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Relationship between Electronic Structure and Cytotoxic Activity of Azulenequinones and Trihaloacetylazulenes

TERUO KURIHARA[1], RIE SATOH[1], TAKASHI MIYAGAWA[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 relationship between the structure and cytotoxic activity of azulenequinones and trihaloacetylazulenes was investigated based on theoretical calculations. Four different dipole moments (μ_G, $\mu_{ESP\text{-}G}$, μ_W and $\mu_{ESP\text{-}W}$) and heats of formation (ΔH_f) of the azulenequinones **[1-27]** and trihaloacetylazulenes **[28a,b-40a,b]** were separately calculated in gas phase and aqueous solution using the conductor-like screening model/parametric method 3 (COSMO/PM3) method. The cytotoxic activity of azulenequinones was well correlated to $\Delta\Delta H_f$, HOMO energy and $\mu_{ESP\text{-}w}$. The cytotoxic activity of trihaloacetylazulenes was correlated to $\Delta\Delta H_f$, LUMO energy and $\mu_{ESP\text{-}W}$. QSAR may be applicable to predict the cytotoxicity of azulenequinones and trihaloacetylazulenes.*

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 diverse 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). Naphthoquinones have shown antifungal, antibiotic, antimalarial and antitumor activities (10-11). Azulenequinone is a nonbenzenoid aromatic quinone (10-13) and also a naphthoquinone isomer. We recently reported the cytotoxic activity of azulenequinones against human oral tumor cell lines (14) and the inhibition of LPS-stimulated NO production in mouse macrophage-like Raw 264.7 cells by azulenequinones (15). We found that 7-isopropyl-3-(4-methylanilino)-2-methyl-1,5-azulenequinone [**13**] and 3-(3-guaiazulenyl)-1,5-azulenequinone [**20**] showed higher tumor-specific cytotoxicity and induced apoptosis in human promyelocytic leukemia HL-60 and oral squamous cell carcinoma HSC-2 cells, possibly *via* the activation of both mitochondria-independent (extrinsic) and -dependent (intrinsic) pathways (14). We also reported the apoptosis-inducing activity of trihaloacetylazulenes against human oral tumor cell lines (16) and the inhibition of NO production in LPS-stimulated mouse macrophage-like Raw 264.7 cells by trihaloacetylazulenes (17). We also found that trichloroacetylazulenes [**28a-40a**] generally showed higher cytotoxicity and higher tumor-specific cytotoxicity as compared with the corresponding trifluoroacetylazulenes [**28b-40b**] (16). Based on a molecular orbital calculation concerning their physicochemical parameters and cytotoxic activities, we investigated the QSAR of azulenequinones and trihaloacetylazulenes.

Correspondence to: Dr. Teruo Kurihara, Department of Chemistry, Faculty of Science, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan. Fax: +81 049 271 7985, e-mail: tkuri@josai.ac.jp

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Materials and Methods

Chemicals. Twenty-seven azulenequinones [**1-27**] and twenty-six trihaloacetylazulenes [**28a,b-40a,b**] were synthesized, as described elsewhere (14, 16) (Figures 1 and 2).

Theoretical calculations. The molecular orbital calculation using the parametric method 3 (PM3) was performed with application of the winMOPAC program (18). The geometries of the azulenequinones [**1-27**] and trihaloacetylazulenes [**28a,b-40a,b**] were optimized with respect to all geometrical parameters using the Broyden-Fletcher-Goldfrab-Shanno algorithm incorporated into the program. The geometries of the azulenequinones [**1-27**] and trihaloacetylazulenes [**28a,b-40a,b**] in aqueous solution were compared with those in gases using 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 value of the number of the geometrical segments per atom (NSPA)=60, and that of the dielectric constant=78.4 at 25˚C (water). The values of

