A Quantitative Structure–Activity Relationship for Antitumor Activity of Long-Chain Phenols from *Ginkgo biloba* L.

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With the aim of obtaining compounds with strong antitumor activity, a quantitative structure–activity relationship (QSAR) of antitumor phenolic compounds (long-chain phenols) was derived using the Hansch–Fujita equation. The ED_{50} values against Chinese hamster V-79 cells were analyzed in terms of log *P* as the hydrophobic parameter and the energy of the lowest unoccupied molecular orbital (E_{LUMO}) calculated by using the modified neglect of differential overlap (MNDO) method as the electronic parameter, by means of multiple regression analysis. It was found that the activities mainly depended on log *P* (an optimum log *P* of 8.3) and a low-lying E_{LUMO} value. 4-Undecylcatechol, selected on the basis of the above results, exhibited strong antitumor activity against Sarcoma 180 ascites and P-388 lymphocytic leukemia.

Keywords structure–activity relationship; quantitative structure–activity relationship; long-chain phenol; *Ginkgo biloba*; antitumor activity; Sarcoma 180A; P-388; V-79; log *P*; lowest unoccuplied molecular orbital energy; highest occupied molecular orbital energy; modified neglect of differential overlap method

We have previously reported on the isolation, chemical structures, and physiological activities of antitumor long-chain phenols, which were isolated during a search for antitumor substances of *Ginkgo biloba* L. (Ginkgoaceae) with the guidance of bio-assay for activity against Sarcoma 180 ascites in mice.[1] These compounds also showed antimicrobial activities[1,2] and toxicity.[3] We report here a cytotoxic activity against Chinese hamster V-79 cells. Further, a bio-assay based on this activity instead of the activity against Sarcoma 180 ascites was employed in a search for antitumor principles by means of the quantitative structure–activity relationship (QSAR) analysis, because there was a good correlation between the results of the biological tests using V-79 cells and Sarcoma 180 ascites in mice.

We considered that the activities of antitumor long-chain phenols were controlled by both hydrophobic and electronic parameters based on the alkyl side chain moiety and the aromatic ring contribution of hydroxyl functions, respectively, because acetates and methyl esters of the long-chain phenols did not show antitumor activity against Sarcoma 180 ascites in mice.[1]

In the present investigation, thirty long-chain phenol derivatives, which were divided into six groups consisting of five compounds having the same aromatic ring contribution and a different alkyl side chain moiety, were synthesized by Grignard reaction of alkyl bromide and hydroxybenzaldehyde in the usual way. Each compound was tested for cytotoxic activity against V-79 cells. For all synthesized compounds (**31**—**60**), the log *P* values (*P* stands for the *n*-octanol–water partition coefficient) were measured by the high performance liquid chromatography (HPLC) method[4] as the hydrophobic parameter. As the electronic parameter, the energy of the lowest unoccupied molecular orbital (E_{LUMO}) was calculated by using the modified neglect of diatomic differential overlap (MNDO)[5] method, because Hammett's substituent constants[6] were not suitable for both *ortho*- and di-substituted aromatic rings.

In this paper, the structural requirements for both the alkyl side chain moiety and the aromatic ring contribution, and the mechanism of antitumor activity of the long-chain phenols are discussed, and a drug–receptor interaction model of the charge transfer type is proposed.

Materials and Methods

Synthesis of Long-Chain Phenols Grignard reagent was prepared from 245 mg of Mg and 0.84 ml of 1-bromohexane in dry tetrahydrofuran (THF) under N_2, and to this was slowly added 1.06 g of 2-benzyloxybenzaldehyde in 20 ml of dry THF with stirring at room temperature. After 3 h, the reaction mixture was worked up in the usual way and 2-(1-hydroxyheptyl)phenyl benzyl ether (**1**) was obtained. Compound **1** (100 mg) was hydrogenated by catalytic reduction over 5% Pd–C (50 mg) in 20 ml of ethyl acetate containing 5 drops of concentrated sulfuric acid to give almost pure 2-heptylphenol (**31**) after work-up in the usual way. The other compounds (**32**—**60**) were prepared by the same procedures.

