

# Estimation of Relationship Between Descriptors and Cytotoxicity of Newly Synthesized 1,2,3,4-Tetrahydroisoquinoline Derivatives

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**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 (CACHÉ 4.9, PM5) was applied to delineate the relationship between the cytotoxicity (evaluated by 50% cytotoxic concentration,  $CC_{50}$ ) of the nineteen derivatives (TDI-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  $CC_{50}$  and the dipole moment for HSC-4 cells ( $r^2=0.273$ ), between the  $CC_{50}$  and  $\log P$  for HL-60 and HSC-3 cells ( $r^2=0.191-0.212$ ), and between the  $CC_{50}$  and distance of C-R<sub>2</sub> (at three dimensional configuration) ( $r^2=0.394$ ) and molecular weight ( $r^2=0.292$ ) for HL-60 cells. On the other hand, there was little or no correlation between the  $CC_{50}$  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-R<sub>2</sub> 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 (QSAR) analysis.

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**Key Words:** 1,2,3,4-Tetrahydroisoquinoline, QSAR, cytotoxicity, semiempirical molecular-orbital method, absolute hardness.

## 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

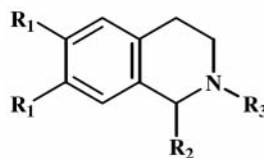
(MTT) (Sigma Chem. Co., St. Louis, MO, USA). All tetrahydroisoquinolines (TD1-19) were provided from Dr. Kawase, Matsuyama University (5).

**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% CO<sub>2</sub> 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 (CC<sub>50</sub>) 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 ( $E_{\text{HOMO}}$ ; eV), **7** lowest unoccupied molecular orbital energy ( $E_{\text{LUMO}}$ ; eV), **8** absolute hardness [ $\eta=(E_{\text{UMO}} - E_{\text{HOMO}})/2$ ; eV], **9** absolute electron negativity [ $\chi=-(E_{\text{LUMO}} + E_{\text{HOMO}})/2$ ; eV], **10** reactivity index ( $\omega=\chi^2/2\eta$ ; eV), **11** maximum length of the molecule (Å), **12** distance between C-R<sub>2</sub> (Å), **13** distance between R<sub>2</sub>-R<sub>3</sub> (Å), **14** distance between R<sub>2</sub>-R<sub>3</sub> (Å), **15** surface area of the molecule (Å<sup>2</sup>), **16** volume of the molecule (Å<sup>3</sup>) (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 CC<sub>50</sub> 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 CC<sub>50</sub> 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 CC<sub>50</sub> 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 CC<sub>50</sub> and the dipole moment for HSC-4 cells ( $r^2=0.273$ ), between the CC<sub>50</sub> and log P for HL-60 and HSC-3 cells ( $r^2=0.191-0.212$ ), and between the CC<sub>50</sub> and distance of C-R<sub>2</sub> (in three dimensional configuration) ( $r^2=0.394$ ) and



Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
1	H	CO <sub>2</sub> H	H
2	H	CO <sub>2</sub> H	COCH <sub>3</sub>
3	H	CO <sub>2</sub> H	COBu <sup>t</sup>
4	H	CO <sub>2</sub> H	COPh
5	H	CO <sub>2</sub> H	COC <sub>6</sub> H <sub>4</sub> Cl-4
6	H	CO <sub>2</sub> H	CO <sub>2</sub> Bn
7	OCH <sub>3</sub>	CO <sub>2</sub> H	COBu <sup>t</sup>
8	OCH <sub>3</sub>	CO <sub>2</sub> H	COC <sub>6</sub> H <sub>2</sub> Me <sub>3</sub> -2,4,6
9	OH	CO <sub>2</sub> H	COBu <sup>t</sup>
10	H	CH <sub>2</sub> CO <sub>2</sub> H	COBu <sup>t</sup>
11	OCH <sub>3</sub>	CH <sub>2</sub> CO <sub>2</sub> H	H
12	OCH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> CO <sub>2</sub> H	COBu <sup>t</sup>
13	H	CO <sub>2</sub> Et	CO <sub>2</sub> Bn
14	OCH <sub>3</sub>	CO <sub>2</sub> Et	COBu <sup>t</sup>
15	OCH <sub>3</sub>	CO <sub>2</sub> Et	COC <sub>6</sub> H <sub>2</sub> Me <sub>3</sub> -2,4,6
16	OCH <sub>3</sub>	CH <sub>2</sub> CO <sub>2</sub> Et	COCH <sub>3</sub>
17	H	CH <sub>2</sub> CO <sub>2</sub> Et	COBu <sup>t</sup>
18	OCH <sub>3</sub>	CH <sub>2</sub> CO <sub>2</sub> CH <sub>3</sub>	COBu <sup>t</sup>
19	OCH <sub>3</sub>	CH <sub>2</sub> CO <sub>2</sub> Et	COBu <sup>t</sup>