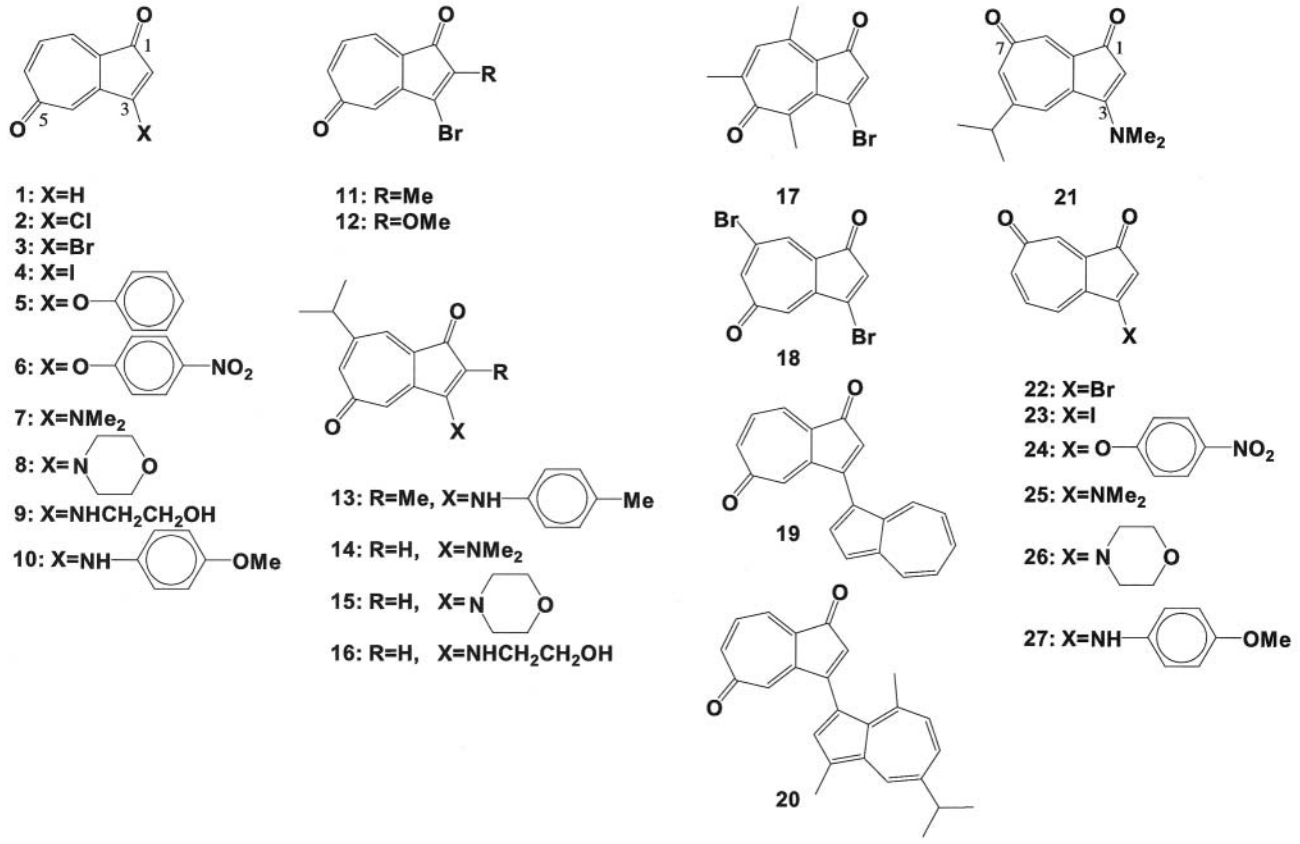

Figure 1. *Structure of 27 azulenequinone derivatives [**1-27**].*

the dipole moment (μ_G and μ_W) in the gas phase and in the aqueous solution of these compounds [**1-40**] were calculated by the ESP/PM3 and COSMO/PM3 methods. For this calculation, a DELL XPS DXG061 personal computer was used.

Results and Discussion

QSAR of azulenequinones. The cytotoxicity of twenty-seven azulenequinones against two human normal oral cells (gingival fibroblast HGF, periodontal ligament fibroblast HPLF) and two human oral squamous cell carcinoma cell lines (HSC-2, HSC-3) was compared with their electronic properties. A partition coefficient log P was used as an index of the QSAR analysis for new drug design. A stereo hydrophobic parameter, dGW, was obtained by the PM3 method. The dGWs were defined as their free-energy changes for the association in the aqueous solution and in the gas phase (19). 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 reported the QSAR between cytotoxic activity and the three QSAR parameters of $\Delta\Delta H_f$, I_{OH} and $\mu_{ESP\text{-}G}$ of 3-benzazepine derivatives (20) and the relationship between the electronic structure and cytotoxic activity of azulenes (21) and tropolones (22). Based on our previous results, the relationship between the cytotoxic activity and the individual QSAR parameters was investigated.

