

Quantitative Structure-cytotoxicity Relationship Analysis of Coumarin and its Derivatives by Semiempirical Molecular Orbital Method

MARIKO ISHIHARA¹, YOSHIKO YOKOTE² and HIROSHI SAKAGAMI³

¹Division of Basic Chemistry, Department of Oral Biology and Tissue Engineering and

³Division of Pharmacology, Department of Diagnostic and Therapeutic Sciences,

Meikai University School of Dentistry, Sakado, Saitama 350-0283;

²Faculty of Science, Josai University, Sakado, Saitama 350-0295, Japan

Abstract. A semiempirical molecular orbital method (C_AC_he) was applied to delineate the relationship between cytotoxicity against the human squamous cell carcinoma line HSC-2 (evaluated by 50% cytotoxic concentration, CC₅₀) of 20 coumarin (2H-pyran-2-one) derivatives and twelve physical parameters (descriptors) calculated by the CONFLEX/PM3 method. There was a highly significant correlation between the CC₅₀ and ionization potential, highest occupied molecular orbital (HOMO) energy, difference between electron energy of HOMO and electron energy of lowest unoccupied molecular orbital (LUMO), or absolute hardness ($r^2=0.756 \sim 0.802$). On the other hand, there was no significant correlation between the CC₅₀ and heat of formation, stability of hydration, dipole moment, electron affinity, or LUMO energy ($r^2=0.13 \sim 0.36$). When the CC₅₀ was plotted vs. log P, a parabolic curve was produced, with a maximum cytotoxicity (or the least CC₅₀ value) at log P of 2.5. The present study demonstrated that hardness and softness, other than the electron accepting and donating properties, are important factors in estimating the cytotoxic activity of coumarin derivatives.

Coumarin (2H-pyran-2-one) and its derivatives (Figure 1) are widely distributed in nature and exhibit a broad pharmacological profile, including anticancer activity (1) and the scavenging activity of superoxide anions generated by activated neutrophils (2). Introduction of substituents

at C-2, C-4 or C-7 of the heterocyclic ring of coumarin induced various biological activities. They are known to induce apoptosis in human leukemia cells by increasing the cytosolic translocation of cytochrome c and activation of the cysteine protease 32 kDa proenzyme (3, 4). However, few efforts (4) have been made to establish the relationship between the structure and cytotoxic activity of coumarins in general. In this study, the correlation between the 50% cytotoxic concentration (CC₅₀) of 20 coumarin derivatives against the HSC-2 cell line and twelve physical parameters (descriptors) calculated by the CONFLEX/PM3 method was investigated.

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 Chemical Co., St. Louis, MO, USA). Coumarin derivatives (Figure 1) were purchased from Tokyo Kasei Co., Tokyo, Japan (5).

Cytotoxic activity. Human squamous cell carcinoma HSC-2 cells (obtained from Prof. M. Nagumo, Showa University, Japan) were cultured in DMEM supplemented with 10% heat-inactivated FBS under a humidified 5% CO₂ atmosphere. HSC-2 cells were inoculated at 5-6x10³ cells/well in 96-microwell plate (Becton Dickinson Labware, NJ, USA). After 48 h, the medium was removed by an aspirator and was replaced with 0.1 mL of fresh medium containing various concentrations of the test compounds. Near confluent cells were incubated for another 24 h, and the relative viable cell number was then determined by the MTT method. In brief, the cells were washed once with phosphate-buffered saline, replaced with fresh culture medium containing 0.2 mg/mL MTT and incubated for another 4 h. The cells were lysed with 0.1 mL of DMSO, and the absorbance at 540 nm of cell lysate was determined, using a microplate reader (Biochromatic Labsystem, Helsinki, Finland). The absorbance at 540 nm of the control cells

Correspondence to: Mariko Ishihara, Division of Basic Chemistry, Department of Oral Biology and Tissue Engineering, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan. Tel: (+81)49-285-5511, ex 563, 690, Fax: (+81)49-285-5171, e-mail: sakagami@dent.meikai.ac.jp or mariko@dent.meikai.ac.jp

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Table I. The cytotoxic activity (expressed as CC_{50}) against HSC-2 cells and twelve descriptors for each coumarin compound.

