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Quantitative Structure–Cytotoxicity Relationship of Chalcones

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Abstract. *Background: Fifteen chalcones were subjected to quantitative structure–activity relationship (QSAR) analysis based on their cytotoxicity and tumor specificity, in order to find their new biological activities. Materials and Methods: Cytotoxicity against four human oral squamous cell carcinoma cell lines and three oral mesenchymal cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Tumor specificity (TS) was evaluated by the ratio of the mean 50% cytotoxic concentration (CC_{50}) against normal cells to that against tumor cell lines. Potency-selectivity expression (PSE) value was calculated by dividing TS by CC_{50} against tumor cells. Apoptosis markers were detected by western blot analysis. Physicochemical, structural and quantum-chemical parameters were calculated based on the conformations optimized by force-field minimization. Results: Among 15 chalcone derivatives, (2E)-1-(2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one had the highest TS and PSE values, comparable with those of doxorubicin and methotrexate, respectively. This compound also stimulated the cleavage of poly(ADP-ribose) polymerase and caspase-3. Chalone TS values were correlated with molecular shape and polarization rather than the types of substituted groups. None of the compounds had any anti-HIV activity. Conclusion: Chemical modification of the lead compound may be a potential choice for designing new types of anticancer drugs.*

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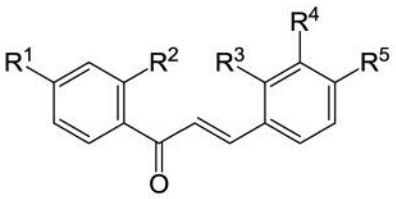
Key Words: Chalcones, QSAR analysis, cytotoxicity, tumor selectivity, apoptosis induction, anti-HIV activity.

Chalcone has a structure of 1,3-diaryl-2-propen-1-one in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system, representing a class of flavonoids that occur naturally in fruits and vegetables. Chalcones are also metabolic precursors of some flavonoids and isoflavonoids (1). Chalcones are promising lead antitumor/chemopreventive drugs due to three different activities: antioxidant, cytotoxic, and apoptosis induction (2). Several studies with murine xenograft models have shown that administration of chalcones significantly reduced the tumor volume by inducing apoptosis (3-10). The tumor specificity of chalcones has been reported in comparing sensitivity of hepatocarcinoma HepG2 cells to normal liver AML12 cells (11); osterosarcoma to bone marrow and small-intestinal epithelial cells (12); murine acute lymphoblastic leukemia cells L-1210 to normal human lymphocytes (13); and human prostate cancer cells PC3 and DU145 to normal human prostate epithelial cells (14). Although chalcones have been reported to induce apoptosis of human oral carcinoma cell line (HSC-3) (15) and cultured primary and metastatic oral cancer cell lines (16), the tumor specificity against these has not been investigated as far as we are aware of.

In order to find new types of anticancer drugs active against human oral cancer, we first investigated the tumor specificity of 15 chalcone derivatives (Figure 1), using four human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) and three human normal oral cells (gingival fibroblast, HGF; periodontal ligament fibroblast, HPLF; pulp cells, HPC) as target cells, and then the apoptosis-inducing activity of the most active compound. The cytotoxicity data were used to perform the quantitative structure–activity relationship (QSAR) analysis. Since very few articles have been published on the antiviral activity of chalcones, we also investigated whether any of these compounds has any anti-human immunodeficiency virus (HIV) activity.



Compound	R^1	R^2	R^3	R^4	R^5
1	H	OH	H	H	H
2	H	OH	H	H	OH
3	H	OH	H	H	OMe
4	H	OH	H	OMe	OMe
5	H	OH	H	H	F
6	H	OH	H	H	Cl
7	H	OH	H	H	Br
8	OMe	OH	H	H	OH
9	OMe	OH	H	H	H
10	OMe	OH	H	H	OMe
11	OMe	OH	H	OMe	OMe
12	OMe	OH	H	H	Br
13	OMe	H	H	H	OMe
14	OMe	H	OMe	H	OMe
15	OMe	OMe	H	H	OMe