Figure 1. The structure of 1,2,3,4-tetrahydroisoquinoline derivatives.

molecular weight ( $r^2=0.292$ ) for HL-60 cells. There was little or no correlation between the CC<sub>50</sub> and the heat of formation, electron affinity, ionization potential,  $E_{\text{HOMO}}$ ,  $E_{\text{LUMO}}$ ,  $\eta$ ,  $\chi$ ,  $\omega$ , maximum length, distance between N-R<sub>3</sub> and distance between R<sub>2</sub>-R<sub>3</sub>, surface area or the volume of the molecule in any of these cells ( $r^2=0.00-0.200$ ). These experimental data suggest that hydrophobicity, distance between C-R<sub>2</sub> 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  $\eta$  value. In contrast, the present results demonstrate the lack of positive correlation between the CC<sub>50</sub> value of 1,2,3,4-tetrahydroisoquinolines and the  $\eta$ ,  $\chi$  and  $\omega$  values

Table I.  $CC_{50}$  and chemical descriptors for 1,2,3,4-tetrahydroisoquinoline derivatives.

Compd.	HL-60 $CC_{50}$ ( $\mu$ M)	HSC-2 $CC_{50}$ ( $\mu$ M)	HSC-3 $CC_{50}$ ( $\mu$ M)	HSC-4 $CC_{50}$ ( $\mu$ M)	Log HL-60 $CC_{50}$	Log HSC-2 $CC_{50}$	Log HSC-3 $CC_{50}$	Log HSC-4 $CC_{50}$	Heat of formation (kcal/mol)	Dipole moment (D)	Electron affinity (eV)	Ionization potential (eV)	Log P
1	400	400	400	400	2.602	2.602	2.602	2.602	-74.514	2.528	0.097	9.312	1.291
2	400	400	400	400	2.602	2.602	2.602	2.602	-118.117	5.945	0.106	9.521	0.955
3	400	400	400	400	2.602	2.602	2.602	2.602	-130.736	5.775	0.019	9.461	2.811
4	394	400	400	400	2.595	2.602	2.602	2.602	-83.685	5.868	0.424	9.526	2.868
5	342	400	400	400	2.534	2.602	2.602	2.602	-94.636	5.295	0.586	9.561	3.386
6	199	400	400	400	2.299	2.602	2.602	2.602	-132.336	5.313	0.737	9.510	3.357
7	400	400	400	400	2.602	2.602	2.602	2.602	-199.086	4.805	0.364	9.173	2.306
8	131	365	400	386	2.117	2.562	2.602	2.587	-180.103	4.971	0.470	9.172	3.764
9	146	364	376	392	2.164	2.561	2.575	2.593	-218.867	4.175	0.212	8.881	2.242
10	360	400	400	400	2.556	2.602	2.602	2.602	-132.670	7.939	0.131	9.579	2.868
11	108	115	275	118	2.033	2.061	2.439	2.072	-153.212	2.165	0.140	9.076	0.842
12	396	400	400	400	2.598	2.602	2.602	2.602	-211.683	4.638	-0.078	8.557	2.614
13	10	61	63	72	1.000	1.785	1.799	1.857	-135.184	2.357	0.570	9.468	3.731
14	88	354	400	400	1.944	2.549	2.602	2.602	-202.419	5.967	0.043	8.578	2.680
15	16	68	64	83	1.204	1.833	1.833	1.919	-182.860	5.351	0.337	9.111	4.139
16	201	400	400	400	2.303	2.602	2.602	2.602	-193.985	6.052	0.046	8.634	0.881
17	38	258	307	300	1.580	2.412	2.487	2.477	-137.369	5.793	-0.211	9.410	3.242
18	112	353	400	390	2.049	2.548	2.602	2.591	-200.240	6.187	-0.071	8.623	2.394
19	84	333	391	373	1.924	2.522	2.592	2.572	-207.892	5.936	-0.101	8.598	2.737

