in vivo *20*: 385-390 (2006)

Relationship between Electronic Structure and Cytotoxic Activity of Azulenes

TERUO KURIHARA[1], MAYUMI NOGUCHI[1], TAKASHI NOGUCHI[1], HIDETSUGU WAKABAYASHI[1], NOBORU MOTOHASHI[2] and HIROSHI SAKAGAMI[3]

[1]*Department of Chemistry, Faculty of Science, Josai University, Sakado, Saitama;* [2]*Meiji Pharmaceutical University, Kiyose, Tokyo;* [3]*Meikai University School of Dentistry, Saitama, Japan*

Abstract. *The structure-activity relationship of the cytotoxic activity of azulene and azulene derivatives was discussed, using theoretically calculated results. In order to clearly divide the azulenes into three groups according to their functional groups, the CC_{50}, four different dipole moments (μ_G, $\mu_{ESP\text{-}G}$, μ_W and $\mu_{ESP\text{-}W}$) and heats of formation (ΔHf) of the azulenes* ***[1-24]*** *were separately calculated in two states, gas-phase and water, by the conductor-like screening model/parametric method 3 (COSMO/PM3). For the halogenated azulenes and isopropyl azulenes, the cytotoxic activity might follow the three quantitative structure-activity relationship (QSAR) parameters: $\Delta\Delta H_f$, HOMO energy and μ_w. Whereas, for the other ten compounds* ***[3-5, 7-8, 10, 15-18]****, the cytotoxic activity might be related to the three QSAR parameters, $\Delta\Delta H_f$, LUMO energy and μ_G.*

Azulene chemistry, including synthesis and their physical and chemical properties, has been extensively studied for more than four decades (1-4). Azulene derivatives have shown interesting biological activities, such as antibacterial (5), anti-ulcer (6) and relaxant activities (7), inhibition of thromboxane A_2-induced vasoconstriction and thrombosis (8), acute toxicity and local anesthetic activity (9).

In our previous paper, the cytotoxicities of 27 azulene derivatives against three human normal cell lines and three human oral tumor cell lines were discussed (10). Among six halogenated azulenes, 1,3-dibromoazulene **[13]** was found to be highly cytotoxic against both human tumor cells [CC_{50} (HSG)=0.31 mM; CC_{50} (HSC-2)=0.19 mM; CC_{50} (HSC-3)=0.15 mM] and normal human cells [CC_{50} (HGF)=0.27 mM; CC_{50} (HPC)=0.26 mM; CC_{50} (HPLF)=0.30 mM]. Among seven isopropylazulene derivatives, methyl 7-isopropyl-2-methoxyazulene-1-carboxylate **[24]** was comparably cytotoxic against the tumor cell lines [CC_{50} for HSG, HSC-2 and HSC-3=0.11 mM], but showed much lower cytotoxicity against normal cells. (10). This tumor-specific cytotoxic activity might be related to the position of functional groups. The azulenes can be conveniently divided into three groups based on their functional groups: halogenated azulene derivatives, isopropylazulene derivatives and other derivatives. Azulene **[1]** is considered to be the reference compound. Based on a molecular orbital calculation concerning the physicochemical parameters and the cytotoxic activities of azulene derivatives, the purpose of this paper was to present some relationships between the electronic structure and the cytotoxic activity of azulene derivatives.

Correspondence to: Dr. Teruo Kurihara, Department of Chemistry, Faculty of Science, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan. Fax: (+)-81-49-271-7985, e-mail: tkuri@josai.ac.jp

Key Words: Azulenes, cytotoxic activity, PM3 calculation method, dipole moment (μ).

Materials and Methods

Chemicals. Twenty-four azulenes **[1-24]** were synthesized, as described previously (10) (Figure 1).