Figure 1. *Structure of fifteen chalcones.*

Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco's modified Eagle's medium (DMEM), from GIBCO BRL, Grand Island, NY, USA; fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), doxorubicin, azidothymidine, 2',3'-dideoxycytidine from Sigma-Aldrich Inc., St. Louis, MO, USA; dimethyl sulfoxide (DMSO), dextran sulfate (molecular mass, 5 kDa) from Wako Pure Chem. Ind., Osaka, Japan; methotrexate from Nacalai Tesque, Inc., Kyoto, Japan; curdlan sulfate (molecular mass: 79 kDa) from Ajinomoto Co. Ltd., Tokyo, Japan. Culture plastic dishes and plates (96-well) were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

Synthesis of test compounds. (*2E*)-1-(2-Hydroxyphenyl)-3-phenyl-2-propen-1-one (**1**), (*2E*)-1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one (**2**), (*2E*)-1-(2-hydroxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**3**), (*2E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**4**), (*2E*)-3-(4-fluorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**5**), (*2E*)-3-(4-chlorophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**6**), (*2E*)-3-(4-bromophenyl)-1-(2-hydroxyphenyl)-2-propen-1-one (**7**), (*2E*)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-hydroxyphenyl)-2-propen-1-one (**8**), (*2E*)-1-(2-hydroxy-4-methoxyphenyl)-3-phenyl-2-propen-1-one (**9**), (*2E*)-1-(2-hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**10**), (*2E*)-3-(3,4-dimethoxyphenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-propen-1-one (**11**), (*2E*)-3-(4-bromophenyl)-1-(2-hydroxy-4-methoxyphenyl)-2-propen-1-one (**12**), (*2E*)-1-(4-methoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**13**), (*2E*)-3-(2,4-dimethoxyphenyl)-1-(4-methoxyphenyl)-2-propen-1-one (**14**), (*2E*)-1-(2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)-2-propen-1-one (**15**) (structures shown in Figure 1) were synthesized by base-catalyzed condensation of the appropriate acetophenone with selected benzaldehyde derivatives according to previous methods (17). All compounds were dissolved in DMSO at 40 mM and stored at –20˚C before use.

Cell culture. Human normal oral mesenchymal cells (HGF, HPLF, HPC), established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl (18), and human OSCC cell lines [Ca9-22 (derived from gingival tissue); HSC-2, HSC-3, HSC-4 (derived from tongue)], purchased from Riken Cell Bank (Tsukuba, Japan), were cultured at 37˚C in DMEM supplemented with 10% heat-inactivated FBS, 100 units/ml, penicillin G and 100 μg/ml streptomycin sulfate under a humidified 5% CO_2 atmosphere. HGF, HPLF and HPC cells at 10-18 population doubling levels were used in the present study.

Assay for cytotoxic activity. Cells were inoculated at 2.5×10^3 cells/0.1 ml in a 96-microwell plate (Becton Dickinson Labware, Franklin Lakes, NJ, USA).After 48 h, the medium was replaced with 0.1 ml of fresh medium containing different concentrations of single test compounds. Cells were incubated further for 48 h and the relative viable cell number was then determined by the MTT method (19). The relative viable cell number was determined by the absorbance of the cell lysate at 562 nm, using a microplate reader (Sunrise Rainbow RC-R; TECAN, Männedorf, Switzerland). Control cells were treated with the same amounts of DMSO and the cell damage induced by DMSO was subtracted from that induced by test agents. The concentration of compound that reduced the viable cell number by 50% (CC_{50}) was determined from the dose–response curve and the mean value of CC_{50} for each cell type was calculated from duplicate assays.

Calculation of tumor-selectivity index (TS). TS was calculated using the following equation: TS=mean CC_{50} against normal cells/mean CC_{50} against tumor cells [(D/B) in Table I]. Since both Ca9-22 and HGF cells were derived from the gingival tissue (20), the relative sensitivity of these cells was also compared [(C/A) in Table I]. We have confirmed that the TS value thus determined reflects the antitumor potential of test samples, although normal and tumor cells are derived from different tissues (mesenchymal or epithelial tissues, respectively) (21). We did not use human normal oral keratinocytes as controls, since doxorubin and 5-fluorouracil showed potent cytotoxicity against these epithelial cells by an as yet unidentified mechanism (19, 22, 23).

