Abstract. We recently demonstrated that the cytotoxicity of nineteen 1,2,3,4-tetrahydroisoquinoline derivatives depends on the molecular size (surface area, volume, width measured at 3-dimensional configuration), but not on most of the other electronic factors (Ishihara et al., Anticancer Res 29: 2265-2272, 2009). However, the information regarding cytotoxicity and molecular size in these compounds is limited. Here, a quantitative structure–activity relationship (QSAR) analysis using nineteen newly synthesized 1,2,3,4-tetrahydroisoquinoline derivatives was carried out. A semiempirical molecular-orbital method (CAChe 4.9, PM5) was applied to delineate the relationship between the cytotoxicity (evaluated by 50% cytotoxic concentration, CC50) of the nineteen derivatives (TD1-19) against human promyelocytic leukemia HL-60 and human oral squamous cell carcinoma (HSC-2, HSC-3, HSC-4) cell lines and sixteen chemical descriptors determined by CONFLEX/PM5 method or the molecular weight. There was some correlation between the CC50 and the dipole moment for HSC-4 cells (r²=0.273), between the CC50 and log P for HL-60 and HSC-3 cells (r²=0.191-0.212), and between the CC50 and distance of C-R₂ (at three dimensional configuration) (r²=0.394) and molecular weight (r²=0.292) for HL-60 cells. On the other hand, there was little or no correlation between the CC50 and other descriptors. The present study demonstrated the dependency of the cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives on hydrophobicity and distance between C-R₂ in the 3-dimensional configuration. These descriptors obtained from the CONFLEX/PM5 method may be utilized as a tool to analyze the biological effect of 1,2,3,4-tetrahydroisoquinolines.

1-Methyl-1,2,3,4-tetrahydroisoquinoline (TIQ) is the only endogenous parkinsonism-preventing agent discovered to date (reviewed (1)). TIQ derivatives have shown diverse biological activity (1). Among these properties, TIQ derivatives have induced cell death via the decline of ATP level due to the mitochondria inhibition of complex 1, and the DNA damage (1) and inactivation of Cu,Zn-superoxide dismutase (2) induced by free radical formation. It has recently been reported that TIQ derivatives possessing bulky alkyl group substituents such as 1-cyclobutyl-, 1-cyclohexyl-, 1-phenyl, or 1-benzyl- at the C-1 position significantly showed cytotoxicity against rat PC12 cells (3). We also recently found a good correlation between the cytotoxicity of TIQ compounds and their molecular size (such as surface area, volume and width), but not with other physicochemical descriptors (such as heat of formation, stability of hydration, dipole moment, electron affinity, ionization potential, highest occupied molecular orbital energy, lowest unoccupied molecular orbital energy, absolute hardness, molecular weight) (4). However, the information between these two factors in these compounds has been limited. Since basic TIQ structure is important in the regulation of various biological functions in the body, we have synthesized newly synthesized TIQ compounds, and performed similar quantitative structure-activity relationship (QASR) analysis.

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco’s modified Eagle medium (DMEM) (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS) (JRH Bioscience, Lenexa, KS, USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Assay for cytotoxicity. Human promyelocytic leukemic cell line (HL-60) and human oral squamous cell carcinoma cell lines (HSC-2, HSC-3, HSC-4) were cultured in RPMI-1640 or DMEM supplemented with 10% heat-inactivated FBS under a humidified 5% CO₂ atmosphere, respectively. These cells were incubated for 48 hours with different concentrations of each compound, and the viable cell number was determined by cell counting after trypan blue exclusion test (for HL-60 cells) or MTT method (for other cell lines) (6). The 50% cytotoxic concentration (CC₅₀) against these cell lines was determined from the dose–response curve.

Calculation. The most stable configuration of the nineteen newly synthesized 1,2,3,4-tetrahydroisoquinolines was calculated by CONFLEX 5 (Conflex Co. Ltd., Tokyo, Japan). The optimization of the structure was achieved using a semiempirical molecular-orbital method (PM5), using a CAChe Worksystem 4.9 (MOPAC, PM5, non-COSMO) (Fujitsu Co. Ltd., Tokyo, Japan). The following descriptors were used: 1 heat of formation (COSMO, non-COSMO; kcal/mole), 2 dipole moment (D), 3 electron affinity (eV), 4 ionization potential (eV), 5 hydrophobicity (log P), 6 highest occupied molecular orbital energy (E_HOMO; eV), 7 lowest unoccupied molecular orbital energy (E_LUMO; eV), 8 absolute hardness \[ \eta = (E_{\text{LUMO}} - E_{\text{HOMO}})/2; \text{eV} \], 9 absolute electron negativity \[ \chi = -(E_{\text{LUMO}} + E_{\text{HOMO}})/2; \text{eV} \], 10 reactivity index \[ \omega = \chi^2/2\eta; \text{eV} \], 11 maximum length of the molecule (Å), 12 distance between C-R₂ (Å), 13 distance between R₂–R₃ (Å), 14 distance between R₃–R₄ (Å), 15 surface area of the molecule (Å²), 16 volume of the molecule (Å³) (7-9). The values of 11, 12, 13 and 14 were measured using 3-dimensional images of the most stable structure of each molecule. The QSAR was investigated from the each descriptor (determined from molecular structure) and CC₅₀ value (plotted as logarithmic scale), using a CAChe Worksystem 4.9 project reader.