Theoretical calculations. The molecular orbital calculation by the parametric method 3 (PM3) was performed with application of the winMOPAC program (11). The geometries of the azulene derivatives **[1-24]** were optimized with respect to all geometrical parameters using the Broyden-Fletcher-Goldfarb-Shanno algorithm incorporated in the program. The geometries of the azulene derivatives **[1-24]** in water-solution were compared with those in gases by the conductor-like screening model orbital (COSMO) and electrostatic potential (ESP) calculations. The COSMO procedure generates a conducting polygonal surface around the system at van der Waal's distance. The standard values used here were the number of geometrical segments per atom (NSPA)=60 and the dielectric constant=78.4 at 25°C (water). The values of the dipole moment (μ_G and μ_W) in the gas-phase and in the water-solution of azulene derivatives **[1-24]** were calculated by the ESP/PM3 and COSMO/PM3 methods. For this calculation, an IBM Intellistation M Pro personal computer was used (11).

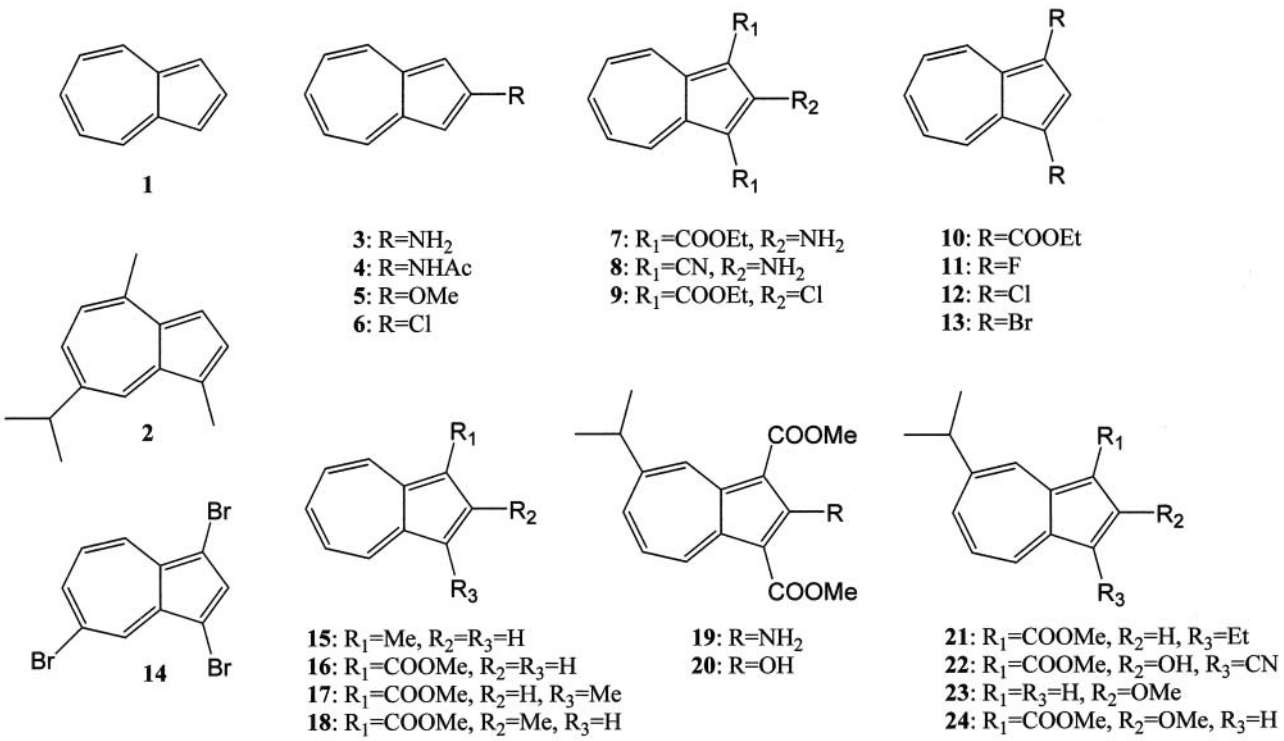

Figure 1. *Structure of 24 azulene derivatives* ***[1-24]****.*

Results and Discussion

Relationship between structure and activity. The relationship between the cytotoxicity of 24 azulene derivatives against two human normal cell lines (HGF, HPLF) and two human oral squamous cell carcinoma cell lines (HSC-2, HSC-3) and their electronic properties were investigated.