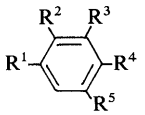
Assay of Activity Against V-79 Cells Cloned Chinese hamster V-79 cells, supplied by Dr. S. Tsukagoshi of the Japan Foundation for Cancer Research, were maintained in RPMI-1640 medium (Nissui Pharm. Co., Ltd.) supplemented with 10% fetal calf serum (Mitsubishi Chemical Industry Co., Ltd.) and kanamycin (100 μg/ml). The cells (3×10^5 cells/well) were cultured in Corning disposable 6-well plates containing 2 ml of growth medium per well and were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Various drug concentrations (10 μl) were added to the cultures at day 1 after the transplantation. The colonies were fixed with a 10% formaldehyde solution (2 ml) for 20 min and stained with 0.05% Crystal Violet (0.75 ml) at day 5. The cytotoxic activity of the drugs was assessed by determining the T (number of stained colonies of test groups)/C (those of control groups) $\times$ 100 values or ED_{50} (drug concentration that inhibits colony growth by 50%) in drug-containing medium relative to colony growth in 0.5% EtOH medium at day 5 after drug treatment.

Assay of Activity Against Sarcoma 180 Ascites ICR male mice, 5 weeks old, supplied by Clea Japan Co., Ltd., were used in groups of 6 animals. Sarcoma 180 ascites, provided by the National Cancer Center Research Institute and maintained in successive generations by us, was implanted i.p. at 1×10^6 cells/mouse. Administration of a test drug was started at 1 d after the implantation and continued for 5 d by the i.p. route. The effectiveness was evaluated by means of the total packed cell volume method; growth ratio (GR%)=(packed cell volume (PCV) of test groups /PCV of control groups) $\times$ 100; GR=0—10% (+++), 11—40% (++), 41—65% (+) and over 66% (−).

Assay of Activity Against P-388 Lymphocytic Leukemia CDF_1 male mice, 5 weeks old, supplied by Japan Charles River Co., Ltd., were used in groups of 6 animals. P-388 lymphocytic leukemia was implanted i.p. at 1×10^6 cell/mouse. A test drug was given i.p. at 1 d after the implantation and continued for 9 d. The effectiveness was evaluated in terms of the increase of life span (ILS, T/C%).

Determination of log *P* with *n*-Octanol–H_2O The hydrophobic coef-

TABLE I. Structures and Parameters for Multiple Regression Analysis



Compound	R^1	R^2	R^3	R^4	R^5	Yield	mp (°C)	MS (M^+)	$-\log ED_{50}$	log P	E_{LUMO}	E_{HOMO}
A-7 (31)	C_7H_{15}	OH	H	H	H	63.5	—	192	1.16	4.45	0.142	−8.86
A-9 (32)	C_9H_{19}	OH	H	H	H	59.0	—	220	1.38	5.76	0.142	−8.86
A-11 (33)	$C_{11}H_{23}$	OH	H	H	H	75.4	32.0—33.0	248	1.39	7.17	0.142	−8.86
A-13 (34)	$C_{13}H_{27}$	OH	H	H	H	70.4	42.5—43.5	276	1.42	8.61	0.142	−8.86
A-15 (35)	$C_{15}H_{31}$	OH	H	H	H	29.3	53.0—54.0	304	1.43	10.07	0.142	−8.86
B-5 (36)	C_5H_{11}	H	OH	H	H	50.0	—	164	—	3.13	0.135	−8.88
B-9 (37)	C_9H_{19}	H	OH	H	H	56.4	—	220	1.16	5.61	0.135	−8.88
B-11 (38)	$C_{11}H_{23}$	H	OH	H	H	57.6	—	248	1.14	6.98	0.135	−8.88
B-13 (39)	$C_{13}H_{27}$	H	OH	H	H	31.7	41.0—42.0	276	1.34	8.84	0.135	−8.88
B-15 (40)	$C_{15}H_{31}$	H	OH	H	H	11.2	50.0—51.0	304	1.33	10.11	0.135	−8.88
C-7 (41)	C_7H_{15}	H	H	OH	H	82.4	—	192	1.43	4.15	0.179	−8.82
C-9 (42)	C_9H_{19}	H	H	OH	H	35.7	41.0—42.5	220	1.29	5.76	0.179	−8.82
C-11 (43)	$C_{11}H_{23}$	H	H	OH	H	86.6	56.5—57.0	248	1.14	7.18	0.179	−8.82
C-12 (44)	$C_{12}H_{25}$	H	H	OH	H	79.8	67.5—68.0	262	1.14	7.91	0.179	−8.82
C-13 (45)	$C_{13}H_{27}$	H	H	OH	H	84.5	68.0—69.0	276	1.39	8.20	0.179	−8.82
D-7 (46)	C_7H_{15}	OH	OH	H	H	48.8	—	208	1.76	3.59	0.020	−8.60
D-9 (47)	C_9H_{19}	OH	OH	H	H	46.6	—	236	2.04	4.86	0.020	−8.60
D-11 (48)	$C_{11}H_{23}$	OH	OH	H	H	63.5	51.8—52.5	264	2.34	6.30	0.020	−8.60
D-13 (49)	$C_{13}H_{27}$	OH	OH	H	H	43.0	56.0—56.5	292	2.08	7.75	0.020	−8.60
D-15 (50)	$C_{15}H_{31}$	OH	OH	H	H	41.0	60.5—61.0	320	2.01	9.27	0.020	−8.60
E-7 (51)	C_7H_{15}	OH	H	OH	H	3.9	70.8—71.3	208	1.10	2.90	0.098	−8.75
E-9 (52)	C_9H_{19}	OH	H	OH	H	7.6	72.0—72.7	236	1.11	4.10	0.098	−8.75
E-11 (53)	$C_{11}H_{23}$	OH	H	OH	H	2.9	72.5—73.0	264	1.43	5.43	0.098	−8.75
E-13 (54)	$C_{13}H_{27}$	OH	H	OH	H	6.2	72.3—73.0	292	1.36	6.84	0.098	−8.75
E-15 (55)	$C_{15}H_{31}$	OH	H	OH	H	2.2	84.5—85.1	320	1.68	8.29	0.098	−8.75
F-5 (56)	C_5H_{11}	H	OH	OH	H	37.5	—	180	1.57	3.13	−0.098	−9.06
F-9 (57)	C_9H_{19}	H	OH	OH	H	35.6	75.5—77.0	236	1.82	5.54	−0.098	−9.06
F-11 (58)	$C_{11}H_{23}$	H	OH	OH	H	73.6	84.0—85.0	264	2.28	6.94	−0.098	−9.06
F-13 (59)	$C_{13}H_{27}$	H	OH	OH	H	80.0	90.0—91.5	292	2.33	8.42	−0.098	−9.06
F-15 (60)	$C_{15}H_{31}$	H	OH	OH	H	40.6	88.5—91.0	320	2.11	9.90	−0.098	−9.06
61	$C_{15}H_{29}$	COOH	OH	H	H		40.0—41.0	346				
62	$C_{15}H_{29}$	H	OH	H	OH		30.0—31.0	318				
63	$C_{15}H_{29}$	H	OH	H	H		—	302				

