta E_{HL}$,$Q_5$,$Q_6$, $MP$, and $MR$) and hydrophobicity (log$P$).

The energies of the frontier orbitals are important properties in several chemical and pharmacological processes. The reason is that these properties provide information on the electron donating and electron accepting character of a compound and consequently on the formation of a charge transfer complex. The energy of the highest occupied molecular orbital ($E_{HOMO}$) measures the electron donating character of a compound and the energy of the lowest unoccupied molecular orbital ($E_{LUMO}$) measures its electron accepting character [25–36]. From these definition, we have: (a) the greater the $E_{HOMO}$ is, the greater the electron donating capability will be; (b) the smaller the $E_{LUMO}$ is, the smaller the resistance to accept electrons will be [37]. For the active compounds studied in this work, we can say that their $E_{LUMO}$ should be big values, whereas the inactive compounds should be small values. This result means that the inactive compounds are more efficient electron acceptor compounds than the active ones, i.e., the inactive compounds may interact through charge transfer mechanism with some compounds before reaching the biological donor.

The HOMO-LUMO gap, i.e., the difference in energy between the $E_{LUMO}$ and $E_{LUMO}$, is an important stability index [38]. A large HOMO-LUMO gap implies high stability for the molecule in the sense of its lower reactivity in chemical reactions [39]. For the active compounds studied in this work, we can see that their $\Delta E_{HL}$ should be small values, whereas the inactive compounds present large values. This result means that the inactive compounds are more stable compounds than the active ones.

On the charge at atom $Q_5$ and $Q_6$, we would like to pay attention to the fact that for the active compounds it is important to have atoms with big charge at position 5 and 6. In other words, electron-donator atoms at $Q_5$ and $Q_6$ are required for the active compounds since they would react with an electron-acceptor biological donor.

The role of MR can be ambivalent, i.e., MR can represent dispersive forces that help the interaction between substituents and the biological receptor. In these cases, a positive coefficient is expected for MR. Other MR characters arise from the fact that MR can represent a measure of the volume. Therefore, it can measure the capacity of the substituent in distorting the conformation of the receptor and thus avoiding the interaction receptor-substratum. Hence a negative signal for MR coefficients reflects stereochemical hindrances [40,41]. For the studied active compounds, the molecular refractivity should be big values, indicating that some substituents in the active compounds can interact with polar groups of the biological receptor or some modifications on the receptor can occur due to the size of these substituents, in order to avoid the interaction between the fluoroquinolone derivatives and biological receptor.

Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes. Drugs with values much greater than 1 are classified as lipophilic, whereas those with partition coefficients much less than 1 are indicative of a hydrophilic drug. From Table 3, we can see that the partition coefficient should be low values for the studied active compounds, which means that the active compounds are more hydrophilic drug than the inactive ones.

Since most of the variances are explained by the first two PCs, their score plot is a reliable representation of the spatial distribution of the points for the studied data set. The plot of the score vectors of the first two principal components (PC1 × PC2) is shown in Fig.3. In Figure 3, when we used the variables $E_{LUMO}$, $\Delta E_{HL}$, $Q_5$, $Q_6$, log$P$, $MR$, and $MP$ to obtain