A partition coefficient logP is used as an index for the structure-activity relationship analysis in new drug design. A stereo hydrophobic parameter dGW was obtained by the PM3 method. The dGWs were defined by their free-energy changes for the association in the aqueous solution and in the gas-phase (12). From the calculations, the structure-activity relationship analysis revealed that the hydrophobicity of the whole molecule ($\Delta\Delta H_f$) and the dipole moment (μ) might control the cytotoxic activities of the azulene derivatives. Recently, we represented the quantitative structure-activity relationship (QSAR) between cytotoxic activity and the three QSAR parameters of $\Delta\Delta H_f$, I_{OH} and $\mu_{ESP\text{-}G}$ on 3-benzazepine derivatives (13). Based on our previous results, the relationship between the cytotoxic activity and the individual QSAR parameters are presented.

The $\Delta\Delta H_f$, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) energy and the dipole moment (μ) of azulene derivatives **[1-24]**, calculated by the PM3 method, are provided in Table I. Four types of dipole moment were calculated by the PM3 method. Among the azulene derivatives **[1-24]**, the value of $\Delta\Delta H_f$ / M.W. increased in the following order: **[14]** ($\Delta\Delta H_f$ / M.W.=0.093 kJ/mol/g) < **[12]** ($\Delta\Delta H_f$ / M.W.=0.113 kJ/mol/g) < **[2]** and **[13]** ($\Delta\Delta H_f$ /M.W.=0.117 kJ/mol/g) < **[15]** ($\Delta\Delta H_f$ / M.W.=0.137 kJ/mol/g). < **[6]** ($\Delta\Delta H_f$ / M.W.=0.139 kJ/mol/g). The value of HOMO energy in the water-solution increased in the following order: **[3]** (–7.75 eV) < **[19]** (–8.10 eV) < **[12]** (–8.28 eV) < **[2]** (–8.30 eV) < **[15]** (–8.31 eV). < **[1]** (–8.39 eV).

The value of the dipole moment (μ_w) in the water-solution also increased as follows: **[3]** (1.92 D) < **[15]** (3.07 D) < **[2]** (3.44 D) < **[23]** (4.19 D) < **[5]** (4.31 D) < **[1]** (4.52 D). The cytotoxic activity of **[24]** against HGF cells was the highest (CC_{50}=0.18 mM), followed by **[13]** (CC_{50}=0.27 mM), **[21]** (CC_{50}=0.35 mM) and **[18]** (CC_{50}=0.39 mM).

The cytotoxic activity of **[24]** against the HSC-2 cells was also the highest (CC_{50}=0.11 mM), followed by **[18]** (CC_{50}=0.14 mM), **[2]** (CC_{50}=0.16 mM) and **[14]** (CC_{50}=0.17 mM). Their cytotoxic activities could not be related to the individual QSAR parameters, such as $\Delta\Delta H_f$ /M.W., HOMO energy or μ_w. The correlation coefficient (r^2) and the Fisher statistic (F) are important in assessing the "correctness" of a regression fit. In order