ED_{50} in mM, E_{LUMO} and E_{HOMO} in eV.

ficients, log P values, of the long-chain phenols were determined by HPLC method.[4] Equation 1 was derived from other compounds whose log P values were taken from the literature.[7] The HPLC conditions were as follows: column, Toyo Soda TSK-gel 80T_M; detection, UV 254 nm; column temperature, 40 °C; mobile phase, acetonitrile–water (8 : 2); elution speed, 1 ml/min.

$$\log P = 5.34(\pm 0.82)\log k' + 1.96(\pm 0.32) \quad (1)$$

$$n = 15,\ r = 0.97,\ s = 0.47,\ F = 202.29$$

In Eq. 1, n represents the number of data points used to derive Eq. 1, r is the correlation coefficient, s is the standard deviation from the regression, and F is the F statistics value. The figures in parentheses are 95% confidence limits.

MNDO Calculation The E_{LUMO} and E_{HOMO} (energy of highest occupied molecular orbital) values were calculated by the MNDO program in MOPAC, distributed by the Quantum Chemical Program Exchange (QCPE), using a HITAC M-280H computer at the Computer Center, the University of Tokyo. These values were represented by the compounds having 13 carbons in the alkyl side chain moiety in each group.

Correlation Analysis The ED_{50} values were analyzed in terms of hydrophobic (log P) and electronic (E_{LUMO} and E_{HOMO}) parameters by the least-squares method (Eq. 2).

$$-\log ED_{50} = a(\log P)^2 + b \log P + c\, E_{LUMO} + d\, E_{HOMO} + e \quad (2)$$

Results and Discussion

The compounds and parameters used in this work are listed in Table I. In the comparison of the cytotoxic activity in each group, the compounds having 11, 13 or 15 carbons in the alkyl side chain moiety usually showed strong activity compared with others in each group, and in a further comparison among groups, groups D (**46**—**50**) and F (**56**—**60**) exhibited ten-fold stronger activity than the other groups. The optimum log P existed in groups A, D and F from the multiple regression analysis of each group, but the activity was modified by electronic effects based on the aromatic ring contributions.

