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Quantitative Structure-Cytotoxicity Relationship Analysis of Phenoxazine Derivatives by Semiempirical Molecular-Orbital Method

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Abstract. *A semiempirical molecular-orbital method (CAChe) was applied to delineate the relationship between the cytotoxicity (evaluated by 50% cytotoxic concentration, CC_{50}) of fifteen phenoxazine derivatives and eleven physical parameters (descriptors). Most of the phenoxazine derivatives had extended and planar structure. The cytotoxic activity of phenoxazines against the human oral squamous cell carcinoma HSC-2 and HSC-4 cells correlated to electron affinity, absolute hardness (η), absolute electron negativity (χ) and octanol-water distribution coefficient (log-P). However, only log-P was correlated to CC_{50} in the HSC-3 cells, whereas only heat of formation and log-P were correlated to CC_{50} in the human promyelocytic leukemia HL-60 cells. The cytotoxic activity of the phenoxazine derivatives became maximum at the log-P=5.9. Their cytotoxicity strongly depended on the χ value, but not on the η value. Compounds with relatively higher cytotoxicity showed higher χ value (χ>5.28), whereas compounds with relatively lower cytotoxicity showed lower χ value (χ<4.27). These data suggest that appropriate chemical descriptors should be selected to estimate the cytotoxicity of phenoxazines, depending on the target cells.*

Phenoxazines are a group of *N*-heterocycles having three six ring structures with nitrogen and oxygen atoms (1). Phenoxazines have shown antitumor (2), antimicrobial (3), antiviral (4), anti-inflammatory (5) and multidrug resistance reversal activity (6), they have been shown to prevent human amyloid disorders (7) and to protect neuronal cells from death by oxidative stress (8). However, the mechanism for the induction of antitumor activity, and the type of cell death induced by phenoxazines are not well understood.

We have recently found that among twenty four phenoxazine derivatives, compounds **[7]** and **[8]** showed the highest tumor-specificity index of 4.3 and 4.8, respectively (9). These compounds did not apparently induce intranucleosomal DNA fragmentation, nor activate caspase-3 in the human oral squamous cell carcinoma cell lines HSC-2, HSC-4 and human glioblastoma T98G cells. The quantitative structure-activity relationship (QSAR) of fifteen phenoxazine derivatives (Figure 1) was investigated, using conventional and recent techniques of the computation chemistry such as the concept of chemical hardness (10-12).

Materials and Methods

Calculation. The most stable conformation of fifteen phenoxazine derivatives was calculated by CONFLEX 5 (Confluex Co. Ltd., Tokyo, Japan). The optimization of the structure was done by semiempirical molecular-orbital method (PM3), using CAChe Worksystem 4.9 MOPAC (PM3) (Fujitsu Co. Ltd., Tokyo, Japan). The following descriptors were used: heat of formation (COSMO, non-COSMO), stability of hydration (ΔH), dipole moment, ionization potential, electron affinity, length of molecule, highest occupied molecular orbital (HOMO) energy (E_{HOMO}), lowest unoccupied molecular orbital (LUMO) energy (E_{LUMO}), absolute hardness (η), absolute electron negativity (χ) and reactivity index (ω) (10) The η, χ and ω were determined by the following equations:

$$\eta = (E_{LUMO} - E_{HOMO})/2$$
$$\chi = -(E_{LUMO} + E_{HOMO})/2$$
$$\omega = \chi^2/2\eta$$

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Key Words: Phenoxazine, cytotoxicity, semiempirical molecular-orbital method.