Table I. *QSAR parameters of azulene derivatives.*

Compound no.	$\Delta\Delta H_f$ (in KJ/mol)/ M.W.	HOMO (eV)		LUMO (eV)		Dipole moment (in Debye units)			
		in gas-phase	in water	in gas-phase	in water	μ_G	μ_{ESP-G}	μ_W	μ_{ESP-W}
1	0.183	–7.99	–8.39	–1.19	–1.47	1.86	1.70	4.52	4.44
2	0.117	–7.89	–8.30	–0.92	–1.36	1.48	1.34	3.44	3.47
3	0.241	–7.51	–7.75	–0.91	–1.22	0.70	0.61	1.92	1.79
4	0.357	–8.33	–8.57	–1.24	–1.48	4.36	3.96	9.21	8.96
5	0.188	–8.25	–8.57	–0.99	–1.39	1.66	1.11	4.31	3.99
6	0.139	–8.34	–8.56	–1.22	–1.52	2.47	1.89	5.59	5.15
7	0.277	–8.19	–8.62	–1.50	–1.56	4.70	4.84	10.63	11.07
8	0.476	–8.71	–8.71	–1.93	–1.67	4.38	4.52	8.79	9.12
9	0.454	–8.77	–8.92	–1.80	–1.75	6.70	5.85	11.87	11.71
10	0.270	–8.70	–8.82	–1.74	–1.63	6.71	6.42	12.97	13.10
11	0.153	–8.37	–8.48	–1.49	–1.63	3.06	3.01	6.48	6.55
12	0.113	–8.08	–8.28	–1.35	–1.60	2.47	2.03	5.74	5.42
13	0.117	–8.41	–8.56	–1.44	–1.65	2.88	2.38	6.39	5.99
14	0.093	–8.60	–8.70	–1.64	–1.76	2.01	1.66	4.54	4.32
15	0.137	–8.00	–8.31	–0.97	–1.34	1.18	1.08	3.07	3.04
16	0.293	–8.48	–8.66	–1.43	–1.54	4.63	4.59	9.67	9.85
17	0.268	–8.34	–8.50	–1.39	–1.53	4.55	4.44	9.52	9.67
18	0.237	–8.44	–8.63	–1.38	–1.55	3.74	3.83	8.77	9.08
19	0.276	–8.00	–8.10	–1.36	–1.43	4.34	4.54	7.18	7.68
20	0.291	–8.77	–8.99	–1.65	–1.61	7.24	7.04	13.57	13.73
21	0.196	–8.28	–8.51	–1.35	–1.55	4.83	4.63	9.50	9.60
22	0.353	–8.80	–8.95	–1.81	–1.73	6.77	6.82	12.80	12.34
23	0.144	–8.17	–8.55	–0.95	–1.39	1.60	1.14	4.19	3.89
24	0.238	–8.56	–8.70	–1.40	–1.57	5.83	4.56	9.68	9.60

$\Delta\Delta H_f$=hydrophobicity of whole molecule

to obtain a more quantitative characteristic of the "correctness" of a model, QSAR uses the well-known Fisher statistic value. The correlation coefficient (r^2) and F value against HGF cells for all the azulenes, except for compounds **[3]** (CC_{50} > 2.79 mM), **[4]** (CC_{50} > 1.99 mM) and **[8]** (CC_{50} > 2.07 mM), using the three electronic parameters of $\Delta\Delta H_f$ /M.W., HOMO energy and μ_w, were calculated as 0.041 and 0.244 (< F (3, 17, 0.05)=3.160), respectively. The r^2 and F value between CC_{50} values against HSC-2 cells for all the azulenes except for compound **[3]** (CC_{50} > 2.79 mM), using the three QSAR parameters $\Delta\Delta H_f$ /M.W., HOMO energy and μ_w, were also calculated as 0.073 and 0.501, respectively. Since the F values of these derivatives for this model were lower than the 5% critical value (F=3.127), the hypothesis was not be acceptable.

The azulenes may thus be conveniently divided according to their three functional groups: halogenated compounds **[6, 9, 11-14]**, isopropylazulene derivatives **[2, 19-24]** and other compounds **[3-5, 7-8, 10, 15-18]**.

Relationship between the cytotoxic activity of halogenated compounds against normal cells (HGF, HPLF) or tumor cells (HSC-2, HSC-3) and $\Delta\Delta H_f$/M.W., HOMO energy and μ_w. Of the six halogenated azulenes, 1,3-dibromoazulene **[13]** showed the highest cytotoxicity against normal human cells. In order to obtain a quantitative correlation between the cytotoxic activity and electronic properties, the coefficient of the multiple determination and the F value were calculated. The structure-activity relationship analysis revealed that the hydrophobicity of the molecule ($\Delta\Delta H_f$), the HOMO energy in the water-solution and the dipole moment (μ_W) in the water-solution might greatly contribute to cytotoxic activity. Consequently, the following correlation equations **1** and **2** were obtained for the HGF and HPLF cells, respectively:

CC_{50}=24.051 + 12.766 x $\Delta\Delta H_f$ / M.W. + 2.623 x E_{HOMO} –0.440 x μ_w (equation **1**)

n=5 (**1, 9, 11, 13, 14**), r^2=0.999, F=17239 > F (3, 1, 0.05)=215.7.

CC_{50}=39.781 + 5.963 x $\Delta\Delta H_f$ / M.W. + 4.353 x E_{HOMO} –0.449 x μ_w (equation **2**)

n=5 (**1, 11-14**), r^2=0.999, F=920.9.

