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# Relationship between Electronic Structure and Cytotoxic Activity of Tropolones

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**Abstract.** A structure-activity relationship of the cytotoxic activity of tropolone derivatives was discussed, using theoretical calculations. In order to clearly divide the tropolones into two structurally analogous groups, four different dipole moments ( $\mu_G$ ,  $\mu_{ESP-G}$ ,  $\mu_W$  and  $\mu_{ESP-W}$ ) and heats of formation ( $\Delta H_f$ ) of the tropolones [1-21] were calculated in the gas-phase and in water-solution by the conductor-like screening model/parametric method 3 (COSMO/PM3). The cytotoxic activities of the tropolones and 2-methoxytropolones seem to be related to the three QSAR parameters  $\Delta\Delta H_f$ , HOMO energy ( $E_H$ ) and  $\mu_w$ . The cytotoxic activity of the five tropolone derivatives [17-21] might depend on the QSAR parameters  $\Delta\Delta H_f$ , LUMO energy ( $E_L$ ) and  $\mu_{ESP-G}$ . 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 tropolone derivatives.

Hinokitiol (compound [1] in Figure 1) and its related compounds with a tropolone skeleton (1-3) have shown a broad spectrum of biological activities, including antimicrobial (4), antifungal (5) and phyto-growth-inhibitory activities (6, 7), a cytotoxic effect on mammalian tumor cells (8, 9) and an inhibitory activity on catechol-*O*-methyltransferase (10) and metalloproteases (4). Hinokitiol acetate did not show the cytotoxic activity (9), antimicrobial activity or metalloprotease inhibition (4), suggesting that the biological effects of hinokitiol-related compounds may result from the metal chelation between the carbonyl group at C-1 and the hydroxyl group at C-2 in the tropolone

skeleton. However, no study of cytotoxicity induction by tropolone derivatives has yet been performed. Recently, the cytotoxicity of 27 tropolone derivatives against three human normal cell lines and three human oral tumor cell lines was reported by our group (11). The tropolone derivatives with a phenolic OH group, hinokitiol and its tosylate and methyl ethers, were found to have a relatively higher tumor specificity. 5-Aminotropolone [6] showed the highest tumor specificity, whereas 2-aminotropone [19] and its derivatives showed little or no tumor specificity. 5-Aminotropolone [6] induced apoptotic cell death, as evidenced by internucleosomal DNA fragmentation and caspase-3 activation in human promyelocytic leukemic HL-60 cells (11). Based on a molecular orbital calculation using the physicochemical property and the cytotoxic activity of the tropolone derivatives, the possible relationship between the electronic structure and cytotoxic activity of tropolone derivatives was investigated.

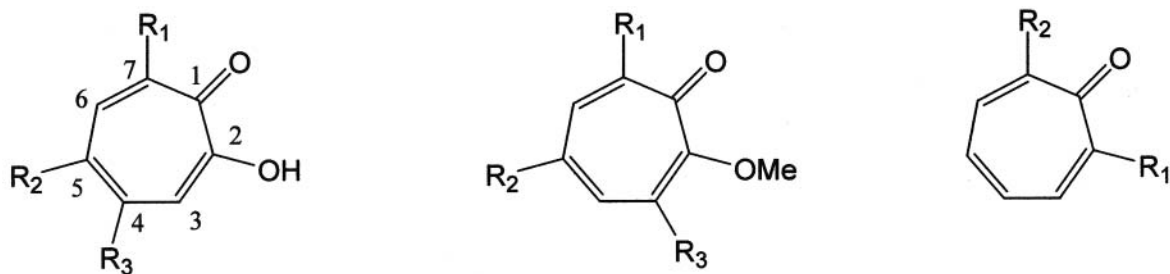
## Materials and Methods

**Chemicals.** All 21 tropolone derivatives [1-21] were synthesized as described previously (11) (Figure 1).

**Theoretical calculations.** The molecular orbital calculation using parametric method 3 (PM3) was performed by applying the winMOPAC program (12). The geometries of the tropolones [1-21] were optimized with respect to all geometrical parameters using the Broyden-Fletcher-Goldfrab-Shanno algorithm incorporated in the program. The geometries of tropolones [1-21] in water-solution were compared with values 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 the geometrical segments per atom (NSPA)=60 and the dielectric constant =78.4 at 25°C (water). The values of the dipole moment ( $\mu_G$  and  $\mu_W$ ) in the gas-phase and in the water-solution of tropolones [1-21] were calculated by the ESP/PM3 and COSMO/PM3 methods. For this calculation, an IMB Intellistation M Pro personal computer was used (12).

