Quantitative Structure—Activity Relationship Model for Prediction of Genotoxic Potential for Quinolone Antibacterials

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Antibiotics are of concern because of their widespread usage, their potential role in the spread and maintenance of bacterial resistance, and because of the selection pressure on microbes. In this study, the genotoxic potential of 20 quinolone antibacterials, including 5 first-generation, 8 second-generation, and 7 third-generation quinolones, was determined. While all of the antibacterials studied showed genotoxic potential, the molar concentration for each antibacterial that produces 10% (EC10) of the maximum response of corresponding antibacterial ranged from 0.61 to 2917.0 nM, and was greatly dependent on chemical structures. A quantitative structure—activity relationship (QSAR) was established by applying a quantum chemical modeling method to determine the factors required for the genotoxic potential of quinolone antibacterials. The octanol—water coefficient (logPow) adjusted by the pH and energies of the highest occupied molecular orbital (EHOMO) and lowest unoccupied molecular orbital (ELUMO) were selected as hydrophobic and electronic chemical descriptors, respectively. The genotoxic potentials of quinolone antibacterials were found to be dependent on their logPpow and EHOMO, while the effects of ELUMO on the genotoxic potentials could not be identified. The QSAR model was also used to predict the genotoxic potentials for 14 quinolone antibacterials, including 1 second-generation, 2 third-generation, and 11 fourth-generation quinolone antibacterials. A correlation between the genotoxic potentials and their minimal inhibition concentrations (MIC50) against Streptococcus pneumoniae from the literature for 18 quinolone antibacterials was observed, providing a potential method to estimate MIC50.

Introduction

Scientists have become increasingly concerned about the potential health and ecological hazards of exposure to pharmaceutical residues in the environment (1). Antibiotics are the most often discussed pharmaceuticals because of their widespread usage against diseases in human and veterinary as well as in industrial farming, and their potential role in the spread and maintenance of resistance of bacterial pathogens, in addition to their ecotoxicity (2–6). Antibiotics are discharged into the environment through domestic sewage, municipal wastewater treatment, and hospital wastewater, and several antibiotics have been detected in wastewater effluents, river waters, and groundwaters (7–9). The introduction of antibiotics into the environment may exert selection pressure on microorganisms, and thus change the antibiotic susceptibility of the microbes and/or change the predominant microbial species.

Quinolone antibacterials, one of the most powerful classes of antibiotics, were initially employed in the treatment of gram-negative urinary tract infections in humans and animals (10, 11). Of the quinolone antibacterials, fluoroquinolones, which are piperazinyl derivatives of quinolone, are currently commonly used in the treatment of a wide variety of diseases due to their broad spectrum of antimicrobial activity; not only are they effective against gram-negative bacteria but they are also moderately active against gram-positive bacteria (12). Extensive clinical use has led to increasing resistance to fluoroquinolones, which is common among Staphylococcus spp and Pseudomonas aeruginosa and some initially more susceptible pathogens such as Escherichia coli and Salmonella spp (13–15). Some centers have reported that the rates of fluoroquinolone resistance in Enterobacteriaceae were above 50%, whereas they were once uniformly susceptible (16). To counteract such bacterial resistance, a new generation of fluoroquinolones such as gatifloxacin and moxifloxacin has been introduced (17). To effectively assess the exposure and effects of these early and recently developed quinolone antibacterials in different environmental compartments, the need for a rapid and sensitive screening technique has become apparent, as over 10 000 molecules have been patented (18). Early work on quinolone antibacterials found that the activity against bacteria is principally based on the inhibition of the bacterial DNA gyrase, thereby blocking DNA replication and inhibiting synthesis and cell division, leading to rapid cell death in susceptible organisms (19, 20). While early work on quinolone antibacterials focused on DNA gyrase, topoisomerase IV was found to be another cellular target for quinolone antibacterials. The inhibition of topoisomerase IV by quinolone antibacterials induces a slow decline in DNA synthesis by damaging DNA, which was evidenced by the induction of the SOS pathway for DNA repair. In the SOS pathway, bacteria can produce many defense proteins, the genes of which are normally in a repressed state when the bacteria are exposed to stress (19–20). Based on this mechanism, quinolone antibacterials should elicit genotoxicity as exemplified by the umuC genotoxieties of ciprofloxacin and norfloxacin (21), and the extent of mutation in the SOS pathway for DNA repair should reflect the antibacterial efficacy. In addition, quantitative structure activity relationship (QSAR) is invaluable as an initial screening tool for these chemicals prior to in vitro or in vivo assays, and there are inherent advantages in the use of such a technique in that QSAR can provide mechanistic information.