Compd.	HSC-2 cells CC_{50} ($\mu\text{M/L}$)	Heat of formation (Kcal/mol)	ΔH	Dipole moment (D)	Electron affinity (eV)	Ionization potential (eV)	$\log P$	ϵ_{HOMO}	ϵ_{LUMO}	$\Delta \epsilon$	η	χ
1	1370	-150.799	12.671	7.400	1.035	9.623	1.39	-9.623	-1.035	-8.588	4.294	5.329
2	1025	-100.588	16.491	6.046	1.007	9.290	1.58	-9.290	-1.007	-8.282	4.141	5.148
3	236	-145.390	19.172	9.478	1.056	9.099	1.84	-9.099	-1.056	-8.043	4.021	5.078
4	1042	-137.096	18.892	5.568	1.043	9.266	1.28	-9.266	-1.043	-8.223	4.111	5.154
5	901	-172.396	20.858	5.564	1.123	9.320	1.71	-9.320	-1.123	-8.198	4.099	5.221
6	950	-109.326	16.482	6.525	1.062	9.313	1.64	-9.313	-1.062	-8.251	4.125	5.187
7	664	-107.911	16.818	8.175	1.130	9.350	2.00	-9.305	-1.030	-8.175	4.087	5.217
8	172	-154.099	18.757	5.874	1.109	9.117	2.08	-9.117	-1.109	-8.008	4.004	5.113
9	417	-154.078	19.078	8.063	1.015	9.221	1.81	-9.221	-1.015	-8.206	4.103	5.118
10	791	-144.433	19.752	8.393	1.193	9.287	1.73	-9.287	-1.193	-8.094	4.047	5.240
11	971	-145.733	20.062	6.055	1.096	9.285	1.49	-9.285	-1.096	-8.189	4.095	5.190
12	443	-108.071	15.812	8.851	1.017	9.158	3.10	-9.158	-1.017	-8.140	4.070	5.087
13	405	-115.471	15.849	9.290	1.041	9.171	2.96	-9.171	-1.041	-8.130	4.065	5.106
14	92	-160.324	18.154	9.641	1.089	9.028	2.67	-9.028	-1.089	-7.939	3.970	5.059
15	105	-150.052	16.792	9.358	1.039	9.114	2.32	-9.114	-1.139	-7.974	3.987	5.126
16	282	-153.639	19.305	5.762	1.079	9.151	2.08	-9.151	-1.079	-8.072	4.036	5.115
17	41	-155.885	20.685	6.531	1.059	8.996	2.54	-8.996	-1.159	-7.838	3.919	5.078
18	325	-156.667	19.367	10.964	1.052	9.035	3.04	-9.035	-1.052	-7.983	3.992	5.044
19	73	-145.465	19.088	8.332	1.247	9.204	2.20	-9.204	-1.247	-7.958	3.979	5.226
20	82	-146.697	18.028	5.757	1.046	9.131	2.68	-9.131	-1.146	-7.985	3.993	5.139

was usually in the range of 0.40 to 0.90. The CC_{50} was determined from the dose-response curve (5).

Calculation. The most stable structure of twenty coumarin derivatives was calculated by CONFLEX (Confluex Co. Ltd., Tokyo, Japan). The optimization of the structure was done by semiempirical molecular orbital method (PM3), using CAChe Worksystem 4.9 (Fujitsu Co. Ltd., Tokyo, Japan). The octanol-water distribution coefficient ($\log P$) was calculated by ACD-Log P (Fujitsu). The QSAR between the CC_{50} and descriptors, delineated from the molecular structure, was investigated by CAChe Worksystem 4.9 project reader. The following descriptors were used: ①heat of formation, ②stability of hydration, ③dipole moment, ④electron affinity, ⑤ionization potential, ⑥hydrophobicity ($\log P$), ⑦highest occupied molecular orbital (HOMO) energy, ⑧lowest unoccupied molecular orbital (LUMO) energy, ⑨ $\Delta \epsilon (\epsilon_{\text{HOMO}} - \epsilon_{\text{LUMO}})$, ⑩absolute hardness (η), ⑪ absolute electron negativity (χ) and ⑫ $\eta - \chi$, where ϵ_{HOMO} , electron energy of HOMO; ϵ_{LUMO} , electron energy of LUMO; $\eta = (\epsilon_{\text{LUMO}} - \epsilon_{\text{HOMO}})/2$; $\chi = -(\epsilon_{\text{LUMO}} + \epsilon_{\text{HOMO}})/2$.