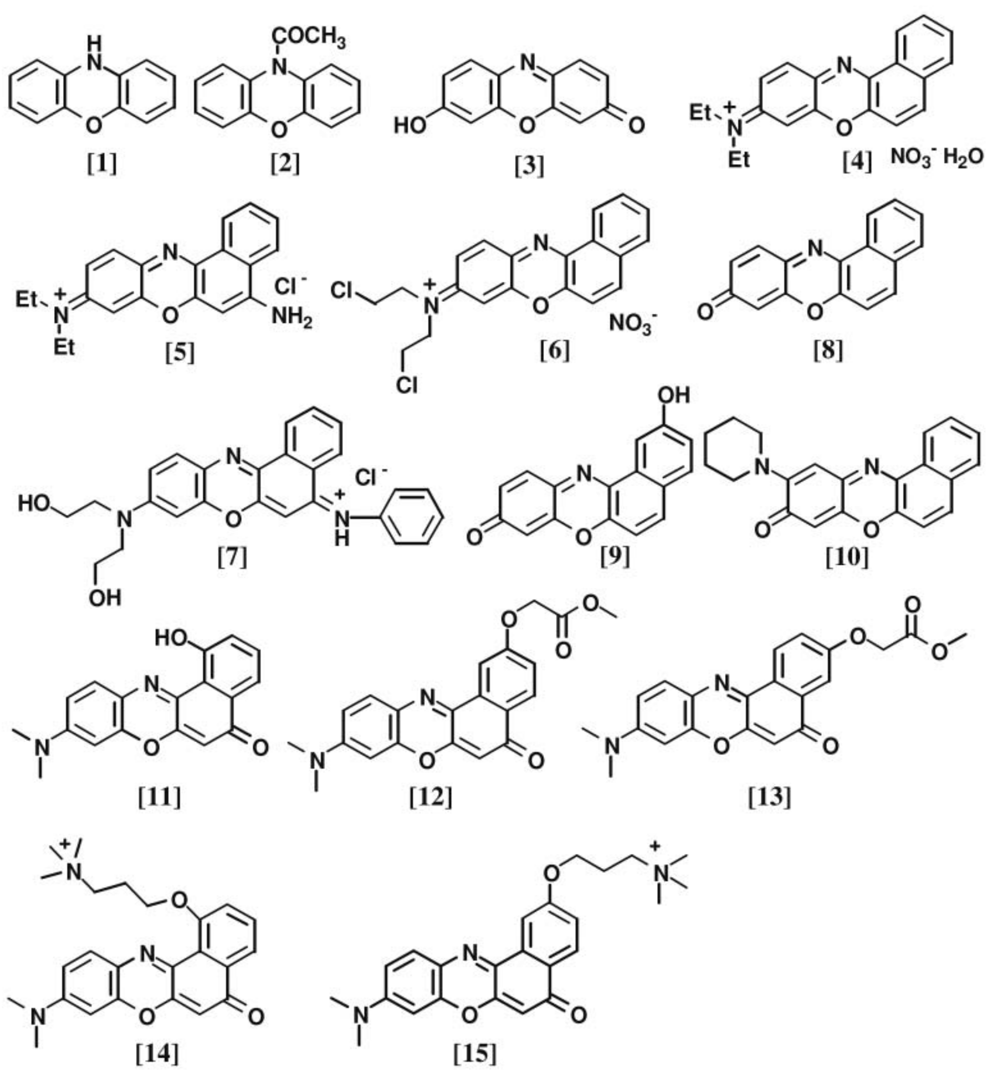

Figure 1. *The structure of the phenoxazine derivatives used.*

The octanol-water distribution coefficient (log-P) was determined by ACD/Log P DB6.0 (Fujitsu). The QSAR between 50% cytotoxic concentration (CC_{50}) and each physical parameter (descriptor) delineated from the molecular structure was investigated by CAChe Worksystem 4.9 project reader. The phenoxazines **[1-15]** were synthesized as described previously (13). The cytotoxicity assay and determination of CC_{50} against the HSC-2, HSC-3, HSC-4 and human promyelocytic leukemia HL-60 cell lines were performed as described in our accompanying paper (9).

Results and Discussion

By determining the most stable conformation of 15 phenoxazine derivatives by CONFLEX 5, their structure was approximated to the molecular form present *in vivo* (biomimetic). The most stable structure was next determined by the CAChe Worksystem 4.9 MOPAC (PM3) method (Figure 2). The CC_{50} value (determined by experiments) of the phenoxazines against HSC-2, HSC-3, HSC-4 and HL-60 cells, and the chemical descriptors (determined by calculations): heat of formation (COSMO), ionization potential, electron affinity, log-P, E_{HOMO}, E_{LUMO}, η, χ, ω and molecular weight (not determined by calculation) are listed in Table I. Using these values, all possible relationships between CC_{50} and each of these descriptors were investigated (Figure 3).