Calculation of potency-selectivity expression (PSE). PSE was calculated using the following equation: PSE=TS/CC_{50} against tumor cells ×100 (24) [that is, (D/B^2) ×100 (HGF, HPLF, HPC *vs.*

Table I. *Cytotoxic activity of 15 chalcones against human oral malignant and non-malignant cells. Each value represents the mean of duplicate determinations.*

	CC_{50} (μM)														
	Human oral squamous cell carcinoma cell lines						Human normal oral cells					TS		PSE	
Compound	(A) Ca9-22	HSC-2	HSC-3	HSC-4	(B) Mean	SD	(C) HGF	HPLF	HPC	(D) Mean	SD	(D/B)	(C/A)	(D/B^2) ×100	(C/A^2) ×100
1	18.2	28.6	19.2	33.2	24.8	7.3	41.5	72.0	71.8	61.8	17.5	2.5	2.3	10	13
2	31.2	53.0	29.4	51.0	41.1	12.6	115.5	281.5	167.0	188.0	85.0	4.6	3.7	11	12
3	21.7	36.2	21.0	42.0	30.2	10.5	56.5	145.0	69.5	90.3	47.8	3.0	2.6	10	12
4	24.2	45.7	26.0	41.1	34.2	10.7	83.0	153.5	112.1	116.2	35.4	3.4	3.4	10	14
5	15.1	22.2	13.4	26.6	19.3	6.2	34.7	69.3	46.2	50.1	17.6	2.6	2.3	13	15
6	8.7	13.2	10.1	16.4	12.1	3.4	21.1	35.0	30.2	28.8	7.1	2.4	2.4	20	28
7	9.5	10.0	9.9	14.3	10.9	2.2	22.8	47.0	36.6	35.5	12.1	3.3	2.4	30	26
8	29.6	32.7	27.8	44.6	33.6	7.6	73.7	151.0	153.0	125.9	45.2	3.7	2.5	11	8
9	9.8	11.2	10.7	20.5	13.0	5.0	31.6	51.8	55.3	46.2	12.8	3.5	3.2	27	33
10	21.5	23.1	21.6	40.2	26.6	9.1	47.4	234.0	197.5	159.6	98.9	6.0	2.2	23	10
11	14.6	20.9	15.4	31.8	20.7	7.9	64.4	97.3	73.4	78.4	17.0	3.8	4.4	18	30
12	10.3	11.0	10.1	19.4	12.7	4.5	38.1	98.5	81.3	72.6	31.1	5.7	3.7	45	36
13	15.8	21.8	18.9	28.7	21.3	5.5	32.5	52.0	70.4	51.6	19.0	2.4	2.1	11	13
14	15.8	30.0	17.9	19.8	20.8	6.3	30.7	68.8	70.1	56.5	22.4	2.7	1.9	13	12
15	4.2	6.6	3.9	<3.1	<4.4	1.5	23.1	50.0	40.8	37.9	13.6	>8.6	5.6	>194	134
DXR	0.089	<0.078	<0.078	<0.078	<0.08	0.006	0.17	0.64	0.54	0.45	0.25	>5.5	1.9	>6865	2102
MTX	10.7	10.9	<7.8	<7.8	<9.3	1.8	203.0	962.5	1000.0	721.8	449.7	>77.8	18.9	>838	177

HGF, Human gingival fibroblast; HPC, human pulp cells; HPLF, human periodontal ligament fibroblast; Ca9-22 (derived from gingival tissue), HSC-2, HSC-3 and HSC-4 (derived from tongue), oral squamous cell carcinoma cell lines; CC_{50}, 50% cytotoxic concentration; DXR, doxorubicin; MTX, methotrexate. TS, tumor-selectivity; PSE, potency-selectivity expression.

Ca9-22, HSC-2, HSC-3, HSC-4) and (C/A^2) ×100 (HGF *vs*. Ca9-22 in Table II).

Estimation of CC_{50} values. Since the CC_{50} values had a distribution pattern close to a logarithmic normal distribution, we used the pCC_{50} (*i.e*., the −log CC_{50}) for the comparison of the cytotoxicity between the compounds. The mean pCC_{50} values for normal cells and tumor cell lines were defined as N and T, respectively (19).

