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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 (μ_G, μ_{ESP-G}, μ_W and μ_{ESP-W}) and heats of formation (ΔH_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-methoxytropones seem to be related to the three QSAR parameters $\Delta\Delta H_f$, HOMO energy (E_H) and μ_w. The cytotoxic activity of the five tropone derivatives **[17-21]** might depend on the QSAR parameters $\Delta\Delta H_f$, LUMO energy (E_L) and μ_{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 phytogrowth- inhibitory activities (6, 7), a cytotoxic effect on mammalian tumor cells (8, 9) and an inhibitory activity on catechol-*O*-methyl-transferase (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.

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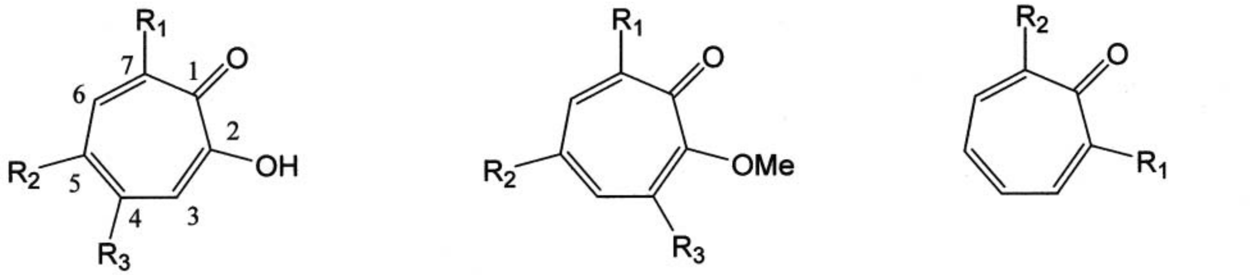
Materials and Methods

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 (μ_G and μ_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).



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_2=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 logP 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 dGWs 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 (μ) 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) and the lowest unoccupied molecular orbital (LUMO) energy and dipole moment (μ) 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]** (–8.12eV)<**[20]** (–8.69 eV)<**[10]**(–8.79 eV)<**[2]**(–8.92 eV)=**[11]** (–8.92 eV).

The value of the dipole moment ($\mu_{ESP\text{-}G}$) 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 $\mu_{ESP\text{-}G}$.

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., $\Delta E_{H\text{-}L}$ and μ_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	μ_G	$\mu_{ESP\text{-}G}$	μ_W	$\mu_{ESP\text{-}W}$
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., $\Delta E_{H\text{-}L}$ and μ_w, were calculated as 0.192 and 1.351, respectively. $\Delta E_{H\text{-}L}$ 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-methoxytropone derivatives **[10-16]** and tropone 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-methoxytropone **[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 ($\Delta\Delta H_f$), $\Delta E_{H\text{-}L}$ in the water-solution and the dipole moment (μ_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 x $\Delta\Delta H_f$ / M.W. + 4.238 x $\Delta E_{H\text{-}L}$ + 0.195 x μ_w (equation **1**)
n=5 (**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 x $\Delta\Delta H_f$ / M.W. + 4.257 x $\Delta E_{H\text{-}L}$L + 0.105 x μ_w (equation **2**)
n=5 (**1, 5-6, 11, 16**), r^2=0.999, F=18734.9.

Of the 16 tropolone derivatives, 2,4,6-tribromo-7-methoxytropone **[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 x $\Delta\Delta H_f$ / M.W. + 4.321 x $\Delta E_{H\text{-}L}$ –0.0118 x μ_w (equation **3**)
n=5 (**1, 9, 11, 12, 14**), r^2=0.999, F=7817.2.

CC_{50}=–72.166 + 1.203 x $\Delta\Delta H_f$ / M.W. + 9.224 x $\Delta E_{H\text{-}L}$ –0.148 x μ_w (equation **4**)
n=5 (**5, 11, 14-16**), r^2=0.999, F=770.6.

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

[a]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 μ_{ESP-G} in the gas-phase were used instead of $\Delta\Delta H_f$ / M.W., ΔE_{H-L} and μ_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 x $\Delta\Delta H_f$ +2.553 x E_{HOMO} + 0.550 x μ_{ESP-G} (equation **5**)

n=5 (**17-21**), r^2=0.999, F=8143.0.

CC_{50}=13.488 + 0.038 x $\Delta\Delta H_f$ + 1.561 x E_{HOMO} –0.150 x μ_{ESP-G} (equation **6**)

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 μ_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 x $\Delta\Delta H_f$ + 4.988 x E_{LUMO} + 0.260 x μ_G (equation **7**)

n=5 (**17-21**), r^2=0.999, F=403.9.

Using the $\Delta\Delta H_f$, $\Delta E_{HOMO-LUMO}$ and μ_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 x $\Delta\Delta H_f$ – 4.459 x ΔE_{H-L} + 0.053 x μ_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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