The SOS/umuC bioassay based on alterations in the induction of the SOS response as a consequence of DNA damage has been used to evaluate the ability of test substances or samples to induce DNA damage (22). In this study, we used a SOS/umuC bioassay to determine the genotoxic potential (the molar concentration of an agonist
which produces 10% of the maximum possible response for that agonist, i.e., EC_{10} of 20 quinolone antibacterials including 4 first-generation, 7 second-generation, and 4 third-generation quinolone antibacterials. In addition, the relationship between the quantitative structure indices and their genotoxic potentials (QSAR) was established by applying a quantum chemical modeling method. Finally, the genotoxic potentials of another 10 quinolone antibacterials including 1 third-generation, and 9 fourth-generation fluoroquinolones that show potential for clinical application were predicted using the developed QSAR model. The relationships between the detected and predicted genotoxic potentials and MIC_{50} (the minimum inhibitory concentration required to inhibit the growth of 50% of organisms) against \textit{S. pneumoniae} of 18 quinolone antibacterials were analyzed for the first time to provide a potential method to estimate the MIC_{50} values of quinolone antibacterials from genotoxic potentials.

**Experimental Section**

**Reagents and Chemicals.** The structures of the 20 chemicals examined are shown in Tables 1 and 2. Cinoxacin (100% pure), lomefloxacin hydrochloride (98.9% pure), pipemidic acid (99% pure), enoxacin (98% pure), ofloxacin (98.6% pure), danofloxacin (98.4% pure), enrofloxacin (99.9% pure), ciprofloxacin (99.9% pure), sarafloxacin (99.9% pure), difloxacin (98.4% pure), sparflaxin (98% pure), and feroxacin (99.9% pure) were purchased from Sigma (St. Louis, MO); norfloxacin (98.5% pure), oxolinic acid (99.0% pure), pefloxacin (99.0% pure), and flumequine (99.0% pure) were obtained from Dr. Ehrenstorfen (GmbH, Germany); levofloxacin (98% pure) was bought from Fluka (Canada); and nalidixic acid (99.5% pure) was from Acros Organics (New Jersey). Ampicillin and dimethylsulfoxide (DMSO) were obtained from Amresco (USA); D-glucose (99.5%) was from Sigma (St. Louis, MO); sodium dodecyl sulfate (98%) and trichloromethane were purchased from the Beijing Chemical Reagent Co. (China). Agar powder was from the Sino-American Biotechnology Co. (China), and tryptone was from Oxoid (England). 2-Nitrophenyl-(D-galactopyranoside) (ONPG) was purchased from the Tokyo Kasei Kogyo Co. (Japan), and 2-mercaptoethanol was from Farco Chemical Supplies (Hong Kong). LB broth was obtained from DIFCO (France). Sodium carbonate, acetone, disodium hydrogen, and phosphate dodecahydrate were from the Beijing Shiji Co. (Beijing, China). Potassium chloride was obtained from the Shuang Huan Shiji Co. (Beijing, China). All reagents used were of the purest grade available. DMSO stock solutions of all chemicals were prepared to 10 mg/L for pefloxacin, 12.5 mg/L for enrofloxacin, 20 mg/L for ofloxacin, 20 mg/L for levofloxacin, 25 mg/L for ciprofloxacin, 25 mg/L for sarafloxacin, 50 mg/L for lomefloxacin, 50 mg/L for norfloxacin, 50 mg/L for danofloxacin, 200 mg/L for nalidixic acid, 200 mg/L for flumequine, 200 mg/L for enoxacin, 250 mg/L for oxolinic acid, 400 mg/L for cinoxacin, 1000 mg/L for pipemidic acid, 20 mg/L for difloxacin, 20 mg/L for sparflaxin, 12 mg/L for feroxacin, 25 mg/L for gatifloxacin, and 300 mg/L for pipromidic acid.