The $\Delta\Delta H_f$, the highest occupied molecular orbital (HOMO), the lowest unoccupied molecular orbital (LUMO) energy and the dipole moment (μ) of azulenequinones [**1-27**] calculated using the PM3 method are provided in Table I.

Four types of dipole moment were calculated using the PM3 method. Among the azulenequinones [**1-27**], the value of ΔΔHf increased in the following order: [**17**] ($\Delta\Delta H_f$=54.11 kJ/mol)<[**2**] ($\Delta\Delta H_f$=64.09 kJ/mol)<[**11**] ($\Delta\Delta H_f$=64.26 kJ/mol)<[**1**] ($\Delta\Delta H_f$=66.06 kJ/mol)<[**22**] ($\Delta\Delta H_f$=66.24 kJ/mol)<[**23**] ($\Delta\Delta H_f$=66.39 kJ/mol). The value of HOMO energy in aqueous-solution increased in the following order: [**20**] (–8.30 eV)<[**19**] (–8.36 eV)<[13] (–8.39 eV)<[**10**] (–8.45 eV)<[**27**] (–8.46 eV)<[**7**] (–8.71 eV).

The value of the dipole moment ($\mu_{ESP\text{-}W}$) in the aqueous solution calculated using the ESP/PM3 method also increased as follows: [**2**] (1.64 D)<[**5**] (1.74 D)<[**1**] (1.90 D)<[**4**] (1.93 D)<[18] (2.06 D)<[**3**] (2.13 D).