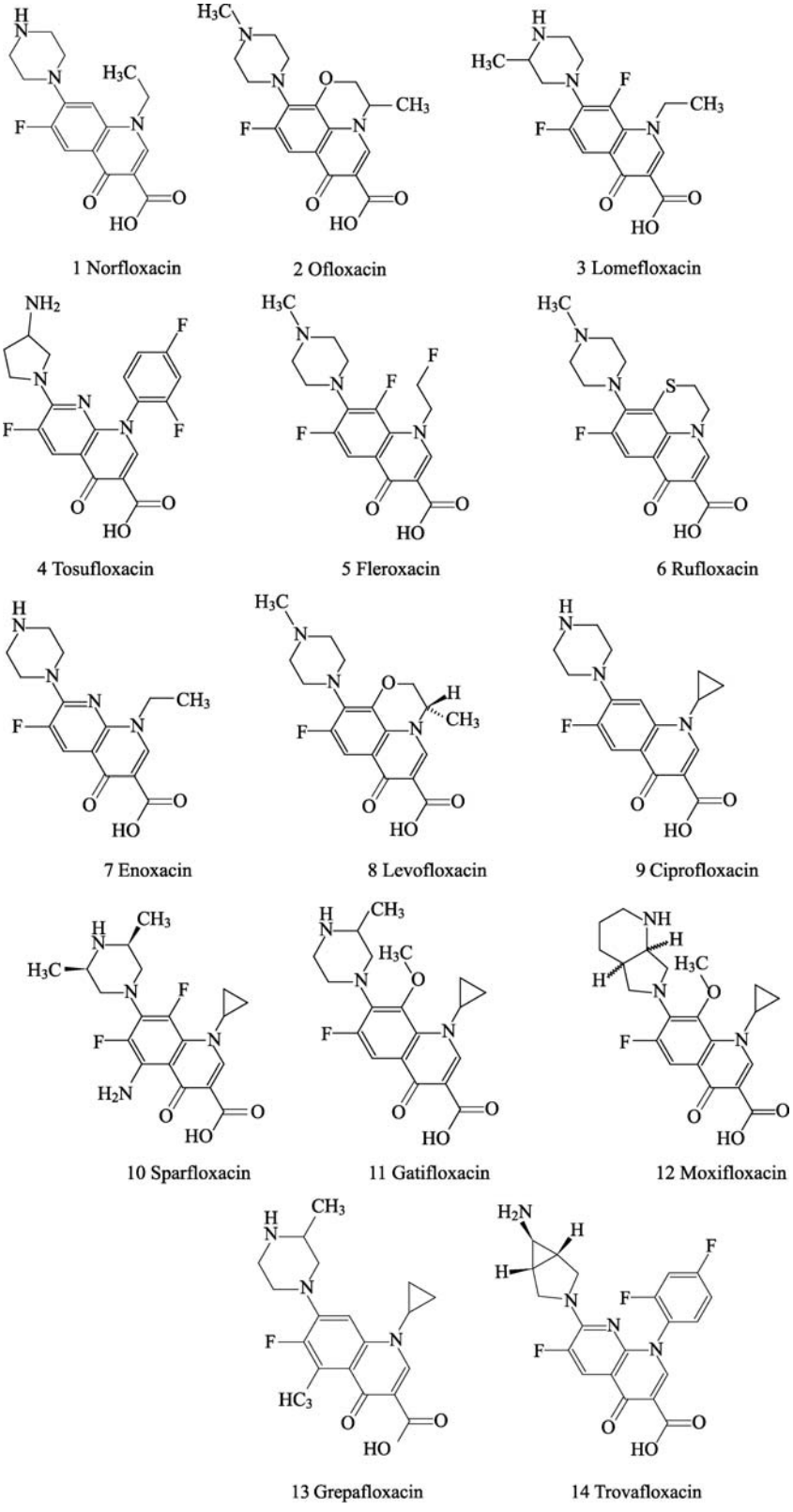



**Figure 2** Molecular structural schematic diagrams of fourteen fluoroquinolones.

**Table 1** Correlation matrix between the selected variables

| | $E_{LUMO}$ | $\Delta E_{HL}$ | $Q_5$ | $Q_6$ | $Q_7$ | $Q_8$ | $Q_A$ | log$P$ | $MR$ | $MP$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $E_{LUMO}$ | 1.000 | −0.002 | 0.403 | 0.275 | −0.479 | 0.563 | −0.557 | −0.300 | 0.557 | 0.105 |
| $\Delta E_{HL}$ | | 1.000 | 0.215 | −0.446 | 0.501 | −0.562 | 0.338 | 0.412 | −0.394 | 0.036 |
| $Q_5$ | | | 1.000 | −0.188 | −0.001 | 0.079 | −0.008 | 0.091 | 0.237 | 0.295 |
| $Q_6$ | | | | 1.000 | −0.929 | 0.628 | −0.840 | −0.233 | 0.617 | 0.007 |
| $Q_7$ | | | | | 1.000 | −0.783 | 0.895 | 0.430 | −0.714 | 0.011 |
| $Q_8$ | | | | | | 1.000 | −0.844 | −0.278 | 0.720 | 0.169 |
| $Q_A$ | | | | | | | 1.000 | 0.094 | −0.638 | −0.167 |
| log$P$ | | | | | | | | 1.000 | −0.340 | 0.485 |
| $MR$ | | | | | | | | | 1.000 | 0.486 |
| $MP$ | | | | | | | | | | 1.000 |