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**Key Words:** Tropolones, cytotoxic activity, PM3 calculation method, dipole moment ( $\mu$ ).



**1:**  $R_1=R_2=H$ ,  $R_3=iPr$

**2:**  $R_1=R_3=H$ ,  $R_2=iPr$

**3:**  $R_1=R_2=R_3=H$

**4:**  $R_1=R_3=H$ ,  $R_2=Br$

**5:**  $R_1=R_3=H$ ,  $R_2=NO_2$

**6:**  $R_1=R_3=H$ ,  $R_2=NH_2$

**7:**  $R_1=R_3=H$ ,  $R_2=NO$

**8:**  $R_2=R_3=H$ ,  $R_1=CN$

**9:**  $R_2=R_3=H$ ,  $R_1=Br$

**10:**  $R_1=R_3=H$ ,  $R_2=iPr$

**11:**  $R_1=R_2=R_3=H$

**12:**  $R_2=R_3=H$ ,  $R_1=Br$

**13:**  $R_1=R_2=H$ ,  $R_3=Br$

**14:**  $R_1=R_3=H$ ,  $R_2=Br$

**15:**  $R_1=R_2=Br$ ,  $R_3=H$

**16:**  $R_1=R_2=R_3=Br$

**17:**  $R_1=Cl$ ,  $R_2=H$

**18:**  $R_1=R_2=Br$

**19:**  $R_1=NH_2$ ,  $R_2=H$

**20:**  $R_1=NHAc$ ,  $R_2=H$

**21:**  $R_1=NHMe$ ,  $R_2=H$

Figure 1. Structure of 21 tropolone derivatives.

## Results and Discussion

**Structure and activity relationship.** The relationship between the cytotoxicity of 21 tropolone derivatives [1-21] against two human normal cells (HGF, HPLF) and two human oral tumor cell lines (HSG, HSC-2) and their electronic properties were investigated.

A partition coefficient  $\log P$  is used as an index of the structure- activity relationship analysis in new drug design. A stereohydrophobic parameter  $dGW$  was obtained by the PM3 method. The  $dGW$ s were defined by their free-energy changes for the association in aqueous solution and in the gas-phase (12). The structure-activity relationship analysis revealed that the hydrophobicity of the whole molecule ( $\Delta\Delta H_f$ ) and dipole moment ( $\mu$ ) might affect the cytotoxic activity. Recently, we reported the theoretical quantitative structure-activity relationship (QSAR) analysis of 3-benzazepine derivatives (13) and azulene derivatives (14). The  $\Delta\Delta H_f$ , the highest occupied molecular orbital (HOMO) energy and the lowest unoccupied molecular orbital (LUMO) energy and dipole moment ( $\mu$ ) of tropolones [1-21] calculated by the PM3 method are given in Table I.

Four types of dipole moment were calculated by the PM3 method. Among the 21 tropolone derivatives [1-21], the value of  $\Delta\Delta H_f / M.W.$  increased in the following order: [16] ( $\Delta\Delta H_f/M.W.=0.126$  kJ/mol/g) < [21] ( $\Delta\Delta H_f/M.W.=0.160$

kJ/mol/g) < [13] ( $\Delta\Delta H_f/mol/g=0.190$  kJ/mol/g) < [18] ( $\Delta\Delta H_f/M.W.=0.206$  kJ/mol/g). < [1] ( $\Delta\Delta H_f/M.W.=0.139$  kJ/mol/g).

The value of HOMO energy in the gas-phase increased in the following order: [21] (-8.04 eV) < [19] (-8.12 eV) < [20] (-8.69 eV) < [10] (-8.79 eV) < [2] (-8.92 eV) = [11] (-8.92 eV).