Of the six halogenated azulenes, 1,3,5-tribromoazulene **[14]** was the most cytotoxic against the HSC cells.

The following correlation equations **3** and **4** were obtained for the HSC-2 and HSC-3 cells, respectively:

Table II. *Observed and estimated activity of azulenes **[1-24]**.*

Compd.	Cytotoxic activity (CC_{50}: mM)							
	Normal human cells				Human tumor cell lines			
	HGF		HPLF		HSC-2		HSC-3	
	obs.	estim.[a]	obs.	estim.[a]	obs.	estim.[a]	obs.	estim.[a]
1	2.38	2.38	2.29	2.30	1.33	1.33	2.33	2.34
2	0.40	0.39	0.19	0.20	0.16	0.15	0.12	0.13
3	>2.79	3.30	>2.79	2.83	>2.79	0.83	>2.79	1.23
4	>1.99	1.93	>1.99	1.38	0.34	0.39	0.98	0.99
5	1.82	1.81	1.71	1.64	0.61	0.64	0.92	0.83
6	2.39	0.92	1.86	0.85	0.42	0.55	0.65	0.81
7	0.98	0.67	0.92	0.58	0.64	0.25	1.17	0.28
8	>2.07	10.03	>2.07	9.04	1.47	1.46	1.00	1.00
9	1.22	1.22	>1.30	–1.69	0.24	0.24	0.25	0.25
10	1.41	–0.64	1.43	–0.49	1.30	0.01	>1.47	–0.19
11	0.91	0.92	0.90	0.88	0.62	0.65	0.58	0.82
12	1.78	1.26	1.85	1.85	1.06	1.04	1.40	1.39
13	0.27	0.27	0.30	0.32	0.19	0.28	0.15	0.17
14	0.42	0.42	0.43	0.42	0.17	0.16	0.30	0.29
15	0.55	0.50	0.54	0.55	0.58	0.50	0.51	0.61
16	0.76	0.89	0.45	0.70	0.45	0.28	0.35	0.45
17	0.41	0.33	0.40	0.24	0.25	0.23	0.49	0.38
18	0.39	0.76	0.38	0.72	0.14	0.34	0.35	0.34
19	0.95	0.45	0.95	0.95	0.28	0.28	0.80	0.79
20	0.95	0.56	0.78	0.78	0.43	0.43	0.38	0.37
21	0.35	0.35	0.30	0.29	0.18	–0.04	0.29	0.15
22	0.96	0.96	0.84	1.30	0.84	0.83	0.78	0.79
23	0.48	0.49	0.43	0.42	0.46	0.47	0.23	0.22
24	0.18	0.57	0.17	0.65	0.11	0.37	0.11	0.36

[a]Estimated from the corresponding equation. obs., observed.

CC_{50}=20.597 + 5.128 x $\Delta\Delta H_f$ / M.W. + 2.314 x E_{HOMO} –0.172 x μ_w (equation **3**)
n=5 (**1, 9, 11, 12, 14**), r^2=0.998, F=218.4.

CC_{50}=30.528 + 11.254 x $\Delta\Delta H_f$ / M.W. + 3.357 x E_{HOMO} –0.458 x μ_w (equation **4**)
n=5 (**1, 9, 12-14**), r^2=0.999, F=2376.1.

Relationship between the cytotoxic activity of isopropylazulenes against normal cells (HGF, HPLF) or tumor cells (HSC-2, HSC-3) and $\Delta\Delta H_f$/M.W., HOMO energy and μ_w. Of the seven isopropylazulene derivatives, methyl 7-isopropyl- 2-methoxyazulene-1-carboxylate **[24]** showed a much lower cytotoxicity against the normal cells (10). In the case of the isopropylazulene derivatives, the following correlation equations **5** and **6** were obtained for the HGF and HPLF cells, respectively:
CC_{50}=–0.201 + 5.416 x $\Delta\Delta H_f$ / M.W. –0.027 x E_{HOMO} –0.078 x μ_w (equation **5**)
n=5 (**2, 19, 21-23**), r^2=0.999, F=779.9.