**SOS/umu Bioassay.** To test the genotoxic potentials of quinolone antibacterials, a SOS/umu bioassay which is standardized for the determination of the genotoxic potential of water and wastewater as an ISO standard was performed as previously described (22). An assay for \textit{umuC} gene expression was carried out according to the procedure described by Oda et al. (23). The test strain \textit{Salmonella}
typhimurium TA1535/PSK1002 was provided by the Osaka Prefectural Institute of Public Health, Japan. In this strain, the multicopy plasmid pSK1002 bearing an umuC/lacZ gene fusion product was introduced into Salmonella typhimurium TA1535, and the umu operon was genetically regulated by the SOS genes recA and lexA. Overnight culture of the bacterial tester strains was carried out by Luria broth (LB) medium 5 μg/mL ampicillin with shaking (150 rpm) at 30 °C for 16 h. The overnight culture was diluted 100-fold with tryptone glucose ampicillin (TGA) medium and incubated at 30 °C until the bacterial density reached an absorbance level of 0.3 at 600 nm. DMSO solutions (3 L) diluted to the desired concentrations were then added to 300 μL of overnight culture. The mixture was incubated at 37 °C for 2 h with shaking. A 150 μL portion of the above culture was fractionated, and its absorbance at 595 nm was detected. The residual culture (100 μL) was added to 1 mL of B buffer solution containing 50 μL of SDS solution and 10 μL of CHCl₃. The enzymatic reaction was started by the addition of 40 μL of 4 mg/mL ONPG, and incubated for 20 min at 30 °C. Then the enzymatic reaction was stopped by adding 1 M Na₂CO₃ (500 μL). After the above solution was centrifuged, 150-μL aliquots were placed into 96 wells of a microplate. Absorbances at 415 and 570 nm were read on a microplate reader (Bio RAD 550, USA) to estimate the β-galactosidase activity (U), which was calculated according to eq:

\[
\beta\text{-galactosidase activity (U)} = \frac{1000(OD_{415} - 1.75 \ OD_{570}) \ t \ \times \ \ OD_{595}}{t}
\]

where \(t\) represents the reaction time (min), \(v\) is the volume of the culture used in the assay (mL), \(OD_{595}\) is the cell density at the start of the assay, \(OD_{415}\) is the absorbance of o-nitrophenol at the end of the reaction, and \(OD_{570}\) is the light scattering at the end of the reaction. In this assay, DMSO was taken as a solvent control, and 4-NQO was taken as positive control. The results represented means of triplicate determinations.

**Molecular Descriptors.** The quantum mechanical properties such as the energies of the highest occupied molecular orbital (\(\epsilon_{\text{HOMO}}\)), the lowest unoccupied molecular orbital (\(\epsilon_{\text{LUMO}}\)), and the logarithm of the octanol–water coefficient (log\(P_{\text{ow}}\)) have been successfully used in QSAR models for predicting mutagenicities of chemicals (24–26). In this study, the above three descriptors were also adopted for QSAR evaluation of the genotoxic potential of quinolone antibacterials (Table 3). Two stereoelectronic parameters, \(\epsilon_{\text{HOMO}}\) and \(\epsilon_{\text{LUMO}}\), were calculated with the semiempirical method using MOPAC (ver. 6; CAChe Scientific, Inc.) software run on an IBM 600E computer. The PM3 parameter (27) served to optimize stable structures. The log\(P_{\text{ow}}\) was calculated by using ACD/logPow ver. 1.0 (Advanced Chemistry Development, Inc.). The molar concentration for each antibacterial that produces 10% (EC₁₀) of the maximum response of corresponding antibacterial was calculated by the Prism 4 for Windows program (GraphPad Software, Inc.). Because quinolones are carboxylic acid compounds, their \(P_{\text{ow}}\)s were greatly dependent on the pH of the solution, and can be adjusted according to the following equation (28):

<table>
<thead>
<tr>
<th>TABLE 2. Structures of Fourteen Quinolone Antibacterials Used for Predicting Their Genotoxic Potentials</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Quinolones with piperazine; B: quinolones with pyrrolidine ring or cyclopropyl at C-7.</td>
</tr>
</tbody>
</table>