Compd.	$E_{HOMO}$ (eV)	$E_{LUMO}$ (eV)	$\eta$	$\chi$	$\omega$	Max. length ( $\text{\AA}$ )	N-R <sub>3</sub> ( $\text{\AA}$ )	C-R <sub>2</sub> ( $\text{\AA}$ )	R <sub>2</sub> -R <sub>3</sub> ( $\text{\AA}$ )	Surface area ( $\text{\AA}^2$ )	Volume ( $\text{\AA}^3$ )	MW
1	-9.312	-0.097	4.607	4.704	2.402	7.219	1.015	3.313	3.770	153.620	102.195	177.00
2	-9.521	-0.106	4.708	4.813	2.461	9.537	3.405	3.298	6.112	187.513	124.410	219.23
3	-9.461	-0.019	4.721	4.740	2.379	10.646	4.654	3.297	7.425	238.386	179.630	261.32
4	-9.526	-0.424	4.551	4.975	2.720	11.864	6.138	2.585	8.553	236.039	160.490	281.30
5	-9.561	-0.586	4.488	5.074	2.868	12.430	6.712	3.299	9.925	249.967	170.595	315.50
6	-9.510	-0.737	4.387	5.124	2.993	13.543	7.892	3.297	11.012	262.534	176.215	311.00
7	-9.173	-0.364	4.405	4.769	2.581	12.528	4.693	2.590	6.968	289.727	217.060	321.38
8	-9.172	-0.470	4.351	4.821	2.670	14.726	6.927	2.587	9.443	339.387	225.580	383.17
9	-8.881	-0.212	4.335	4.546	2.494	10.562	4.686	3.300	8.700	254.972	193.320	293.00
10	-9.617	-0.153	4.732	4.885	2.522	11.916	6.386	3.421	10.053	256.355	169.270	275.00
11	-9.076	-0.140	4.468	4.608	2.376	8.908	1.016	4.380	6.388	222.404	144.705	251.00
12	-8.568	-0.019	4.294	4.274	2.081	12.450	4.701	5.757	9.464	323.188	247.190	349.41
13	-9.468	-0.570	4.449	5.019	2.831	12.455	7.484	5.705	6.206	299.244	199.145	339.38
14	-8.578	-0.043	4.268	4.311	2.177	12.324	4.655	5.512	8.451	326.719	246.450	349.41
15	-9.111	-0.337	4.387	4.724	2.543	14.460	6.942	5.729	10.475	N.D.	N.D.	411.00
16	-8.634	-0.046	4.294	4.340	2.194	10.821	2.779	6.217	10.821	292.287	190.475	321.30
17	-9.410	0.211	4.810	4.600	2.199	11.571	4.239	6.477	8.760	291.762	221.270	303.41
18	-8.623	0.071	4.347	4.276	2.103	12.315	4.068	5.147	7.744	325.882	246.300	349.41
19	-8.598	0.101	4.350	4.248	2.075	13.694	4.672	6.481	8.818	343.333	261.580	363.30

(Table II, Figure 3). There was some correlation between the  $CC_{50}$  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-R<sub>2</sub> 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

This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (Sakagami, No. 19592156, Kawase, No., Kawase, No. 20590114).