In the HSC-2 (shown in Figure 3) and HSC-4 cells, there was correlation between CC_{50} and electron affinity r^2=0.580, 0.515, respectively), E_{LOMO} (r^2=0.580, 0.515, respectively), absolute hardness that indicates the extent of excitation (η) (r^2=0.404, 0.40, respectively), absolute electron negativity (χ) (r^2=0.521, 0.436, respectively) and reactivity index (ω) (r^2=0.437, 0.405, respectively). However, there was no correlation between CC_{50} and heat of formation, E_{HOMO}, ionization potential, molecular

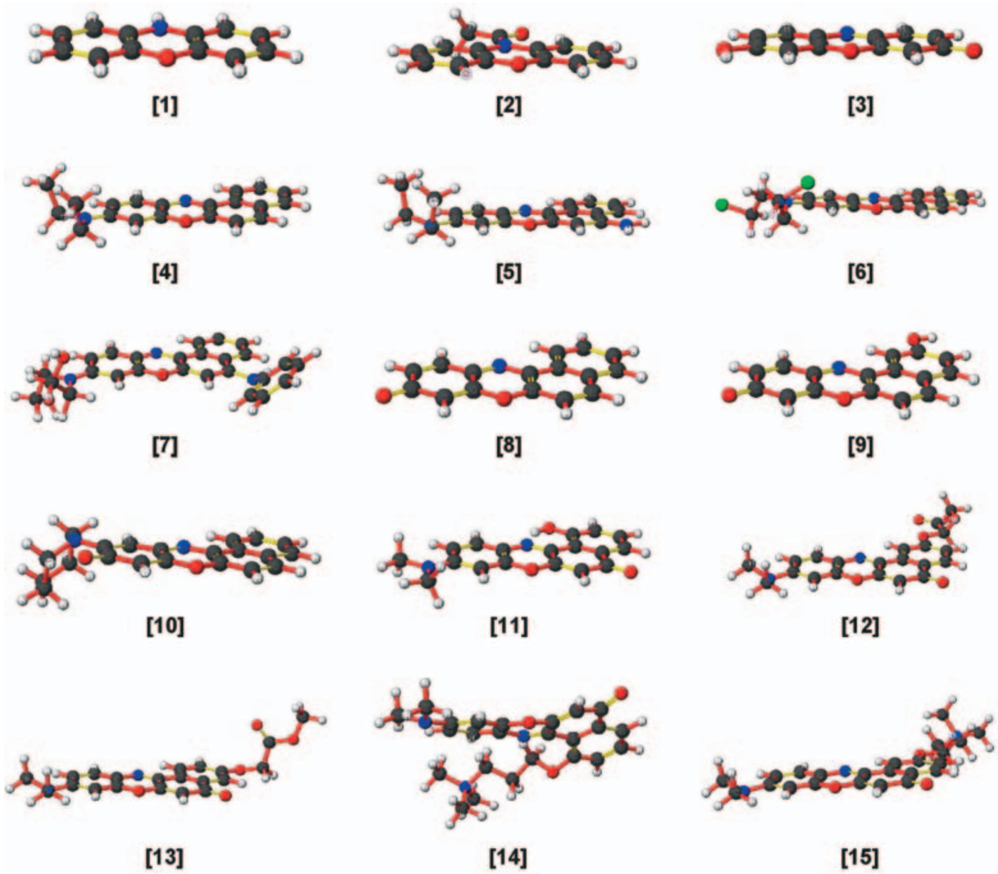

Figure 2. *The most stable conformation of the phenoxazine derivatives.*

Table I. *CC_{50} and chemical descriptors for phenoxazines.*

Compd.	HL-60 cells CC_{50} (μM)	HSC-2 cells CC_{50} (μM)	HSC-3 cells CC_{50} (μM)	HSC-4 cells CC_{50} (μM)	Heat of formation (Kcal/mol)	Electron affinity (eV)	Ionization potential (eV)	log-P	E_{HOMO}	E_{LUMO}	η	χ	ω	M.W.	Length (A)
[1]	6.9	154.0	137.0	164.0	22.957	0.104	7.757	2.681	–7.757	–0.104	3.827	3.930	2.018	183.21	9.432
[2]	42.0	181.0	109.0	157.0	–10.092	0.264	8.284	2.206	–8.284	–0.264	4.010	4.274	2.278	225.24	9.444
[3]	60.0	40.0	28.0	115.0	–33.951	1.721	8.963	1.396	–8.963	–1.721	3.621	5.342	3.940	213.19	9.864
[4]	1.6	3.5	2.6	4.4	60.007	3.900	7.869	9.118	–7.869	–3.900	1.984	5.884	8.726	383.39	13.544
[5]	1.6	4.5	2.0	4.5	34.226	2.239	8.788	3.645	–8.788	–2.239	3.275	5.513	4.641	353.85	13.392
[6]	16.0	48.0	56.0	78.0	39.007	3.437	9.202	9.840	–9.202	–3.437	2.883	6.319	6.926	434.27	14.055
[7]	1.6	25.0	3.8	28.0	1.653	2.540	8.183	4.108	–8.183	–2.540	2.821	5.361	5.094	461.94	15.873
[8]	1.6	18.0	31.0	17.0	29.457	1.802	8.767	2.683	–8.767	–1.802	3.483	5.285	4.010	247.24	11.514
[9]	1.6	3.2	6.9	8.0	–15.490	1.760	8.710	2.398	–8.710	–1.760	8.475	5.235	3.943	263.24	11.531
[10]	49.0	–	–	–	18.102	1.679	8.294	3.669	–8.294	–1.679	3.307	4.986	3.758	330.37	12.691
[11]	147.0	–	–	–	–24.755	1.553	8.249	2.663	–8.249	–1.553	3.348	4.901	3.588	306.00	13.675
[12]	148.0	–	–	–	–96.408	1.510	8.520	2.268	–8.520	–1.510	3.505	5.015	3.588	378.00	17.092
[13]	165.0	–	–	–	–98.723	1.509	8.462	2.268	–8.462	–1.509	3.477	4.985	3.575	378.00	17.986
[14]	–	1.6	6.2	3.1	136.03	4.225	10.580	2.251	–10.580	–4.225	3.177	7.402	8.623	406.00	11.996
[15]	–	1.6	100.0	19.0	132.172	4.465	9.779	2.251	–9.779	–4.465	2.657	7.122	9.545	406.00	18.394

HL-60, human promyelocytic leukemia; HSC-2, -3, -4, human oral squamous cell carcinoma; log-P, octanol-water distribution coefficient; E_{HOMO}, highest occupied molecular orbital energy; E_{LUMO}, lowest unoccupied molecular orbital energy; η, absolute hardness; χ, absolute electron negativity; ω, reactivity index; M.W. molecular weight.