The quantum mechanical properties such as the energies of the highest occupied molecular orbital (\(\epsilon_{\text{HOMO}}\)), the lowest unoccupied molecular orbital (\(\epsilon_{\text{LUMO}}\)), and the logarithm of the octanol–water coefficient (log\(P_{\text{ow}}\)) have been successfully used in QSAR models for predicting mutagenicities of chemicals (24–26). In this study, the above three descriptors were also adopted for QSAR evaluation of the genotoxic potential of quinolone antibacterials (Table 3). Two stereoelectronic parameters, \(\epsilon_{\text{HOMO}}\) and \(\epsilon_{\text{LUMO}}\), were calculated with the semiempirical method using MOPAC (ver. 6; CAChe Scientific, Inc.) software run on an IBM 600E computer. The PM3 parameter (27) served to optimize stable structures. The log\(P_{\text{ow}}\) was calculated by using ACD/logPow ver. 1.0 (Advanced Chemistry Development, Inc.). The molar concentration for each antibacterial that produces 10% (EC₁₀) of the maximum response of corresponding antibacterial was calculated by the Prism 4 for Windows program (GraphPad Software, Inc.). Because quinolones are carboxylic acid compounds, their \(P_{\text{ow}}\)s were greatly dependent on the pH of the solution, and can be adjusted according to the following equation (28):

\[
\beta\text{-galactosidase activity (U)} = \frac{1000(OD_{415} - 1.75 \ OD_{570}) \ t \ \times \ \ OD_{595}}{t}
\]
Comparison with Their Minimum Inhibitory Concentrations (MIC 50) against *Streptococcus pneumoniae*

To evaluate the genotoxic potential of quinolone antibacterials, the EC 10 was calculated by non-linear least-squares regression as shown in Table 3. All of the genotoxic potentials of the 15 quinolone antibacterials, the EC10 values for which ranged from 6.2 to 26.6 nM, and the genotoxic potential of sparfloxacin was found to be the highest. The above results suggest that the genotoxic potentials of quinolone antibacterials are greatly dependent on their structures.

**Chemometric Methods.** All possible combinations of the two descriptors were verified for the choice of the best modeling variables, and Multiple Linear Regression analysis and variable selection were performed using SPSS for Windows Release 11.5.0 (SPSS Inc., Chicago, IL) using the Ordinary Least-Square regression (OLS) method. The internal predictivity of the model was evaluated by calculating the explained variance by leave-one-out cross-validation (Q^2_{LOO}). The real predictive capability of a QSAR model, developed on a training set, was verified on test sets by checking the explained variance (Q^2_{EXT}).

Results and Discussion

**Genotoxic Potential.** To evaluate the genotoxic potential of quinolone antibacterials, the 15 compounds listed in Table 1 were selected, and the genotoxic potentials were determined. Figure 1 shows the dose–response curves of the 15 quinolone antibacterials. The EC_{10} was calculated by non-linear least-squares regression as shown in Table 3. All of the compounds showed significant genotoxic potential, and their EC_{10} values ranged widely, from 0.61 to 2917.0 nM. Basically, the genotoxic potential of the earliest quinolone antibacterials, i.e., first generation in Table 1, were found to be lower than those of fluoroquinolone antibiotics; EC_{10} values for the former ranged from 188.1 to 2917.0 nM and those for the latter ranged from 16.8 to 140.0 nM. Of the 4 first-generation quinolone antibacterials, cinoxacin, which has a cinnoline ring, was found to have the weakest genotoxic potential, followed by pefloxacin, which has a hydrophilic ring in its molecular structure, was found to have the lowest genotoxic potential, followed by enoxacin, which does have a piperazinyl ring. The highest genotoxic potential, followed by pefloxacin (40.6 nM). The third-generation quinolone antibacterials showed the highest genotoxic potential, which ranged from 6.2 to 26.6 nM, and the genotoxic potential of sparfloxacin was found to be the highest. The above results suggest that the genotoxic potentials of quinolone antibacterials are greatly dependent on their structures.

**QSAR for Prediction of Genotoxic Potential.** A large amount of biological data indicates that the functional targets of quinolone antibacterials are DNA gyrase and topoisomerase IV; however, they do not directly bind to the two type-2 DNA topoisomerases but form a noncovalent complex with DNA. According to the binding mode of the quinolone antibacterials with various DNA (29), four drug molecules bind to DNA through hydrogen bonds between the carbonyl group on the quinoline rings and the DNA bases, and two adjacent quinoline rings interact via hydrophobic interactions between the drug molecules. Based on this mode of quinolone antibacterial binding with DNA, the genotoxic potential of a quinolone antibacterial is described as a linear expression with terms for a hydrophobic effect, and reactivity (eq 3). For the genotoxic potential, the specific terms and corresponding descriptors are as follows: polarity, logP_{ow}, reactivity, E_{HOMO} or E_{LUMO}.

\[
\log \text{EC}_{10} = \text{hydrophobicity} + \text{reactivity} \quad (3)
\]