Then we used the E_{LUMO} and E_{HOMO} values as electronic parameters. They are related to the drug–receptor interaction processes, and the E_{LUMO} value is noted as a measure of the relative electron-acceptor property of a molecule.[8] Many workers have discussed the charge-transfer interaction between a drug and its receptor using the E_{LUMO} value.[8] When the correlation between the activity ($-\log ED_{50}$) and the E_{LUMO} value was examined by single regression analysis, a good correlative equation (Eq. 3) was derived from compounds having 13 carbons at the alkyl side chain moiety, whose activity was usually stronger than others in each group.

$$-\log ED_{50} = -4.47\, E_{LUMO} + 1.97 \quad (3)$$

$$n = 6,\ r = 0.96,\ s = 0.14,\ F = 50.03$$

TABLE II. Multiple Regression Generated from Table I

$-\log ED_{50} = a(\log P)^2 + b \log P + cE_{LUMO} + dE_{HOMO} + e$

Eq.	a	b	c	d	e	$n^{a)}$	$r^{b)}$	$s^{c)}$	$F^{d)}$
4	−0.016	0.282	−4.108	1.107	10.582	29	0.905	0.184	27.10
	(0.001)	(0.107)	(0.407)	(0.296)	(0.058)				
5	−0.017	0.278	−3.485		0.818	29	0.845	0.227	20.74
	(0.001)	(0.132)	(0.458)		(0.062)				
6	0.003		−3.332		1.657	29	0.814	0.241	25.56
	(0.001)		(0.482)		(0.060)				
7			−3.311		1.809	29	0.784	0.253	43.13
			(0.504)		(0.055)				
8		0.048	−3.361		1.493	29	0.823	0.236	27.36
		(0.021)	(0.470)		(0.057)				
9		0.062	−3.997	1.121	11.353	29	0.887	0.196	30.61
		(0.018)	(0.430)	(0.315)	(0.057)				
10			−3.805	0.891	9.714	29	0.829	0.232	28.47
			(0.506)	(0.366)	(0.060)				
11	0.004		−3.947	1.098	11.356	29	0.876	0.204	27.37
	(0.001)		(0.448)	(0.330)	(0.061)				
12	−0.006	0.120		−0.116	0.032	29	0.226	0.412	0.45
	(0.018)	(0.238)		(0.606)	(0.113)				

a) Number of compounds used for correlations. *b*) Correlation coefficient. *c*) Standard deviation. *d*) *F* values. The figures in parentheses are 95% confidence limits.

TABLE III. Antitumor Activity against Sarcoma 180 Ascites in Mice

Compound	Dose (mg/kg)	BWC	PCV/TV	GR (%)	Assessment
48	10.0	6.05	0.43	65.48	−
59	10.0	5.57	0.42	38.32	++
63	10.0	4.83	0.37	101.09	−

The effectiveness was evaluated by means of the total packed cell volume method. BWC, body weight change = (day 7 weight − TV)/day 0 weight. PCV, packed cell volume; TV, total volume. GR, growth ratio = PCV (test groups)/PCV (control groups) × 100.

TABLE IV. Antitumor Activity against P-388 Lymphocytic Leukemia

Compound	Dose (mg/kg)	T/C (%)
59	100.0	141
61	100.0	93
62	100.0	96
63	100.0	89

The effectiveness was evaluated in terms of the ILS (T/C%).

Since the sign of the E_{LUMO} coefficient in Eq. 3 was negative, we found that a drug with lower-lying E_{LUMO} value interacted strongly with the receptor.

The results of the multiple regression analysis based on Eq. 2 are summarized in Table II. In Eq. 4, the optimum log *P* existed at 8.29, and lower-lying E_{LUMO} and higher lying E_{HOMO} values were necessary for the appearance of significant activity. However, the E_{HOMO} value in Eq. 12 could not explain the activity without the E_{LUMO} value, whereas the E_{LUMO} value in Eq. 7 could independently explain the activity and was a significant parameter. In conclusion, the cytotoxic activity mainly depended on the log *P* and a low-lying E_{LUMO}. It has been suggested that receptor protein tryptophan residues containing an aromatic ring moiety should be the best electron donor for the charge transfer interactions with phenols because of the high E_{HOMO} value.[9]

Among the synthesized long-chain phenols (**31**—**60**), the activity of **59** was stronger by about 10 times than others against V-79 cells, and **59** also showed antitumor activity against Sarcoma 180 ascites in mice at a low dose, 10 mg/kg/d. Natural compounds (**61**—**63**) from *G. biloba* did not show activity at the same dose, as shown in Table III. Furthermore, **59** exhibited significant activity against P-388 lymphocytic leukemia in mice at 100 mg/kg, as shown in Table IV.

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