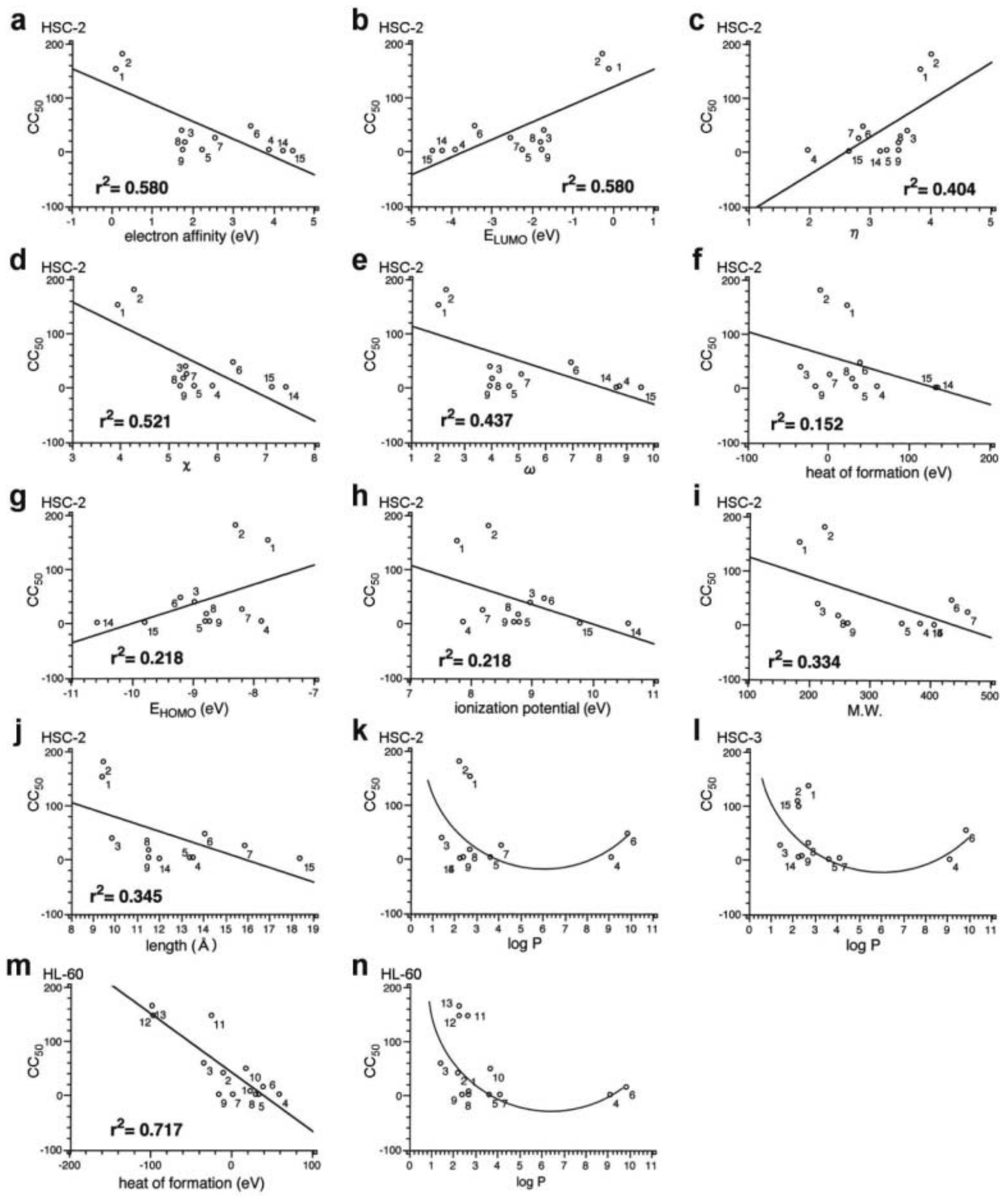

Figure 3. *Correlation between CC_{50} value and each chemical descriptor of phenoxazine derivatives against HSC-2 (a-k), HSC-3 (l) and HL-60 cells (m, n). HSC-2, -3, -4, human oral squamous cell carcinoma; HL-60, human promyelocytic leukemia; E_{HOMO}, highest occupied molecular orbital energy; E_{LUMO}, lowest unoccupied molecular orbital energy; η, absolute hardness; χ, absolute electron negativity; ω, reactivity index; log-P, octanol-water distribution coefficient; M.W. molecular weight.*

weight or molecular length (r^2=0.152~0.345). Generally, the interaction between the cells and the drugs was generated by the shape of the molecule. The molecular structure of phenoxazine derivatives determined by calculation was generally extended and planar, while compound **[14]** was bent at the centre and overlapping (see Figure 2). The cytotoxic activity of the phenoxazine derivatives became maximum at log-P=5.9, slightly higher than the optimal log-P values reported for prenylalcohol-, vitamin K_2- (14), gallic acid- (15) and coumarin- (16) derivatives (log-P of 2-3).