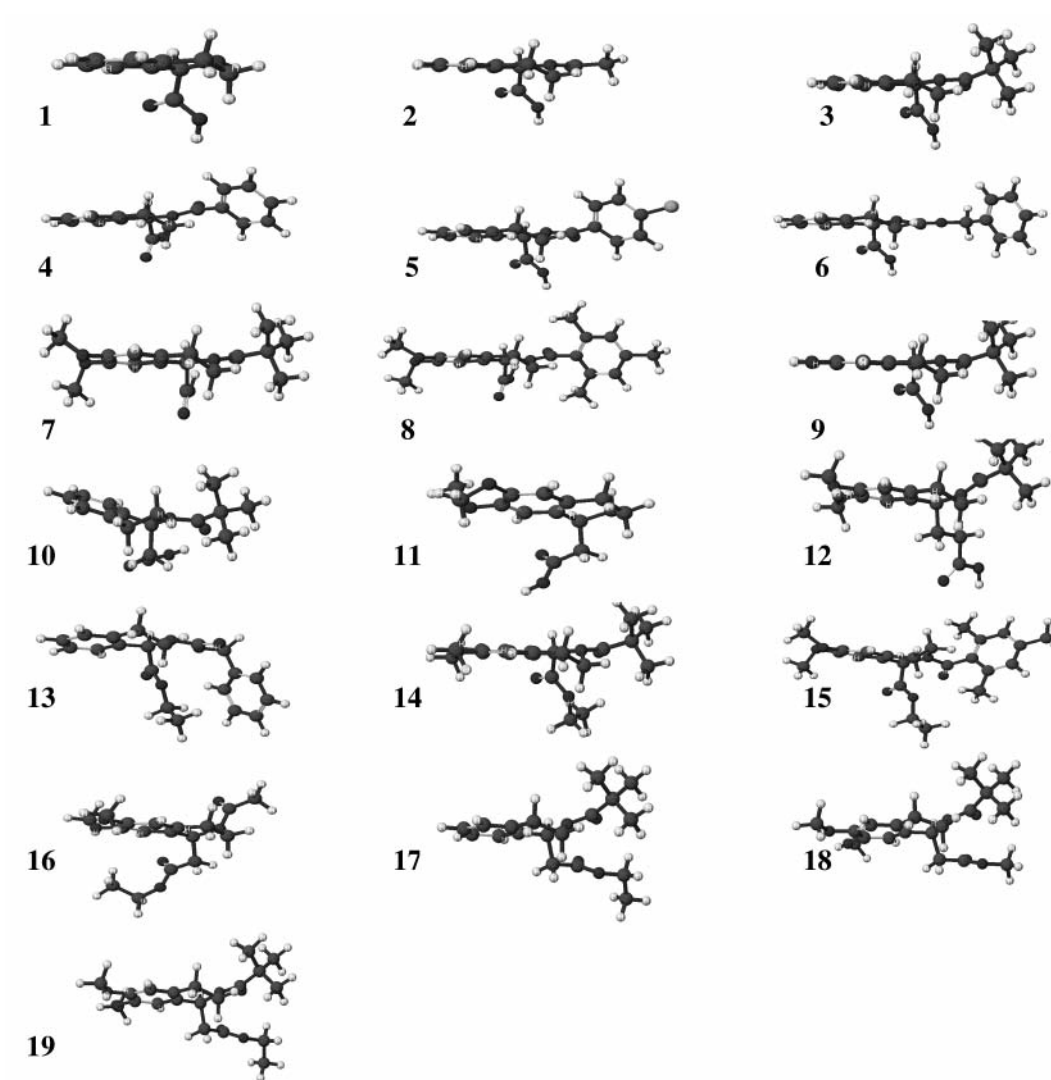


Figure 2. The most stable conformation of 1,2,3,4-tetrahydroisoquinoline derivatives used [1-19].

 Table II. Correlation coefficients between  $CC_{50}$  and each chemical descriptor in four different cell lines.

Cell line	Heat of formation (kcal/mol)	Dipole moment (D)	Electron affinity (eV)	Ionization potential (eV)	Log P	$E_{HOMO}$ (eV)	$E_{LUMO}$ (eV)	$\eta$	$\chi$
HL-60	0.063	0.069	0.004	0.016	0.212	0.016	0.004	0.038	0.000
HSC-2	0.001	0.244	0.042	0.006	0.083	0.006	0.042	0.006	0.022
HSC-3	0.000	0.134	0.083	0.019	0.191	0.019	0.083	0.005	0.052
HSC-4	0.000	0.273	0.056	0.013	0.063	0.013	0.056	0.002	0.003
	$\omega$	Max. length (Å)	N-R <sub>3</sub> (Å <sup>2</sup> )	C-R <sub>2</sub> (Å <sup>2</sup> )	R <sub>2</sub> -R <sub>3</sub> (Å <sup>2</sup> )	Surface area (Å <sup>2</sup> )	Volume (Å <sup>3</sup> )	Molecular weight	
HL-60	0.000	0.173	0.102	0.394	0.010	0.200	0.137	0.292	
HSC-2	0.033	0.025	0.034	0.167	0.006	0.013	0.001	0.090	
HSC-3	0.072	0.071	0.131	0.145	0.000	0.016	0.000	0.134	
HSC-4	0.048	0.013	0.027	0.133	0.011	0.002	0.011	0.064	