Results and Discussion

The CC_{50} and twelve descriptors for each compound are shown in Table I. The correlation between the CC_{50} and each descriptor is shown in Figure 2①-⑫. There was no correlation between the CC_{50} and ①, ②, ③, ④ or ⑧ ($r^2=0.13 \sim 0.36$). When the CC_{50} was plotted vs. $\log P$, a parabolic curve was produced, with a maximum cytotoxicity (the least CC_{50} value) at $\log P$ of 2.5 (Figure 2⑥). There was a highly significant correlation between the CC_{50} and ⑤, ⑦, ⑨ and ⑩ ($r^2=0.756 \sim 0.802$), suggesting that these four

Compd.	R_1	R_2	R_3	R_4	R_5	R_6
1	H	H	H	H	H	H
2	H	H	H	H	OH	H
3	H	H	H	OH	OH	H
4	H	H	H	OCH ₃	OH	H
5	H	H	OCH ₃	OCH ₃	OH	H
6	H	CH ₃	H	H	OH	H
7	H	CH ₃	H	OH	H	H
8	H	CH ₃	H	OH	OH	H
9	H	CH ₃	OH	H	OH	H
10	H	CH ₃	H	OH	OCH ₃	H
11	H	CH ₃	H	OCH ₃	OH	H
12	CH ₃	H	H	H	OH	H
13	CH ₃	CH ₃	H	H	OH	H
14	CH ₃	CH ₃	H	OH	OH	H
15	CH ₃	CH ₃	H	OH	OCH ₃	H
16	CH ₃	CH ₃	H	OCH ₃	OH	H
17	-(CH ₂) ₃ -	H	OH	OH	H	
18	-(CH ₂) ₃ -	OH	H	OH	H	
19	-(CH ₂) ₃ -	H	OH	OCH ₃	H	
20	-(CH ₂) ₃ -	H	OCH ₃	OH	H	

Figure 1. Chemical structures of the coumarin derivatives.