In this study, the correlations between the genotoxic potential of the quinolone antibacterials and two molecular descriptors selected from logP_{ow} or logP_{ow} at pH 6.98, and E_{HOMO} or E_{LUMO} were determined. It was found that while a good regression \( R^2 = 0.83 \) was acquired when \( \log \text{EC}_{10} \) regressed simultaneously with \( \log P_{ow} \) and \( E_{HOMO} \) as shown in eqs 4, a better regression \( R^2 = 0.95 \) was obtained by regressing simultaneously with \( \log P_{ow} \) at pH 6.78 and \( E_{HOMO} \) (eq 5).

\[
\log \text{EC}_{10} = -0.400(\pm 0.182) \log P_{ow} - 5.171(\pm 2.157)E_{HOMO} - 43.1(\pm 19.15) \quad (4)
\]

\[
R^2 = 0.88; \, n = 15; \, s = 0.36; \, p = 2.5 \times 10^{-6} \quad (4)
\]

\[
\log \text{EC}_{10} = -0.256(\pm 0.07) \log P_{ow}\text{pH6.98} - 5.636(\pm 1.37)E_{HOMO} - 47.57(\pm 12.13) \quad (5)
\]

\[
R^2 = 0.935; \, n = 15; \, s = 0.23; \, p = 2.3 \times 10^{-8} \quad (5)
\]

From the above equations, it can be found that the genotoxic potential increased with increasing hydrophobicity (logP_{ow}) and reactivity (E_{HOMO}); this trend is similar to that of

### TABLE 3. Physiochemical Properties of the Fifteen Quinolone Antibacterials and Their Experimental Genotoxic Potentials (EC_{10}) against *Streptococcus pneumoniae*

<table>
<thead>
<tr>
<th>chemical</th>
<th>MW</th>
<th>pK_a</th>
<th>logP_{ow}</th>
<th>logP_{ow pH 6.98}</th>
<th>&lt;E_{HOMO} &gt; (eV)</th>
<th>&lt;E_{LUMO} &gt; (eV)</th>
<th>EC_{10} (nM)</th>
<th>MIC_{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cinoxacin</td>
<td>262.22</td>
<td>4.31</td>
<td>-0.53</td>
<td>-3.14</td>
<td>-8.934</td>
<td>-0.963</td>
<td>2917.0</td>
<td>&gt; 488139.7^a</td>
</tr>
<tr>
<td>pipemidic acid</td>
<td>303.32</td>
<td>8.75</td>
<td>1.06</td>
<td>1.05</td>
<td>-9.073</td>
<td>-0.84</td>
<td>2758.4</td>
<td>-</td>
</tr>
<tr>
<td>nalidixic acid</td>
<td>232.24</td>
<td>5.03</td>
<td>0.18</td>
<td>-1.72</td>
<td>-8.906</td>
<td>-0.829</td>
<td>1448.0</td>
<td>551154.0^a</td>
</tr>
<tr>
<td>oxolinic acid</td>
<td>261.23</td>
<td>3.44</td>
<td>0.94</td>
<td>-2.54</td>
<td>-8.728</td>
<td>-0.729</td>
<td>188.1</td>
<td>&gt; 122495.7^a</td>
</tr>
<tr>
<td>2nd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>flumequine</td>
<td>261.25</td>
<td>3.96</td>
<td>2.42</td>
<td>-0.54</td>
<td>-8.826</td>
<td>-0.791</td>
<td>140.0</td>
<td>-</td>
</tr>
<tr>
<td>enoxacin</td>
<td>320.32</td>
<td>7.94</td>
<td>0.67</td>
<td>0.63</td>
<td>-8.829</td>
<td>-0.904</td>
<td>91.0</td>
<td>39023.5^a</td>
</tr>
<tr>
<td>norfloxacin</td>
<td>319.33</td>
<td>8.76</td>
<td>1.49</td>
<td>1.48</td>
<td>-8.808</td>
<td>-0.752</td>
<td>89.0</td>
<td>12526.2^b</td>
</tr>
<tr>
<td>lomefloxacin</td>
<td>351.36</td>
<td>8.8</td>
<td>2.35</td>
<td>2.34</td>
<td>-8.919</td>
<td>-0.965</td>
<td>56.4</td>
<td>11384.3^a</td>
</tr>
<tr>
<td>ofloxacin</td>
<td>361.37</td>
<td>8.61</td>
<td>0.97</td>
<td>0.61</td>
<td>-8.73</td>
<td>-0.762</td>
<td>43.7</td>
<td>5534.5^a</td>
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<tr>
<td>pefloxacin</td>
<td>333.36</td>
<td>7.03</td>
<td>2.16</td>
<td>1.91</td>
<td>-8.774</td>
<td>-0.744</td>
<td>40.6</td>
<td>11998.0^b</td>
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<tr>
<td>ciprofloxacin</td>
<td>331.35</td>
<td>8.76</td>
<td>1.33</td>
<td>1.32</td>
<td>-8.771</td>
<td>-0.754</td>
<td>16.8</td>
<td>3018.0^d</td>
</tr>
<tr>
<td>3rd</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>levofloxacin</td>
<td>361.37</td>
<td>6.81</td>
<td>0.97</td>
<td>0.61</td>
<td>-8.73</td>
<td>-0.762</td>
<td>26.6</td>
<td>1383.6^d</td>
</tr>
<tr>
<td>sarafloxacin</td>
<td>385.40</td>
<td>8.73</td>
<td>3.01</td>
<td>3.00</td>
<td>-8.787</td>
<td>-1.174</td>
<td>26.5</td>
<td>2370.6^e</td>
</tr>
<tr>
<td>danofloxacin</td>
<td>371.40</td>
<td>8.46</td>
<td>2.42</td>
<td>2.41</td>
<td>-8.724</td>
<td>-0.718</td>
<td>18.4</td>
<td>-</td>
</tr>
<tr>
<td>sparfloxacin</td>
<td>392.40</td>
<td>8.88</td>
<td>4.56</td>
<td>4.55</td>
<td>-8.635</td>
<td>-0.906</td>
<td>0.61</td>
<td>560.0^d</td>
</tr>
</tbody>
</table>