The correlation between the electron structure and the cytotoxicity of phenoxazine derivatives was next investigated, using the absolute hardness (η) – absolute electron negativity (χ) activity diagram (Figure 4). In the HSC-2 cells, compounds with higher cytotoxicity (lower CC_{50}) were distributed within the area surrounded by the box in Figure 4. Their cytotoxicity strongly depended on the χ value, but not on the η value. Phenoxazine derivatives with relatively higher cytotoxicity (lower CC_{50}) showed higher χ values (χ>5.28), and the compounds with relatively lower cytotoxicity (higher CC_{50})

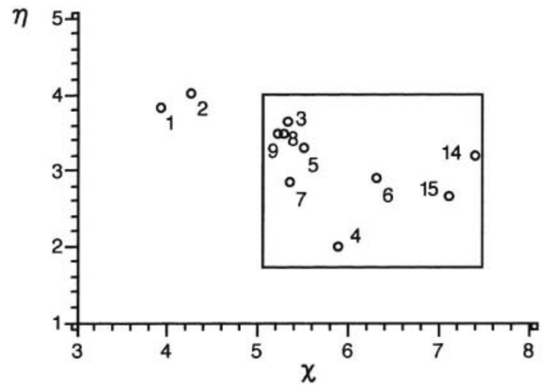

Figure 4. *η-χ activity diagram between the electron structure and the cytotoxicity of phenoxazine derivatives in HSC-2 cells. η, absolute hardness; χ, absolute electron negativity.*

showed lower χ values (χ<4.27). The value of χ determined by this method may be useful to estimate the cytotoxic activity of newly synthesized phenoxazine derivatives.

In the HSC-3 cells, correlation was found only between the CC_{50} and log-P (l in Figure 3). In the HL-60 cells, CC_{50} was correlated only to the heat of formation (r^2=0.717) and log-P (m and n in Figure 3), but not to the other descriptors (data not shown).