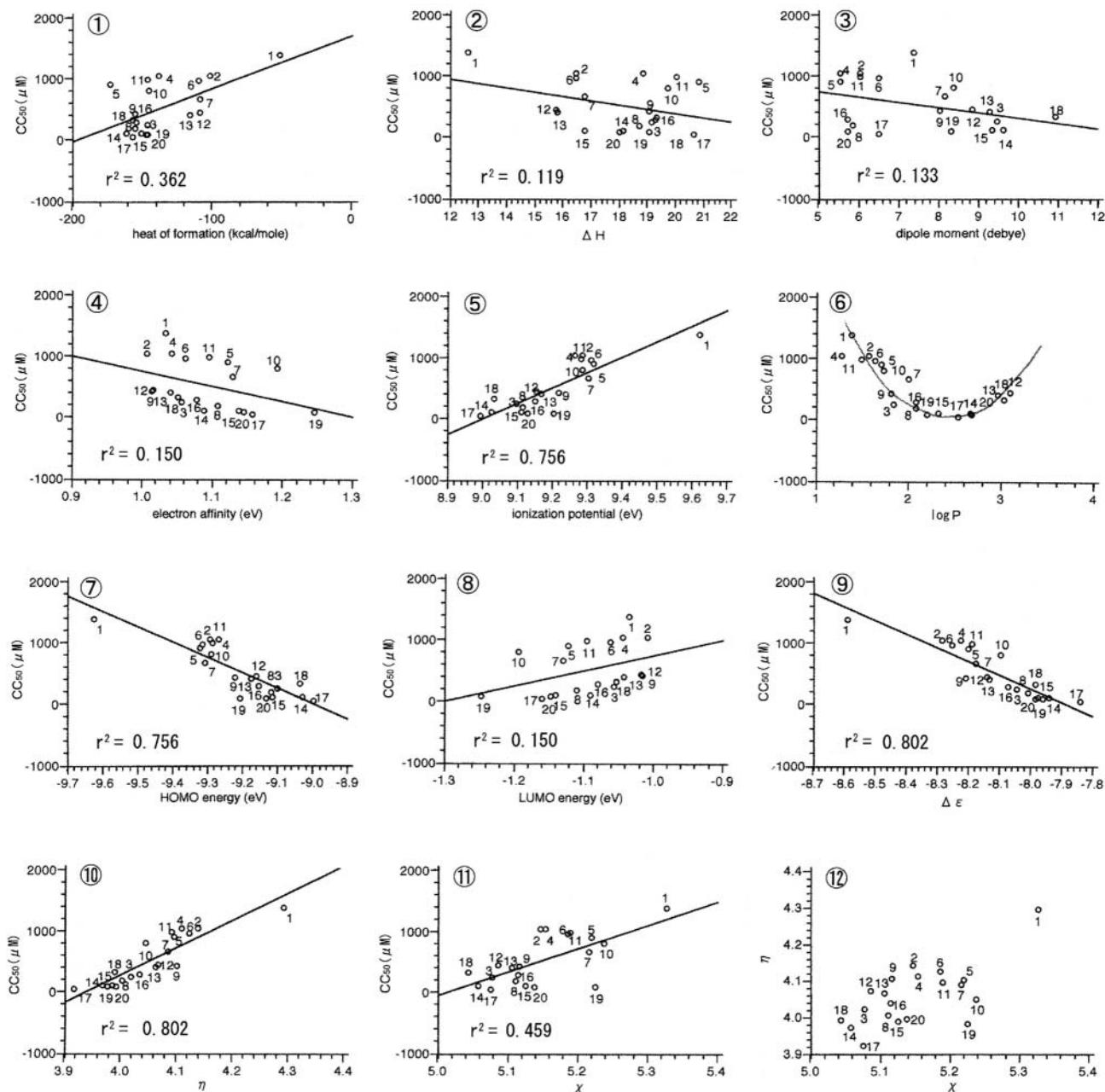


Figure 2. Relationship between the CC_{50} values of coumarin derivatives against HSC-2 cells and each descriptor.

descriptors can be utilized for the estimation of the cytotoxic activity of coumarins. The HOMO and LUMO energy values can be used as markers for electron donating and accepting capabilities, respectively. With an increase in HOMO energy, the molecule has an increasing electron donating property. On the other hand, a decrease in the LUMO energy would lead to an increase in the electron accepting property. The HOMO energy reflects the ionization potential. The correlation between the CC_{50} and

ionization potential ($r^2=0.756$) indicates that the cytotoxicity of coumarin derivatives is produced from this electron donating property.

Recently, the hazardous effects of endocrine disruptors (environmental hormones) on the human body, such as bisphenol A and nonyl phenol, have been reported and, as a result, many studies concerning the detection and structural determination of these compounds, which are present in tiny amounts, were initiated (6). As one of the QSAR analyses of

environmental hormones, the correlation between their biological activity and chemical hardness was reported (7). By applying these analytical methods, the molecular toxicity and estrogen-like activity of environmental hormones were found to correlate strongly with the η value. This method was used to investigate the correlation between the CC_{50} and η values in this study. We found that the cytotoxic activity of coumarin derivatives against HSC-2 cells produced the well-fitted straight line vs. the $\eta-\chi$ value ($r^2=0.802$, Figure 2@). The $\eta-\chi$ diagram was subsequently determined as one of the methods to investigate the electron state of the molecule. By applying this $\eta-\chi$ diagram to the coumarin derivatives, their cytotoxicity was found to depend on the the η value (Figure 2@), but not on the χ (Figure 2 @) nor the $\eta-\chi$ values (Figure 2 @). Compounds with relatively higher cytotoxicity showed lower η values (<4.04). Compounds with relatively lower cytotoxicity (except for compound 18) showed higher η values (>4.04). This $\eta-\chi$ activity diagram may be useful for the study of cytotoxic activity.

In conclusion, the present study demonstrated that the cytotoxic activity of the coumarin derivatives showed a strong linear correlation with the absolute hardness, η . Hardness and softness, other than the electron accepting and donating properties of the molecule, are important factors for estimating their cytotoxic activity (8, 9). From the η value, the CC_{50} value of the novel coumarin compounds can be estimated. CONFLEX is useful in calculating the hardness and softness of the molecule using the PM3 method.

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