**Table 2** Values obtained for the seven most important properties that classified the 14 compounds of the training set as active and inactive molecules and the values of $MIC_{90}$ for the compounds

| compound | $E_{LUMO}$ | $\Delta E_{HL}$ | $Q_5$ | $Q_6$ | log$P$ | $MR$ | $MP$ | $MIC_{90}/(ug \cdot mL^{-1})$ [23] |
|---|---|---|---|---|---|---|---|---|
| 1 | −0.042 | 0.16 | −0.197 | 0.285 | 4.76 | 47.98 | 31.81 | 16 |
| 2 | −0.040 | 0.14 | −0.205 | 0.284 | 4.77 | 57.40 | 35.35 | 8 |
| 3 | −0.045 | 0.15 | −0.198 | 0.296 | 5.42 | 52.30 | 33.56 | 16 |
| 4 | −0.050 | 0.16 | −0.148 | 0.238 | 6.05 | 40.68 | 36.91 | 2 |
| 5 | −0.051 | 0.14 | −0.198 | 0.298 | 5.19 | 53.03 | 33.47 | 32 |
| 6 | −0.052 | 0.14 | −0.203 | 0.314 | 4.8 | 59.15 | 35.88 | –* |
| 7 | −0.048 | 0.15 | −0.145 | 0.229 | 4.43 | 47.08 | 31.11 | 32 |
| 8 | −0.040 | 0.14 | −0.205 | 0.287 | 4.77 | 57.40 | 35.35 | 8 |
| 9 | −0.041 | 0.15 | −0.198 | 0.281 | 4.81 | 50.44 | 32.88 | 4 |
| 10 | −0.037 | 0.15 | 0.347 | 0.267 | 5.02 | 62.51 | 37.81 | 16 |
| 11 | −0.042 | 0.14 | −0.206 | 0.284 | 4.99 | 61.28 | 37.18 | 8 |
| 12 | −0.041 | 0.15 | −0.195 | 0.286 | 5.11 | 68.68 | 40.08 | 16 |
| 13 | −0.037 | 0.15 | −0.035 | 0.299 | 5.54 | 59.47 | 36.55 | 16 |
| 14 | −0.052 | 0.15 | −0.148 | 0.238 | 5.81 | 43.38 | 37.98 | 16 |

*: no experimental value

**Table 3** The Loading Vectors for PC1, PC2, PC3, and PC4

| | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| $E_{LUMO}$ | 0.640 | 0.353 | −0.500 | 0.269 |
| $\Delta E_{HL}$ | −0.591 | 0.451 | −0.330 | 0.492 |
| $Q_5$ | 0.141 | 0.721 | −0.453 | −0.304 |
| log$P$ | −0.549 | 0.546 | 0.498 | 0.207 |
| $MP$ | 0.180 | 0.771 | 0.530 | −0.149 |
| $Q_6$ | 0.741 | −0.214 | 0.322 | 0.428 |
| $MR$ | 0.910 | 0.284 | 0.142 | −0.017 |

the separation, the studied fluoroquinolones are separated into two groups, the inactive group and the active group against anti-*P. aeruginosa*. It can be also seen that PC1 alone is responsible for the separation of the compounds with higher and lower anti-*P. aeruginosa*. In Figure 3, we can see that the 14 studied fluoroquinolones are separated into two groups: the active compounds (compounds 2, 6, 8, 10, 11, 12, and 13 in Figure 2) and the inactive compounds (compounds 1, 3, 4, 5, 7, 9, and 14 in Figure 2).

### 3.3 Hierarchical cluster analysis

In this study, we chose Ward's method [23] to separate the compounds. The method provided an informative dendro-

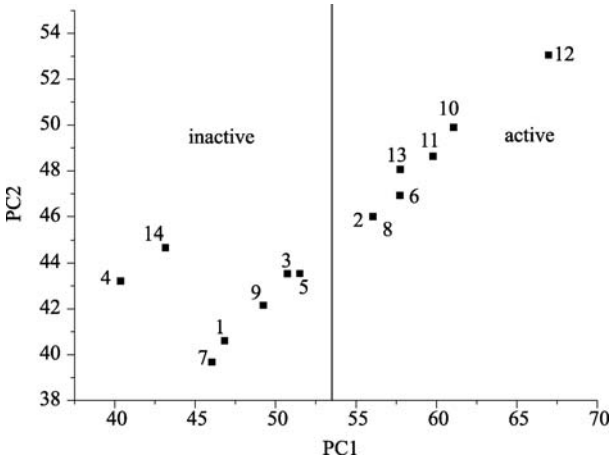



**Figure 3** PCA score (PC1 and PC2) for the 14 fluoroquinolones with anti-*P. aeruginosa* activity. The PC analysis leads to a separation into two groups: inactive group and active group.

gram as shown in Figure 4. The HCA and PCA methods are complementary, and the HCA separated the compounds in a similar way to the separation of PCA for the 14 fluoroquinolones studied in this work. We can see that the two groups I and II in Figure 4 are the same groups as the inactive and the active in Figure 3 in the PCA. That is to say that HCA and PCA classified the 14 fluoroquinolones under study in exactly the same way.