The value of the dipole moment ( $\mu_{ESP-G}$ ) in the gas-phase also increased in the following order: [20] (0.56 D) < [7] (1.06 D) < [16] (1.55 D) < [13] (2.08 D) < [4] (2.10 D).

On the other hand, the cytotoxic activity of [18] against the HGF cells was the highest ( $CC_{50}=0.0076$  mM), followed by that of [16] ( $CC_{50}=0.027$  mM), [13] ( $CC_{50}=0.11$  mM) and [7] ( $CC_{50}=0.13$  mM). However, against the HSC-2 cells the cytotoxic activity of [16] was the highest ( $CC_{50}=0.008$  mM), followed by that of [18] ( $CC_{50}=0.027$  mM), [7] ( $CC_{50}=0.046$  mM) and [6] ( $CC_{50}=0.058$  mM). The cytotoxic activity observed might not be related to the individual QSAR parameters,  $\Delta\Delta H_f / M.W.$ , HOMO energy and  $\mu_{ESP-G}$ .

The multiple correlation coefficient ( $r^2$ ) and the Fisher statistic (F) are important in assessing the "correctness" of a regression fit. In order to obtain a more quantitative characteristic of the "correctness" of a model, QSAR uses the well-known Fisher's statistic value, F. For HGF cells, the correlation coefficient ( $r^2$ ) and F value between  $CC_{50}$  values of the tropolone derivatives [1-21], using the three QSAR parameters  $\Delta\Delta H_f / M.W.$ ,  $\Delta E_{H-L}$  and  $\mu_w$ , were calculated as

Table I. QSAR parameters of tropolone derivatives.

Compound no.	$\Delta\Delta H_f$ (in KJ/mol)	$\Delta\Delta H_f$ (in KJ/mol)/M.W.	HOMO (eV)		LUMO (eV)		Dipole moment (in Debye units)			
			in gas-phase	in water	in gas-phase	in water	$\mu_G$	$\mu_{ESP-G}$	$\mu_W$	$\mu_{ESP-W}$
1	34.330	0.209	-8.96	-9.15	-0.95	-1.06	3.45	3.20	6.18	5.97
2	45.355	0.276	-8.92	-9.11	-0.92	-1.02	3.49	3.19	6.15	5.99
3	46.027	0.377	-9.04	-9.18	-0.98	-1.01	3.12	2.88	5.97	5.90
4	48.445	0.241	-9.22	-9.27	-1.28	-1.20	1.74	2.10	3.70	4.13
5	123.520	0.739	-9.86	-9.50	-2.01	-1.66	2.77	2.75	3.27	3.41
6	71.019	0.519	-9.01	-8.64	-1.04	-1.04	2.84	2.34	6.96	4.91
7	59.402	0.393	-9.43	-9.33	-1.66	-1.58	0.76	1.06	2.22	2.54
8	70.981	0.480	-9.41	-9.27	-1.66	-1.40	0.84	1.36	1.20	1.71
9	51.437	0.256	-9.17	-9.22	-1.20	-1.17	3.22	2.85	6.05	5.85
10	53.647	0.301	-8.79	-9.07	-0.87	-0.98	2.81	2.60	7.67	6.83
11	54.592	0.401	-8.92	-9.13	-0.79	-0.96	3.97	2.93	7.56	6.79
12	58.857	0.274	-9.06	-9.20	-1.04	-1.15	4.73	3.23	8.50	7.33
13	40.858	0.190	-9.07	-9.18	-1.09	-1.10	2.28	2.08	4.29	4.26
14	56.559	0.263	-9.11	-9.23	-1.09	-1.16	3.08	2.36	5.87	5.41
15	60.255	0.205	-9.21	-9.28	-1.31	-1.34	3.95	2.68	7.02	6.09
16	46.842	0.126	-9.31	-9.32	-1.56	-1.47	1.19	1.55	2.57	3.03
17	45.141	0.321	-9.25	-9.39	-1.14	-1.16	4.09	3.36	7.69	7.17
18	54.370	0.206	-9.62	-9.66	-1.40	-1.332	4.67	3.52	8.45	7.56
19	51.573	0.430	-8.12	-7.97	-0.61	-0.88	2.64	2.26	6.41	6.18
20	69.513	0.430	-8.69	-8.87	-0.94	-1.11	0.47	0.56	1.98	2.03
21	21.658	0.160	-8.04	-8.04	-0.61	-0.90	2.80	2.42	4.68	4.28