CC_{50}=–3.317 + 7.372 x $\Delta\Delta H_f$ / M.W. –0.358 x E_{HOMO} –0.093 x μ_w (equation **6**)
n=5 (**2, 19, 21-23**), r^2=0.999, F=669.4.

Of the isopropylazulene derivatives, compound **[22]** showed a much lower cytotoxicity against tumor cells (Table II) (10).

In the case of these derivatives, the following correlation equations **7** and **8** were obtained for the HSC-2 and HSC-3 cells, respectively:
CC_{50}=–8.941 + 5.456 x $\Delta\Delta H_f$ / M.W. –1.076 x E_{HOMO} –0.139 x μ_w (equation **7**)
n=5 (**2, 19, 20, 22-23**), r^2=0.999, F=340.3.

CC_{50}=0.315 + 5.715 x __Hf / M.W. + 0.074 x E_{HOMO} –0.069 x μ_w (equation **8**)
n=5 (**2, 19, 20, 22-23**), r^2=0.999, F=308.3.

Relationship between the cytotoxic activity of other azulenes against normal cells (HGF, HPLF) or tumor cells (HSC-2, HSC-3) and $\Delta\Delta H_f$/M.W., HOMO energy and μ_w. The multiple

linear-regression analysis for the azulene derivatives **[4, 5, 7, 8, 10, 15-18]**, using the above equations **1-8**, did not correlate with the QSAR parameters $\Delta\Delta H_f$ / M.W., HOMO energy and μ_W. The r^2 and F values between the CC_{50} values against the HGF and HPLF cells for the nine azulenes, using the three QSAR parameters of $\Delta\Delta H_f$ / M.W., HOMO energy and μ_w, were 0.441 and 0.788, respectively. The r^2 and F values between the CC_{50} values against the HSC-2 and HSC-3 cell lines and the same QSAR parameters were 0.874 and 0.661, 6.913 and 1.953, respectively. However, since the F values of these azulene derivatives for this model were less than the 5% critical value (F=9.28), the hypothesis was not acceptable. Then, we defined that the LUMO energy in the gas-phase and $\mu_{ESP\text{-}G}$ were used instead of HOMO energy and μ_W.

Of the azulene derivatives **[4, 5, 7, 8, 10, 15-18]**, the cytotoxic activity of **[18]** was the highest (CC_{50}=0.39 mM), followed by that of **[17]** (CC_{50}=0.41 mM), **[15]** (CC_{50}=0.55 mM) and **[16]** (CC_{50}=0.76 mM) for the HGF cells. Methyl 2-methylazulene-1-carboxylate **[18]** showed a much lower cytotoxicity against normal cells. In the case of the other azulene derivatives, the following correlation equations **9** and **10** were obtained for the HGF and HPLF cells, respectively:

CC_{50}=–9.520 + 23.128 x $\Delta\Delta H_f$ / M.W. –9.417 x E_{LUMO} –2.137 x $\mu_{ESP\text{-}G}$ (equation **9**)

n=7 (**1, 5, 7, 15-18**), r^2=0.927, F=12.68 > F (3, 3, 0.05)=9.27.

CC_{50}=–9.009 + 18.753 x $\Delta\Delta H_f$ / M.W. –9.414 x E_{LUMO} –2.008 x $\mu_{ESP\text{-}G}$ (equation **10**)

n=7 (**1, 5, 7, 15-18**), r^2=0.904, F=9.51.

Of the azulene derivatives **[4, 5, 7, 8, 10, 15-18]**, the cytotoxic activity of **[18]** was the highest (CC_{50}=0.14 mM), followed by that of **[4]** (CC_{50}=0.34 mM), **[16]** (CC_{50}=0.45 mM) and **[15]** (CC_{50}=0.58 mM) for HSC-2 cells (Table II). A similar tendency was also found for the HSC-3 cells.

The following correlation equations **11** and **12** were obtained for the HSC-2 and HSC-3 cells, respectively:

CC_{50}=–0.818 + 2.442 x $\Delta\Delta H_f$ / M.W. –1.399 x E_{LUMO} –0.352 x $\mu_{ESP\text{-}G}$ (equation **11**)

n=7 (**4, 5, 8, 15-18**), r^2=0.930, F=13.33.