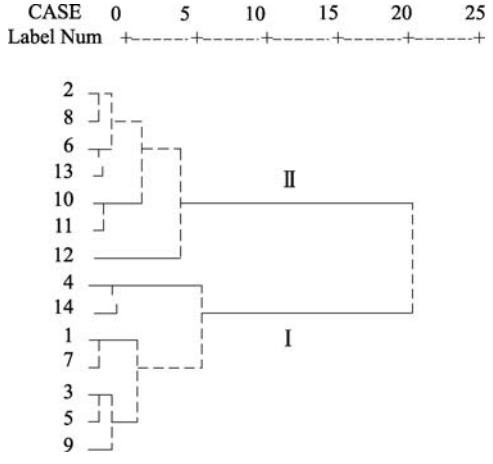



**Figure 4** Dendrogram of the training set, separated into two groups: I (inactive) and II (active).

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

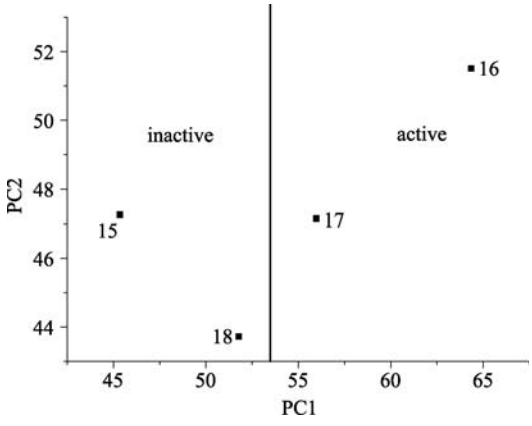


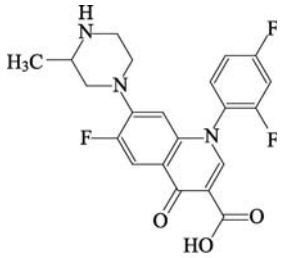
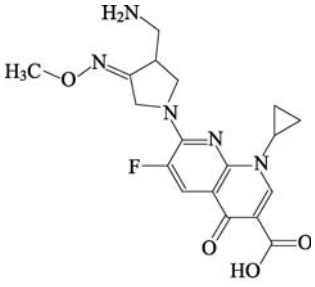
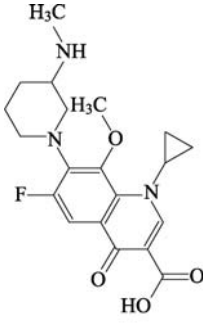
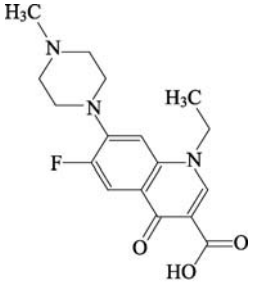

**Figure 5** PCA score (PC1 and PC2) for the four fluoroquinolones (test set) with anti-*P. aeruginosa*.

With the application of the HCA method, the result is same as that of PCA. Two compounds were classified as active by using the two methods. The results of this classification are summarized in Table 4. The clinic experiment results show that the activities [42–45] against *P. aeruginosa* of the four compounds are 0.03 $mL \cdot \mu g^{-1}$ (Temafloxacin), 0.125 $mL \cdot \mu g^{-1}$ (Balofloxacin), 0.125 $mL \cdot \mu g^{-1}$ (Gemifloxacin), and 0.06 $mL \cdot \mu g^{-1}$ (Pefloxacin), respectively. The data is consistent with our classification result.

## 4 Conclusions

The application of PCA and HCA in fluoroquinolones with activity against *P. aeruginosa* showed that the compounds studied in this work can be correctly classified into two groups: the active and the inactive molecules. The PCA results showed that the variables $E_{LUMO}$, $\Delta E_{HL}$, $Q_5$, $Q_6$, log*P*, *MR*, and *MP* are responsible for the separation between active and inactive compounds. The HCA results were similar to those obtained using PCA. Both methods classified the 14 fluoroquinolones under study in exactly the same way. With the application of the PCA and HCA to other four synthetic compounds, the results of PCA and HCA are the same: compounds 16 and 17 are active, and 15 and 18 are inactive. The result is consistent with that of clinic experiment. Therefore, the methodologies of PCA and HCA provide a reliable rule for classifying new fluoroquinolones with anti-*P. aeruginosa* activity. This study also provides a theoretical basis for reasonable pharmic molecular design.

**Table 4** The results of two pattern recognitions for four synthetic compounds: active compound (+) and inactive compound (−)

| compound | PCA | HCA | compound | PCA | HCA |
|---|---|---|---|---|---|
|  15 Temafloxacin | − | − |  17 Gemifloxacin | + | + |
|  16 Balofloxacin | + | + |  18 Pefloxacin | − | − |

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