Results and Discussion

Calculation with CONFLEX soft demonstrated that the most stable structure of all nineteen 1,2,3,4-tetrahydroisoquinoline derivatives showed the protrusion of substituents on the planar backbone (Figures 1 and 2). We first performed the QSAR analysis using HL-60, HSC-2, HSC-3 and HSC-4 cells. The CC₅₀ values of the nineteen 1,2,3,4-tetrahydroisoquinolines, the 16 descriptors and molecular weight of each compound are shown in Table I. The relation between the logarithmically plotted CC₅₀ against HL-60 cells and each descriptor are shown in Figure 3 1-17. The correlation coefficient for each QSAR is shown in Table II. There was some correlation between CC₅₀ and the dipole moment for HSC-4 cells (r²=0.273), between the CC₅₀ and log P for HL-60 and HSC-3 cells (r²=0.191-0.212), and between the CC₅₀ and distance of C-R₂ (in three dimensional configuration) (r²=0.394) and molecular weight (r²=0.292) for HL-60 cells. There was little or no correlation between the CC₅₀ and the heat of formation, electron affinity, ionization potential, E_HOMO, E_LUMO, η, χ, ω, maximum length, distance between C-R₂ and distance between R₂–R₃, surface area or the volume of the molecule in any of these cells (r²=0.00-0.200). These experimental data suggest that hydrophobicity, distance between C-R₂ in the 3-dimensional configuration can be utilized to estimate the cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives.

In a QSAR analysis of endocrine disruptors, positive correlation was reported between the biological activity and the chemical hardness (10). These papers showed the biological activity of endocrine disruptors are well fitted to the η value. In contrast, the present results demonstrate the lack of positive correlation between the CC₅₀ value of 1,2,3,4-tetrahydroisoquinolines and the η, χ and ω values.
There was some correlation between the CC50 value and dipole moment in HSC-2 cells ($r^2=0.244$) and HSC-4 cells ($r^2=0.273$), and the hydrophobicity in HL-60 cells ($r^2=0.212$).

In conclusion, these experiments demonstrated the dependency of the cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives on hydrophobicity and distance between C-R2 in the 3-dimensional configuration. These descriptors obtained from the CONFLEX/PM5 method may be utilized as a tool to analyze the biological effect of 1,2,3,4-tetrahydroisoquinolines.

**Acknowledgements**

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### Table I. CC50 and chemical descriptors for 1,2,3,4-tetrahydroisoquinoline derivatives.

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<th>Compd.</th>
<th>HSC-2 CC50 (μM)</th>
<th>HSC-3 CC50 (μM)</th>
<th>HSC-4 CC50 (μM)</th>
<th>Log HSC-2 CC50</th>
<th>Log HSC-3 CC50</th>
<th>Log HSC-4 CC50</th>
<th>Heat of formation (kcal/mol)</th>
<th>Dipole moment (D)</th>
<th>Electron affinity (eV)</th>
<th>Ionization potential (eV)</th>
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(Table II, Figure 3). There was some correlation between the CC50 value and dipole moment in HSC-2 cells ($r^2=0.244$) and HSC-4 cells ($r^2=0.273$), and the hydrophobicity in HL-60 cells ($r^2=0.212$).

In conclusion, these experiments demonstrated the dependency of the cytotoxicity of 1,2,3,4-tetrahydroisoquinoline derivatives on hydrophobicity and distance between C-R2 in the 3-dimensional configuration. These descriptors obtained from the CONFLEX/PM5 method may be utilized as a tool to analyze the biological effect of 1,2,3,4-tetrahydroisoquinolines.
Table II. Correlation coefficients between CC₅₀ and each chemical descriptor in four different cell lines.

<table>
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<tr>
<th>Cell line</th>
<th>Heat of formation (kcal/mol)</th>
<th>Dipole moment (D)</th>
<th>Electron affinity (eV)</th>
<th>Ionization potential (eV)</th>
<th>Log P</th>
<th>$E_{\text{HOMO}}$ (eV)</th>
<th>$E_{\text{LUMO}}$ (eV)</th>
<th>$\eta$</th>
<th>$\chi$</th>
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<td>0.069</td>
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<td>0.016</td>
<td>0.212</td>
<td>0.016</td>
<td>0.016</td>
<td>0.038</td>
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<td>0.006</td>
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<td>0.006</td>
<td>0.042</td>
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<td>0.056</td>
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Figure 2. The most stable conformation of 1,2,3,4-tetrahydroisoquinoline derivatives used [1-19].
Figure 3. Correlation between CC_{50} value (in log scale) and each descriptor of 1,2,3,4-tetrahydroisoquinoline derivatives against HL-60 cells. The investigated descriptors are 1 heat of formation, 2 dipole moment, 3 electron affinity, 4 ionization potential, 5 hydrophobicity (log P), 6 $E_{HOMO}$, 7 $E_{LUMO}$, 8 absolute hardness, 9 absolute electron negativity, 10 reactivity index ($\omega$), 11 maximum length, 12 distance between N-R$_3$, 13 distance between C-R$_3$, 14 distance between R$_2$-R$_3$, 15 surface area, 16 volume of the molecule, and 17 molecular weight.
References


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