\[
P_{ow} = P_{ow}/(1 + 10^{pH-pK_a}) \quad (2)
\]

where \( P_{ow} \) represents the \( P_{ow} \) values of a quinolone antibacterial in neutral species. The dissociation constants (\( pK_a \)) of chemicals were calculated by ACD/pKa Calculator 4.0 (Advanced Chemistry Development, Inc.). Considering that the exposure experiment in the SOS/umu bioassay was carried out at a pH of 6.98, the log\( P_{ow} \) of each quinolone antibacterial at pH 6.98 according to eq 2 was estimated as shown in Table 3.
mutagenicities for aromatic and heteroaromatic amines (26). LUMO orbital energy has been well used as an important explanatory descriptor in mutagenicities of several compounds such as nitroaromatic compounds and aromatic and heteroaromatic nitro-compounds (24–26). In this study, however, it is the HOMO orbital energy that appears in the significant regression (eqs 4 and 5, indicating that DNA accepts the electron from the quinolone antibacterial when interacting with DNA or protein (30).

The internal validation of the QSAR was preformed, and the cross-validation parameter \( Q_{\text{LOO}}^2 \) was 0.84 and 0.91 for eqs 4 and 5, respectively. To externally validate QSAR modeling of quinolone genotoxicity, five quinolones (piromidic acid, fleroxacin, enrofloxacin, gatifloxacin, and difloxacin (Table 2)) which can be commercially obtained were selected as test chemicals. Figure S1 (Supporting Information) shows the dose–response curves of the five test antibacterials, and their EC\(_{10}\) values are listed in Table 4 to compare with the values predicted by eq 5. The differences between observed and predicted logEC\(_{10}\) values for the five chemicals ranged from 0.34 to 0.68, and the external explained variance (\( Q_{\text{ext}}^2 \)) was estimated to be 0.86. Thus, the internal and external validations both supported that eq 5 is predictive.

In addition to evaluation of goodness-of-fit, robustness, and predictivity of the model, the determination of its applicability domain (AD) is also important. Dimitrov et al. (31) proposed a stepwise approach for determining the model AD which included four stages, i.e., defining the range of variation of physicochemical properties of the model, structural similarity, mechanistic check, and metabolic check. Because the chemicals used in the training of this model were classified into three groups according to their structure as shown in Table 1, the role of the structural domain was not analyzed. The AD of this model was defined by making use of the interpolation space of the descriptors, and the optimal interpolation space which was defined by excluding...
the sparsely populated periphery of the descriptor space containing no more than 5% of the training chemicals is shown in Figure 2.