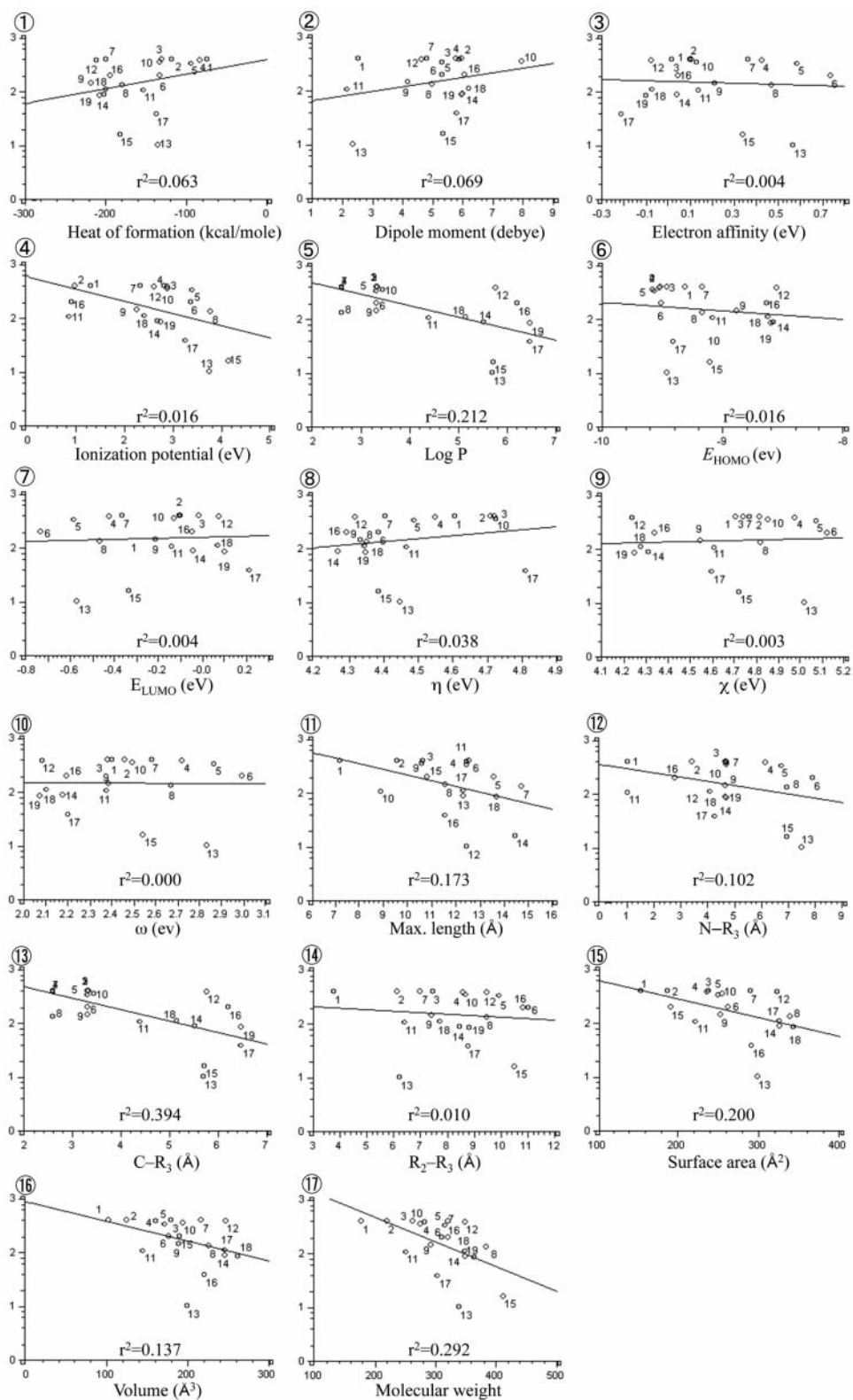


Figure 3. Correlation between  $CC_{50}$  value (in log scale) and each descriptor of 1,2,3,4-tetrahydroisoquinoline derivatives against HL-60 cells. The investigated descriptors are 1 heat of formation, 2 dipole moment, 3 electron affinity, 4 ionization potential, 5 hydrophobicity (log P), 6  $E_{HOMO}$ , 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.

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*Received April 8, 2009*

*Revised July 22, 2009*

*Accepted September 1, 2009*