Calculation of chemical descriptors. The 3D-structure of each chemical structure (drawn by Marvin) was optimized by CORINA Classic (Molecular Networks GmbH, Germany) and force-field calculations (amber-10: EHT) in Molecular Operating Environment (MOE) version 2014.09 (Chemical Computing Group Inc., Quebec, Canada). The number of structural descriptors calculated from MOE and Dragon 7.0 (Kode srl., Pisa, Italy) after the elimination of overlapped descriptors were 295 and 2797, respectively.

The following 12 Dragon descriptors and 4 MOE descriptors were significantly correlated with T, N and T-N.

Dragon descriptors (25): (a) B10[O-O]: Presence/absence of O - O at topological distance 10; (b) CATS3D_10_DA: CATS3D Donor-acceptor BIN 10 (10.000-11.000 Å); (c) F10[O-O]: Frequency of O-O at topological distance 10; (d) VE2_H2: average coefficient of the last eigenvector (absolute values) from reciprocal squared distance matrix (2D matrix-based descriptors); (e) L3m: 3rd component size directional WHIM index/weighted by mass (WHIM descriptors); (f) L3s: 3rd component size directional WHIM

Table II. *Anti-HIV activity of chalcones and chemotherapeutic agents. Each value represents the mean of triplicate determinations.*

Compound	CC_{50} (μM)	EC_{50} (μM)	SI
1	323.627	>400	<1
2	178.21	>400	<1
3	>400	>400	><1
4	387.87	>400	<1
5	80.73	>400	<1
6	200.04	>400	<1
7	34.44	>400	<1
8	38.78	>400	<1
9	205.35	>400	<1
10	191.73	>400	<1
11	369.64	>400	<1
12	32.54	>400	<1
13	235.92	>400	<1
14	142.54	>400	<1
15	>400	>400	><1
Positive controls			
Dextran sulfate (μg/ml)	232.68	0.777	300
Curdlan sulfate (μg/ml)	>1000	0.172	>5805
Azidothymidine	53.004	0.026	2017
2',3'-Dideoxycytidine	1858.629	1.113	1670

CC_{50}, 50% Cytotoxic concentration; EC_{50}, 50% effective concentration; SI: selectivity index (=CC_{50}/EC_{50}).

index/weighted by atomic ionization state (WHIM descriptors); (g) HATS6p: leverage-weighted autocorrelation of lag 6/weighted by polarizability (GETAWAY descriptors); (h) R5v+: R maximal autocorrelation of lag 5/weighted by van der Waals volume (GETAWAY descriptors); (i) R6p R autocorrelation of lag 6/weighted by polarizability (GETAWAY descriptors); (j) R6v: R autocorrelation of lag 6/weighted by van der Waal's volume (GETAWAY descriptors); (k) RDF010s: Radial distribution function - 010/weighted by atomic ionization state (RDF descriptors); (l) RDF035u: Radial distribution function - 035/unweighted (RDF descriptors); MOE descriptors: (m) vsurf_IW6: Hydrophilic integy moment 6 in the vsurf_ descriptors which are similar to the VolSurf descriptors (26); (n) h_logS: Log of the aqueous solubility (mol/L) using a 7 parameter model based on Hueckel theory (27); (o) PEOE_VSA-6: Sum of vi where qi is less than –0.30 in the partial equalization of orbital electronegativities (PEOE) method of calculating atomic partial charges (27); (p) Q_VSA_PNEG: Total negative polar van der Waals surface area (28).

Western blot analysis. The cells were washed with PBS and processed for western blot analysis, as described previously (29). Antibodies against cleaved caspase-3 (Cell Signaling Technology Inc., Beverly, MP, USA), poly(ADP-ribose) polymerase (PARP) (Cell Signaling Technology Inc.) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Trevigen, Gaithersburg, MD, USA) were used as primary antibodies. As secondary antibodies, we used α-rabbit IgG (DAKO Japan) antibodies which were conjugated with horseradish peroxidase.