The QSAR of newly synthesized phenoxazine derivatives was investigated, based on the concept of chemical hardness. The cytotoxic activity of the phenoxazines against HSC-2 and HSC-4 cells could be estimated from the following chemical descriptors: electron affinity, η, χ and log-P. However, only log-P was correlated to CC_{50} in the HSC-3 cells, whereas only heat of formation and log-P were connected to CC_{50} in the HL-60 cells. The similarity observed between the HSC-2 and HSC-4 cells, and the difference from the HSC-3 cells, may be due to difference in the expression of outer cell membrane proteins, since we have found an abundance of multidrug resistance proteins (MDR, MRP) in HSC-2 and HSC-4 cells, as compared with HSC-3 cells (Hashimoto *et al.*, in preparation).

Acknowledgements

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References

1 Motohashi N, Mitscher LA and Meyer R: Potential antitumor phenoxazines. Med Res Rev *11*: 239-294, 1991.
2 Hara K, Okamoto M, Aki T, Yagita H, Tanaka H, Mizukami Y, Nakamura H, Tomoda A, Hamasaki N and Kang D: Synergistic enhancement of TRAIL- and tumor necrosis factor alpha-induced cell death by a phenoxazine derivative. Mol Cancer Ther *4*: 1121-1127, 2005.
3 Motohashi N: Interaction between 9-substituted benzo[*a*] phenoxazine derivatives and DNA and relationships to their antibacterial activities. (In Japanese) Yakugaku Zasshi *102*: 646-650, 1982.
4 Iwata A, Yamaguchi T, Sato K, Izumi R and Tomoda A: Antiviral activity of 2-amino-4,4alpha-dihydro-4alpha-7-dimethyl-3H-phenoxazine-3-one on poliovirus. Tohoku J Exp Med *200*: 161-165, 2003.
5 Blank B and Baxter LL: Synthesis and antiinflammatory screening of phenoxazine-1-carboxylic acids. J Med Chem *11*: 807-811, 1968.
6 Wesolowska O, Molnar J, Westman G, Samuelsson K, Kawase M, Ocsovszki I, Motohashi N and Michalak K: Benzo[α]phenoxazines: a new group of potent P-glycoprotein inhibitors. In Vivo *20*: 109-114, 2006.
7 Klabunde T, Petrassi HM, Oza VB, Raman P, Kelly JW and Sacchettini JC: Rational design of potent human transthyretin amyloid disease inhibitors. Nat Struct Biol *7*: 312-321, 2000.
8 Moosmann B, Skutella T, Beyer K and Behl C: Protective activity of aromatic amines and imines against oxidative nerve cell death. Biol Chem *382*: 1601-1612, 2001.
9 Suzuki F, Hashimoto K, Kikuchi H, Kawase M, Motohashi N and Sakagami H: Tumor-specificity and type of cell death induced by phenoxazines. Anticancer Res *27*: 4231-4236, 2007.
10 Kobayashi S, Tanaka A and Sameshima K: Relationship between electron structure toxicity and biological activity of environmental hormones. (In Japanese) Chemistry Chemical Ind *52*: 41-44, 1999.
11 Kobayashi S, Sameshima K, Ishii Y and Tanaka A: Toxicity of dioxins: role of absolute hardness-absolute electronegativity diagram (η-χ diagram) as a new measure in risk assessment. Chem Pharm Bull *43*: 1780-1790, 1995.
12 Kobayashi S, Hamashima H, Kurihara M, Miyata N and Tanaka A: Hardness controlled enzymes and electronegativity controlled enzymes: role of an absolute hardness-electronegativity (η-χ) activity diagram as a coordinate for biological activities. Pharm Bull *46*: 1108-1115, 1998.
13 Motohashi N: Test for antitumor activities of phenothiazines and phenoxazines. (In Japanese) Yakugaku Zasshi *103*: 364-371, 1983.
14 Ishihara M, Takayama F, Toguchi M, Nakano K, Yasumoto E, Nakayachi T, Satoh K and Sakagami H: Cytotoxic activity of polyprenylalcohols and vitamin K_2 derivatives. Anticancer Res *20*: 4307-4314, 2000.
15 Ishihara M and Sakagami H: Application of semiempirical method to estimate the cytotoxic activity of gallic acid and its related compounds. Anticancer Res *23*: 2549-2552, 2003.
16 Ishihara M, Yokote Y and Sakagami H: Quantitative structure-cytotoxicity relationship analysis of coumarin and its derivatives by semiempirical molecular-orbital method. Anticancer Res *26*: 2883-2886, 2006.

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