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

0.220 and 1.602, respectively. For HSC-2 cells, the  $r^2$  and F value between  $CC_{50}$  values of the tropolone derivatives [1-21], using the three QSAR parameters  $\Delta\Delta H_f / M.W.$ ,  $\Delta E_{H-L}$  and  $\mu_w$ , were calculated as 0.192 and 1.351, respectively.  $\Delta E_{H-L}$  represents the differences in energy between the HOMO and LUMO orbitals. Since the F values of these derivatives [1-21] for this model were lower than the 5% critical value, the hypothesis was not acceptable.

Therefore, the tropolone derivatives [1-21] can be conveniently divided into two groups: tropolones [1-9] together with 2-methoxytropolone derivatives [10-16] and tropolone derivatives [17-21].

*Relationship between cytotoxic activity and QSAR parameters for normal (HGF and HPLF) and tumor cells (HSG and HSC-2).* Of the 16 tropolone [1-16] derivatives, 2,4,6-tribromo-7-methoxytropolone [16] was highly cytotoxic against normal human cells (Table I). In order to obtain a quantitative correlation between the cytotoxic activity and electronic properties, the coefficient of the multiple determination and F value were calculated. The structure-activity relationship analysis suggested that the hydrophobicity of the molecule ( $\Delta\Delta H_f$ ),  $\Delta E_{H-L}$  in the water-solution and the dipole moment ( $\mu_w$ ) in the water-solution might greatly contribute to the cytotoxic activity.

The following correlation equations 1 and 2 were obtained for the HGF and HPLF cells, respectively:

$$CC_{50} = -33.989 + 2.037 \times \Delta\Delta H_f / M.W. + 4.238 \times \Delta E_{H-L} + 0.195 \times \mu_w \quad (\text{equation 1})$$

$$n=5 \text{ (1, 6-7, 11, 16)}, r^2=0.999, F=1960.0 > F(3, 1, 0.05)=215.7.$$

$$CC_{50} = -34.089 + 3.595 \times \Delta\Delta H_f / M.W. + 4.257 \times \Delta E_{H-L} + 0.105 \times \mu_w \quad (\text{equation 2})$$

$$n=5 \text{ (1, 5-6, 11, 16)}, r^2=0.999, F=18734.9.$$

Of the 16 tropolone derivatives, 2,4,6-tribromo-7-methoxytropolone [16] showed the highest cytotoxic against the HSG and HSC-2 cell lines. The following correlation equations 3 and 4 were subsequently obtained for the HSG and HSC-2 cells, respectively:

$$CC_{50} = -34.285 + 3.231 \times \Delta\Delta H_f / M.W. + 4.321 \times \Delta E_{H-L} - 0.0118 \times \mu_w \quad (\text{equation 3})$$

$$n=5 \text{ (1, 9, 11, 12, 14)}, r^2=0.999, F=7817.2.$$

$$CC_{50} = -72.166 + 1.203 \times \Delta\Delta H_f / M.W. + 9.224 \times \Delta E_{H-L} - 0.148 \times \mu_w \quad (\text{equation 4})$$

$$n=5 \text{ (5, 11, 14-16)}, r^2=0.999, F=770.6.$$

*Relationship between cytotoxic activity and QSAR parameters of tropolones.* 2,7-Dibromotropolone [18] showed the lowest cytotoxicity against normal cells of the tropolone derivatives (11) (Table II). However, the multiple linear-regression analysis for the tropolone derivatives [17-21] using the above equations 1-4 did not correlate with the QSAR parameters  $\Delta\Delta H_f / M.W.$ ,  $\Delta E_{H-L}$  and  $\mu_w$ . Since the F values of the derivatives [17-21]

Table II. Cytotoxic activity and estimated cytotoxic activity of tropolones [1-24].