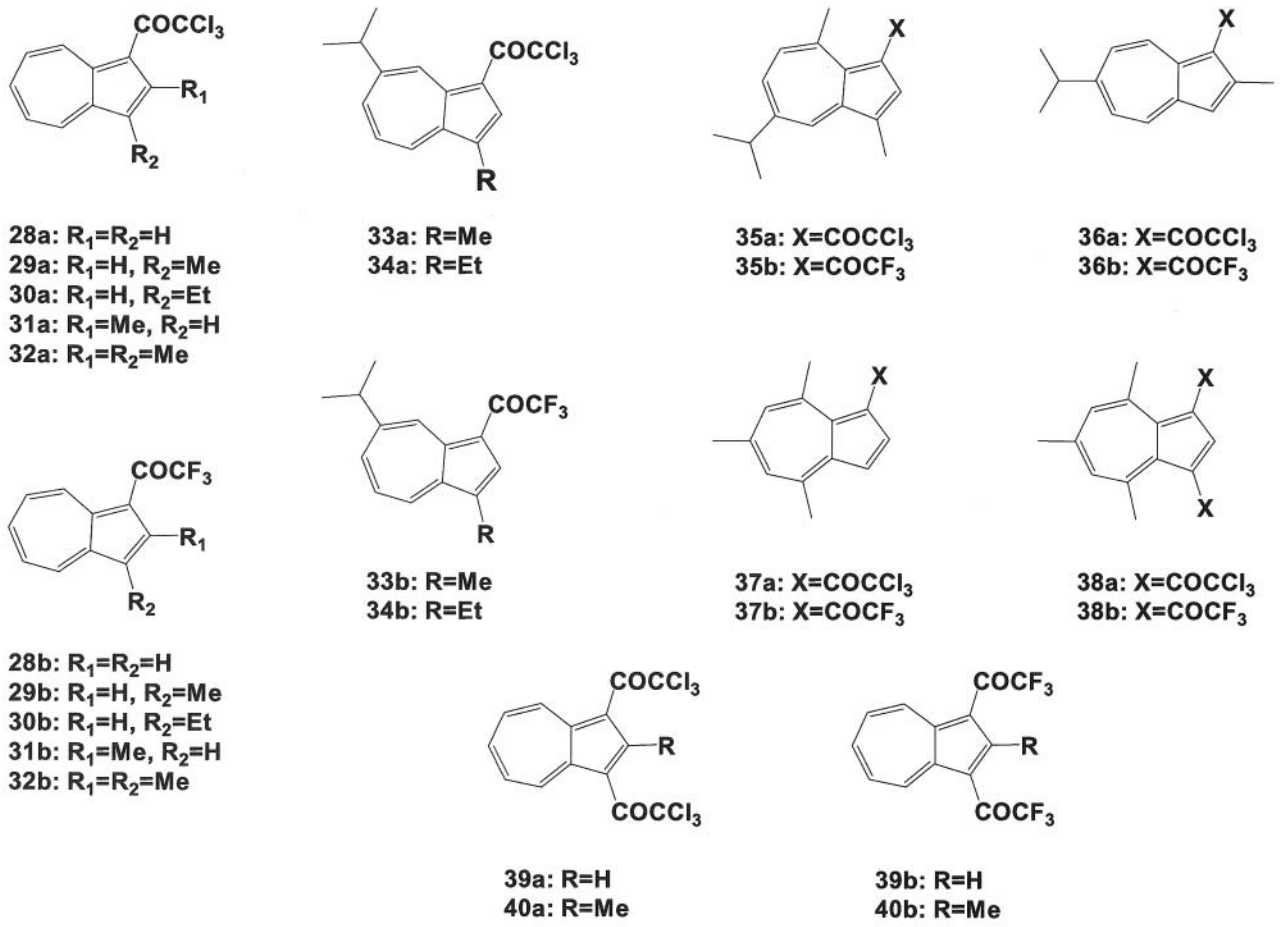

Figure 2. *Structure of 26 trihaloacetylazulene derivatives [28a,b-40a,b].*

The cytotoxic activity of [**5**] against HSC-2 cells was also the highest (CC_{50}=2.2 μM), followed by [**6**] (CC_{50}<3.9 μM), [**2**] (CC_{50}<4.0 μM) and [**1**] (CC_{50}<6.2 μM). Their cytotoxic activity could not be related to the individual QSAR parameters, such as $\Delta\Delta H_f$, HOMO energy or $\mu_{ESP\text{-}W}$. The correlation coefficient (r^2) and the Fisher statistic (F) are important in assessing the "goodness" of a regression fit. In order to obtain more quantitative characteristic of the "goodness" of a model, the well-known Fisher statistic value was used. Of the twenty-seven azulenequinones, 3-phenoxy-1,5-azulenenquinone [**5**] showed the highest cytotoxicity against human tumor cell lines. In order to obtain a quantitative correlation between the cytotoxic activity and electronic properties, the coefficient of the multiple determinations 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 ($E_{H(W)}$) in aqueous solution and the dipole moment ($\mu_{ESP\text{-}W}$) in aqueous solution might significantly contribute to cytotoxic activity. Consequently, the following correlation (Equation **1**) was obtained for the cytotoxicity against HSC-2 cells.

$$CC_{50} = -421.31 + 0.502 \times \Delta\Delta H_f - 36.22 \times E_{H(W)} + 27.97 \times \mu_{ESP\text{-}W} \quad \text{(Equation 1)}$$

n=5 (**4, 5, 8, 19, 25**), r^2=0.999, F=474.9>F (3, 1; 0.05)=215.7

QSAR of trihaloacetylazulenes. The ΔΔHf, HOMO, LUMO energy and μ of trichloroacetylazulenes and trifluoroacetylazulenes are summarized in Table II. The trifluoroacetylazulenes [**28b-40b**] showed lower cytotoxicity compared to the trichloroacetylazulenes [**28a-40a**] (Table III).

Of the thirteen trichloroacetylazulenes, 2,3-dimethyl-1-trichloroacetylazulene [**32a**] and 1,3-ditrichloroacetyl-4,6,8-trimethylazulene [**38a**] were more cytotoxic against the tumor cell lines than against normal cells, yielding the highest tumor-specific cytotoxicity indices (TS) of >35.6 and >44.1, respectively. The correlation coefficient (r^2) and F value against HL-60 cell lines for twenty-six trihalo-