CC_{50}=0.781 + 4.833 x $\Delta\Delta H_f$ / M.W. + 0.675 x E_{LUMO} –0.171 x $\mu_{ESP\text{-}G}$ (equation **12**)

n=7 (**4, 5, 8, 15-18**), r^2=0.923, F=12.11.

The CC_{50} values estimated from the corresponding equations are shown in Table II. The results of the present study suggest the applicability of theoretical calculations, such as frontier molecular orbital, dipole moments and $\Delta\Delta H_f$, in the prediction of the cytotoxic activity of azulene and azulene derivatives.

References

1 Nozoe T and Asao T: Azulenes and heptafulvenes. *In*: Dai-Yuki Kagaku. Kotake M (ed.). Asakura-Shoten. Tokyo (in Japanese). Vol. 13, pp. 439-534, 1960.

2 Nozoe T, Breslow R, Hafner K, Ito S and Murata I (eds.): Topics in Nonbenzenoid Aromatic Chemistry. Hirokawa Publ Co., Tokyo, Vol. I, pp. 1-295, 1973.

3 Nozoe T, Breslow R, Hafner K, Ito S and Murata I (eds.): Topics in Nonbenzenoid Aromatic Chemistry. Hirokawa Publ Co. Tokyo (in Japanese). Vol. II, pp. 1-379, 1977.

4 Zeller KP: Methoden der oranischen chemie. Houben-Weyl, Band V/2c, pp. 127-418, 1985.

5 Frigola J, Pares J, Corbera J, Vano D, Merce R, Torrens A, Mas J and Valenti E: 7-Azetidinylquinolones as antibacterial agents. Synthesis and structure-activity relationship. J Med Chem *36*: 801-810, 1993.

6 Wakabayashi S, Sekiguchi H, Kosakai K, Mochizuki S, Kashima M and Tomiyama A: General pharmacological properties of an anti-ulcer drug, azuletil sodium (KT1-21) (in Japanese). Folia Pharmacol Japan *96*: 185-204, 1990.

7 Tanaka M, Yamaki F, Saitoh M, Nakazawa T, Tanaka H, Noguchi K, Hashimoto K and Shigenobu K: Relaxant action of azulene-1-carboxamidine derivative N^1,N^1-dimethyl-N^2-(2-pyridyl methyl)-5-isopropyl-3,8-dimethylazulene-1-carboxamidine (HNS-32) in pig coronary artery. Pharm Pharmacol Commun *6*: 397-404, 2000.

8 Tomiyama T, Yokota M, Wakabayashi S, Kosakai K and Yanagisawa T: Design, synthesis, and pharmacology of 3-substituted sodium azulene-1-sulfonates and related compounds: non-prostanoid thromboxane A2 receptor antagonists. J Med Chem *36*: 791-800, 1993.

9 Doukas PH, Speaker TJ and Thompson RS: Azulene analogs of pharmacological agents III. Acute toxicity and local anesthetic activity of azulylamides and azulenecarboxamides. J Pharm Sci *64*: 158-161, 1975.

10 Wakabayashi H, Hashiba K, Yokoyama K, Hashimoto K, Kikuchi H, Nishikawa H, Kurihara T, Satoh K, Shioda S, Saito S, Kusano S, Nakashima H, Motohashi N and Sakagami H: Cytotoxic activity of azulenes against human oral tumor cell lines. Anticancer Res *23*: 4747-4756, 2003.

11 PM3 semi-emprical MO calculations were performed by MOPAC version 6 on an Intellistation M Pro personal computer.

12 Ohkura K and Hori H: Analysis of structure-permeability correlation of nitrophenol analogs in newborn rat abdominal skin using semi empirical molecular orbital calculation. Bioorg Med Chem *7*: 309-314, 1998.

13 Kurihara T, Yamada T, Yamamoto A, Kawase M, Motohashi N, Sakagami H and Molnar J: Relationship between electronic structure and cytotoxic activity of dopamine and 3-benzazepine derivatives. In Vivo *18*: 443-448, 2004.

Received March 16, 2006
Revised April 7, 2006
Accepted April 12, 2006