Compd.	Cytotoxic activity (CC <sub>50</sub> ; mM)							
	Normal human cells				Human tumor cell lines			
	HGF		HPLF		HSC-2		HSC-3	
	obs.	estim. <sup>a</sup>	obs.	estim. <sup>a</sup>	obs.	estim. <sup>a</sup>	obs.	estim. <sup>a</sup>
1	1.93	1.91	1.73	1.72	1.52	1.25	0.17	1.75
2	1.79	2.02	1.55	1.94	1.22	1.45	0.13	1.79
3	2.10	2.57	1.23	2.67	0.81	2.17	0.14	2.77
4	1.40	1.40	1.49	1.49	0.96	1.30	0.39	1.98
5	2.05	1.37	2.27	2.27	1.93	1.93	0.53	0.53
6	0.63	0.63	0.85	0.85	0.14	0.14	0.058	-2.47
7	0.13	0.12	0.12	0.57	0.02	0.47	0.046	-0.47
8	>2.72	0.59	>2.72	1.27	>2.72	1.27	0.082	0.85
9	1.91	1.82	1.59	1.72	1.30	1.24	0.17	1.48
10	>2.24	2.39	1.68	2.21	1.01	1.53	1.39	1.64
11	>2.94	2.95	>2.94	2.95	2.24	2.25	2.56	2.61
12	>1.86	2.35	>1.86	2.06	1.30	1.29	0.38	1.18
13	0.11	1.47	0.14	1.42	0.074	1.18	0.074	1.93
14	>1.86	1.87	>1.86	1.80	2.20	1.34	1.71	1.67
15	0.22	1.45	0.16	1.17	0.011	0.60	0.27	0.27
16	0.027	0.044	0.048	0.051	0.013	0.014	0.008	0.022
17	0.23	0.30	0.085	0.15	0.05	0.036	0.19	0.25
18	0.0076	-0.037	>0.034	-0.016	0.045	0.056	0.027	-0.017
19	3.12	3.12	2.97	2.99	2.94	2.94	2.42	2.42
20	>2.45	2.45	>2.45	2.44	2.26	2.26	>2.45	2.45
21	0.62	0.60	2.23	2.21	0.34	0.34	1.40	1.39

<sup>a</sup>Estimated from the corresponding equation. obs., observed.

for this model were less than the 5% critical value, the hypothesis was not acceptable. Then, we defined that  $\Delta\Delta H_f$ , HOMO energy ( $E_{HOMO}$ ) and  $\mu_{ESP-G}$  in the gas-phase were used instead of  $\Delta\Delta H_f / M.W.$ ,  $\Delta E_{H-L}$  and  $\mu_W$ .

The following correlation equations **5** and **6** were obtained for the HSG and HSC-2 cell lines, respectively:  
 $CC_{50} = 17.471 + 0.096 \times \Delta\Delta H_f + 2.553 \times E_{HOMO} + 0.550 \times \mu_{ESP-G}$   
 n=5 (**17-21**),  $r^2=0.999$ , F=8143.0.

$CC_{50} = 13.488 + 0.038 \times \Delta\Delta H_f + 1.561 \times E_{HOMO} - 0.150 \times \mu_{ESP-G}$   
 n=5 (**17-21**),  $r^2=0.999$ , F=340.7.

In the case of the HGF and HPLF cells, the QSAR parameters were not consistent. Using the  $\Delta\Delta H_f$ , LUMO energy ( $E_{LUMO}$ ) and  $\mu_G$  in the gas-phase as QSAR parameters, the following correlation equation **7** was obtained for the HGF cells:  
 $CC_{50} = 1.046 + 0.086 \times \Delta\Delta H_f + 4.988 \times E_{LUMO} + 0.260 \times \mu_G$   
 n=5 (**17-21**),  $r^2=0.999$ , F=403.9.

Using the  $\Delta\Delta H_f$ ,  $\Delta E_{HOMO-LUMO}$  and  $\mu_G$  in the gas-phase

as the QSAR parameters for the HPLF cells, the following correlation equation **8** was obtained:

$$CC_{50} = 34.418 + 0.037 \times \Delta\Delta H_f - 4.459 \times \Delta E_{H-L} + 0.053 \times \mu_G$$

(equation **8**)

n=5 (**17-21**),  $r^2=0.999$ , F 362.6.

The  $CC_{50}$  values estimated from the corresponding equation 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  $\Delta\Delta H_f$  for the prediction of the cytotoxic activity of tropolone derivatives.

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