**Prediction of Genotoxic Potential for New-Generation Quinolone Antibacterials.** The fluoroquinolone antibacterials have had broad acceptance in the treatment of hospitalized patients and outpatients, and newer fluoroquinolones are now incorporated into guidelines for the treatment of patients with lower respiratory tract infections due to rising resistance to β-lactams and other agents in *S. pneumoniae* (32). Of the newly developed fluoroquinolones, temafloxacin, grepafloxacin, and trovafloxacin have been withdrawn or restricted due to adverse events such as hemolytic anemia, renal impairment, hepatotoxicity, disseminated intravascular coagulation, and hypoglycemia associated with the use of temafloxacin, severe cardiovascular events among patients taking grepafloxacin, and hepatic eosinophilia and hypoglycemia associated with the use of trovafloxacin (33). However, new fluoroquinolones such as moxifloxacin continue to be developed and approved. The QSAR model developed in this study was used to predict the genotoxic potential of 10 quinolone antibacterials including 3 fluoroquinolones with piperazine rings at C-7 (grepafloxacin, temafloxacin, and amifloxacin (fourth generation)) and 7 fourth-generation fluoroquinolones with pyrrolidine rings or cyclopropyl at C-7, as shown in Table 1. It should be noted that the predictors of these chemicals are all in the interpolation space of the descriptors calculated by the Ambit Disclosure Software developed by Jaworska, J. S., and Nikolova, N. (http://ambit.acad.bg, accessed 1 April 2007.).

**FIGURE 2.** Interpolation space of the descriptors calculated by the Ambit Disclosure Software developed by Jaworska, J. S., and Nikolova, N. (http://ambit.acad.bg, accessed 1 April 2007.).

### Relationship between MIC <sub>50</sub> and Genotoxic Potential

Although the mechanisms by which quinolones kill bacteria are still not fully understood, the SOS response is induced in bacteria by quinolone antibiotics which damage DNA, and the induction of the SOS response may play a role in the mechanism of quinolone action. From the results described above, it is clear that there are differences in SOS/umuC induction for different quinolone antibiotics. To investigate whether the induction of a SOS/umuC response was related to the antibacterial efficacy, we collated the MIC<sub>50</sub> values of the antibacterials examined in this study from the literature (34–41). Tables 3 and 4 compare the observed or predicted EC<sub>10</sub> with MIC<sub>50</sub> against *S. pneumoniae* for some of the quinolone antibacterials. Except for the newest fourth-generation quinolone antibacterials with pyrrolidine rings or cyclopropyl at C-7, the higher the EC<sub>10</sub> of a quinolone antibacterial, the higher the MIC<sub>50</sub> (Figure 3), and a linear relationship (eq 6) was shown between the logMIC<sub>50</sub> and logEC<sub>10</sub> values. This finding indicates that the genotoxic potentials predicted based on the SOS/umuC assay could reflect the antibacterial efficacy for quinolone antibacterials with structures similar to those shown in Table 1.

\[
\log \text{MIC}_{50} = 1.026(\pm0.262) \times \log \text{EC}_{10} + 2.255(\pm0.478); \quad R^2 = 0.82; \quad n = 18; \quad s = 0.4; \quad p < 0.00003
\]  

As for the quinolone antibacterials with pyrrolidine rings or cyclopropyl at C-7, no relationship between MIC<sub>50</sub> and EC<sub>10</sub> was observed. As shown in Figure 3, although the MIC<sub>50</sub> values against *S. pneumoniae* were high, their genotoxic potentials predicted by the QSAR model were relatively low. This phenomenon could be due to the fact that the training group for developing the QSAR model did not include these chemicals with pyrrolidine rings or cyclopropyl at C-7 as their standards cannot be obtained commercially. Further studies are necessary.

In conclusion, the QSAR model for predicting genotoxic potentials of quinolone antibacterials was established. Their MIC<sub>50</sub> against *S. pneumoniae* was found to be dependent on the genotoxic potentials except for the newest group of fluoroquinolones. There has been a considerable increase in the number of quinolone antibacterials that are in development and to date over 10 000 molecules have been patented. We hope this study will help to screen the genotoxic potential and antibacterial activities of continuously developing quinolone antibacterials to assess the impact of exposure to these agents.

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Supporting Information Available

Dose—response curves of genotoxicity for the five quinolones used as test chemicals. This material is available free of charge via the Internet at http://pubs.acs.org.

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