Table I. *QSAR parameters of azulenequinone derivatives.*

Compound No.	$\Delta\Delta H_f$ (kJ/mol)	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	66.06	–9.68	–9.71	–1.38	–1.48	0.88	0.82	1.93	1.90
2	64.09	–9.75	–9.75	–1.49	–1.59	0.79	0.60	1.88	1.64
3	67.22	–9.78	–9.78	–1.52	–1.60	0.99	0.63	2.52	2.13
4	66.98	–9.50	–9.28	–1.45	–1.58	0.81	1.13	1.99	1.93
5	72.40	–9.45	–9.49	–1.35	–1.53	1.49	1.19	2.19	1.74
6	155.55	–9.92	–9.76	–1.68	–1.57	4.33	4.20	5.80	5.76
7	81.50	–8.67	–8.71	–1.18	–1.47	2.53	2.24	5.31	5.02
8	89.89	–8.80	–8.77	–1.26	–1.49	1.02	1.34	2.59	2.81
9	104.88	–8.87	–8.80	–1.24	–1.42	1.85	1.57	4.17	3.97
10	95.49	–8.40	–8.45	–1.28	–1.52	1.68	1.81	3.25	3.38
11	64.26	–9.67	–9.72	–1.47	–1.59	1.47	1.06	3.17	2.66
12	77.96	–9.34	–9.50	–1.53	–1.58	2.97	2.05	5.59	4.73
13	76.39	–8.36	–8.39	–1.15	–1.49	1.42	1.22	3.28	3.07
14	79.48	–8.64	–8.72	–1.10	–1.50	2.06	1.81	5.09	4.92
15	90.07	–8.74	–8.81	–1.17	–1.52	1.28	1.19	2.96	3.20
16	102.19	–8.60	–8.78	–1.05	–1.51	3.53	2.62	7.26	6.71
17	54.11	–9.39	–9.50	–1.10	–1.31	3.08	2.86	4.56	4.52
18	67.55	–9.97	–9.90	–1.72	–1.72	1.21	1.00	2.26	2.06
19	81.88	–8.35	–8.36	–1.55	–1.71	3.78	3.67	7.11	7.06
20	75.25	–8.20	–8.30	–1.24	–1.53	2.75	2.64	3.69	3.67
21	83.57	–8.66	–8.73	–1.25	–1.53	7.91	6.88	15.35	14.63
22	66.24	–9.60	–9.72	–1.63	–1.61	4.48	4.49	7.90	8.17
23	66.39	–9.44	–9.24	–1.55	–1.59	5.12	5.30	9.02	9.50
24	156.15	–9.74	–9.50	–1.79	–1.58	4.47	4.40	7.64	7.68
25	85.44	–8.69	–8.73	–1.32	–1.49	7.66	6.73	15.22	14.60
26	96.85	–8.78	–8.81	–1.37	–1.51	6.80	6.24	13.55	13.23
27	98.55	–8.43	–8.46	–1.44	–1.54	6.13	5.85	12.74	12.68

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

acetylazulenes [**28a,b-40a,b**], using the three electronic parameters of $\Delta\Delta H_f$, LOMO energy ($EL_{(W)}$) and μ_w in aqueous solution, were calculated as 0.688 and 16.138 (>F(3, 26; 0.05)=3.049), respectively. Since the correlation coefficient (r^2) of these compounds for this model was low, this model was not accepted.

The trihaloacetylazulenes may thus be conveniently divided into two groups according to their functional groups: trichloroacetylazulenes [**28a-40a**] and trifluoroacetylazulenes [**28b-40b**].

The r^2 and F-values between the CC_{50} values against the HSC-3 cells for the thirteen trichloroacetylazulenes, using the three QSAR parameters of $\Delta\Delta H_f$, LUMO energy ($E_{L(G)}$) and μ_{ESP-G} in gas phase, were 0.999 and 2565.6, respectively. The r^2 and F-values between the CC_{50} values against the HL-60 cell lines and the same QSAR parameters were 0.958 and 7.676, respectively. Since the F-values of trichloroacetylazulenes were less than the 5% critical value (F=215.71), this model was not accepted. Therefore, the LUMO energy ($E_{L(W)}$) and μ_{ESP-W} in aqueous solution were used, instead of $E_{L(G)}$ and μ_{ESP-G}. In the case of trichloroacetylazulenes, the following correlation equations [**2** and **3**] were obtained for the HSC-3 and HL-60 cell lines, respectively:

$$CC_{50}=-55.15+0.834\times\Delta\Delta H_f+15.25\times E_{L(G)}+53.06\times\mu_{ESP-G} \quad \text{(Equation 2)}$$

n=5 (**28a, 29a, 34a, 39a, 40a**), r^2=0.999, F=2565.6

$$CC_{50}=-55.15+0.834\times\Delta\Delta H_f+53.06\times E_{L(W)}+52.25\times\mu_{ESP-W} \quad \text{(Equation 3)}$$

n=5 (**29a, 30a, 32a, 34a, 39a**), r^2=0.999, F=2108.4

Of the trifluoroazulenes [**28b-40b**], cytotoxic activity of [**38b**] against the HL-60 cell lines was the highest (CC_{50}=20.4 μM), followed by that of [**37b**] (CC_{50}=27.5 μM), [**32b**] (CC_{50}=31.7 μM) and [**33b**] (CC_{50}=31.8 μM). The r^2 and F-values between the CC_{50} values against the HSC-3 cell lines, using the three QSAR parameters of $\Delta\Delta H_f$, LUMO energy ($E_{L(G)}$) and μ_{ESP-G} in the gas phase were 0.999 and 1045.0, respectively. The r^2 and F-values between the CC_{50} values against the HL-60 cell lines and the same QSAR parameters

Table II. *QSAR parameters of trihaloacetylazene derivatives.*

Compound No.	$\Delta\Delta H_f$ (kJ/mol)	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}
28a	42.66	–8.50	–8.68	–1.65	–1.64	4.69	4.24	8.43	8.14
29a	38.44	–8.34	–8.51	–1.59	–1.62	4.44	3.81	7.86	7.51
30a	42.62	–8.26	–8.42	–1.56	–1.60	4.36	4.05	8.67	8.59
31a	41.12	–8.41	–8.59	–1.55	–1.60	3.91	3.74	6.99	7.05
32a	39.29	–8.33	–8.49	–1.39	–1.52	3.44	3.18	6.93	6.85
33a	41.29	–8.24	–8.45	–1.56	–1.64	4.79	4.21	8.03	7.68
34a	40.91	–8.39	–8.55	–1.45	–1.58	4.73	4.17	8.41	8.08
35a	43.69	–8.18	–8.39	–1.49	–1.62	4.70	4.22	7.38	7.23
36a	39.30	–8.34	–8.68	–1.52	–1.59	4.46	4.25	7.82	7.80
37a	39.80	–8.34	–8.63	–1.51	–1.68	4.98	4.48	7.90	7.60
38a	54.43	–8.78	–8.92	–1.86	–1.83	5.13	4.33	7.86	7.26
39a	63.53	–8.95	–8.94	–2.01	–1.75	4.49	3.90	7.50	7.09
40a	60.74	–8.81	–8.84	–1.91	–1.72	4.36	4.32	6.65	6.56
28b	48.59	–8.71	–8.73	–1.83	–1.66	6.50	6.52	10.50	10.77
29b	47.95	–8.55	–8.53	–1.66	–1.63	6.48	6.45	10.61	10.83
30b	47.83	–8.52	–8.54	–1.79	–1.66	6.44	6.38	10.62	10.84
31b	45.40	–8.61	–8.67	–1.74	–1.63	5.44	5.56	8.84	9.18
32b	44.20	–8.42	–8.48	–1.70	–1.63	5.50	5.50	8.91	9.16
33b	44.56	–8.44	–8.53	–1.74	–1.69	6.50	6.52	10.54	10.86
34b	44.07	–8.46	–8.54	–1.74	–1.69	6.48	6.45	10.55	10.83
35b	38.77	–8.31	–8.47	–1.62	–1.67	5.74	5.65	8.65	8.72
36b	44.54	–8.54	–8.70	–1.70	–1.70	5.92	6.05	9.03	9.35
37b	43.12	–8.50	–8.70	–1.66	–1.70	6.62	6.52	10.19	10.32
38b	60.85	–9.01	–8.98	–2.08	–1.86	7.18	6.92	10.83	10.82
39b	73.07	–9.36	–9.09	–2.34	–1.78	7.73	8.15	11.72	12.42
40b	64.76	–9.17	–9.00	–2.20	–1.79	5.68	5.87	9.30	9.71

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

were 0.994 and 55.658, respectively. However, the F-values of trifluoro-acetylazulenes for this model were less than the 5% critical value (F=215.71), this model was not accepted. Therefore, the LUMO energy ($E_{L(W)}$) and μ_{ESP-W} in aqueous solution were used, instead of $E_{L(G)}$ and μ_{ESP-G}. In the case of the trifluoroacetylazulenes, the following correlation equations [**4** and **5**] were obtained for the HSC-3 and HL-60 cell lines, respectively:

$$CC_{50}=192.96+2.29\times\Delta\Delta H_f+319.48\times E_{L(G)}+64.48\times\mu_{ESP-G} \quad \text{(Equation 4)}$$

n=5 (**29b, 31b, 33b, 35b, 39b**), r^2=0.999, F=1045.0

$$CC_{50}=-286.82+5.59\times\Delta\Delta H_f-52.31\times E_{L(W)}-1.33\times\mu_{EPS-W} \quad \text{(Equation 5)}$$

n=5 (**30b, 34b, 37b, 39b, 40b**), r^2=0.999, F=475.9

The CC_{50} values estimated from the corresponding equations are shown in Table III. The expected CC_{50} values of most of the azulenequinones against HSC-2 cells generally matched those for the CC_{50} values for the corresponding compounds except [**6, 12, 20**]. The expected CC_{50} values for most of the trihaloacetylazulenes against HSC-3 cells also matched those for the CC_{50} values for the corresponding compounds very well except for [**31a, 32a, 37a**]. The expected CC_{50} values for most of the trihaloacetylazulenes against HL-60 cells matched those for the CC_{50} values for the corresponding compounds very well except for [**35b, 38b, 40b**]. Depending on the type of cells, different compounds among one series of derivatives strayed from the regression lines. However, the theoretical calculations such as frontier molecular orbital, dipole moments and $\Delta\Delta H_f$ may be applicable in predicting the cytotoxic activity of azulenequinones and trihaloacetylazulenes.

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Table III. *Observed and estimated cytotoxic activity of azulenequinones [1-27] and trihaloacetylazulenes [28a,b-40a,b].*

Cytotoxic activity (CC_{50}: μM)							
	Human tumor cell line						
	HSC-2			HSC-3		HL-60	
Compound	obs.	estim.	Compound	obs.	estim.	obs.	estim.
1	<6.2	16.7	**28a**	40.4	39.9	16.5	17.5
2	<4.0	10.0	**29a**	133.7	134.2	5.4	5.4
3	<17.6	26.1	**30a**	115.5	83.2	26.4	26.5
4	6.7	2.1	**31a**	36.5	150.4	1.6	2.0
5	2.2	7.1	**32a**	7.3	277.1	1.6	1.7
6	<3.9	171.5	**33a**	65.3	50.1	7.6	9.2
7	58.8	75.5	**34a**	65.0	64.8	18.8	18.6
8	22.3	20.0	**35a**	6.5	50.7	9.7	5.8
9	52.3	61.3	**36a**	34.9	44.1	14.8	12.4
10	27.2	27.2	**37a**	9.2	–3.8	4.9	5.1
11	22.1	37.5	**38a**	<6.1	9.2	2.3	4.2
12	10.9	94.3	**39a**	92.1	91.6	13.1	13.1
13	6.5	7.0	**40a**	8.9	9.5	1.9	4.6
14	112.0	71.9	**28b**	106.3	139.5	59.6	57.6
15	21.6	32.5	**29b**	>187.7	187.8	59.5	52.2
16	207.0	135.5	**30b**	>178.0	142.0	62.0	53.1
17	28.4	76.1	**31b**	101.6	100.9	43.3	40.3
18	21.3	28.9	**32b**	153.5	105.0	31.7	33.3
19	117.0	120.0	**33b**	>161.0	160.4	31.8	36.6
20	117.0	19.6	**34b**	142.1	154.8	54.9	33.6
21	289.0	346.2	**35b**	128.0	128.8	69.9	5.6
22	33.2	185.2	**36b**	>174.9	142.9	40.6	38.7
23	36.2	212.4	**37b**	>200.0	183.0	27.5	29.5
24	69.8	215.9	**38b**	140.2	113.6	20.4	136.1
25	347.0	346.0	**39b**	139.6	139.8	198.1	198.5
26	232.0	316.0	**40b**	167.2	15.7	38.6	155.9
27	15.4	289.4					

obs., observed (ref. 14-17); estim., estimated from the corresponding equation.

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