Assay for anti-HIV activity. HTLV-I-carrying human T-cell line MT-4 cells, highly sensitive to human immunodeficiency virus-1 (HIV-1), were infected with HIV-1_{IIIB} at a multiplicity of infection of 0.01. HIV- and mock-infected (control) MT-4 cells were incubated for 5 days with different concentrations of samples and the relative viable cell number was determined by the MTT assay. The CC_{50} and 50% effective concentration (EC_{50}) were determined from the dose–response curve for mock-infected and HIV-infected cells, respectively (30). All data represent the mean values of triplicate measurements. The anti-HIV activity was evaluated by selectivity index (SI) (=CC_{50}/EC_{50}).

Statistical treatment. The relation among cytotoxicity, tumor specificity index, anti-UV activity and chemical descriptors was investigated using simple regression analyses by JMP Pro version 12.2.0 (SAS Institute Inc., Cary, NC, USA). The significance level was set at $p<0.05$.

Results

Cytotoxicity. A total of 15 chalcone derivatives (Figure 1) generally showed higher cytotoxicity against human OSCC lines (Ca9-22, HSC-2, HSC-3, HSC-4) (mean CC_{50}=4.4-41.1 μM, mean 21.7 μM) (B) than against human mesenchymal normal oral cells (HGF, HPLF and HPC) (CC_{50}=28.8-188.0 μM, mean 80.0 μM), yielding an averaged TS of 3.7 (Table I). Among them, compounds **10**, **12** and **15** had higher TS (5.7-8.6) than other compounds, comparable to that of anticancer drugs, doxorubicin (5.5). When tumor selectivity was calculated using cells both derived from gingival tissue (Ca9-22 *vs*. HGF), 14 compounds **1-13** and **15** had much higher TS (2.1-5.6), exceeding that of doxorubicin (1.9). Compound **15** had the highest TS values in both cases.

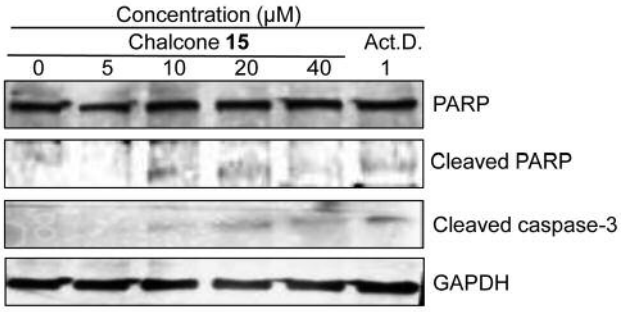


Figure 2. *Apoptosis induction in HSC-2 human oral squamous cell carcinoma cell line by compound **15**.*

In order to identify compounds which have both good potency and are selectively toxic to neoplasms, the PSE values for the compounds were calculated. When all three normal cells (HGF, HPLF and HPC) and all four OSCC cell lines (Ca9-22, HSC-2, HSC-3 and HSC-4) were used, doxorubicin had the highest PSE value (>6,865), followed by methotrexate > compound **15** > compounds **1-14** (Table I). When HGF and Ca9-22 cells (both derived from gingival tissues) were used, the same pattern was found. Compound **15** had the highest PSE value among the 15 chalcones, approaching that of methotrexate. Western blot analysis demonstrated that compound **15** stimulated the cleavage of PARP and caspase-3, suggesting the induction of apoptosis (Figure 2).

Anti-HIV activity of chalcones. In contrast to popular anti-HIV agents (dextran sulfate, curdlan sulfate, azidothymidine, 2',3'-dideoxycytidine) (SI=300-5,805), none of the chalcones protected cells from the cytopathic effect of HIV infection (SI<1) (Table II). Based on these data, the subsequent QSAR analysis was focused on the cytotoxicity of chalcones.

Computational analysis. We next performed the QSAR analysis of chalcone derivatives in regards to their cytotoxicity against tumor cells and normal cells. Among a total of 3,092 descriptors (295 MOE and 2797 Dragon descriptors), 16 descriptors described below correlated well with cytotoxicity and tumor specificity. Cytotoxicity of chalcones against human OSCC cell lines was correlated with HATS6p (polarizability) (r^2=0.541, p=0.0018), vsurf_IW6 (hydrophilic interaction energy moment 6) (r^2=0.537, p=0.0019), R6v (van der Waal's volume) (r^2=0.524, p=0.0023), R6p (polarizability) (r^2=0.491, p=0.0036), h_logS

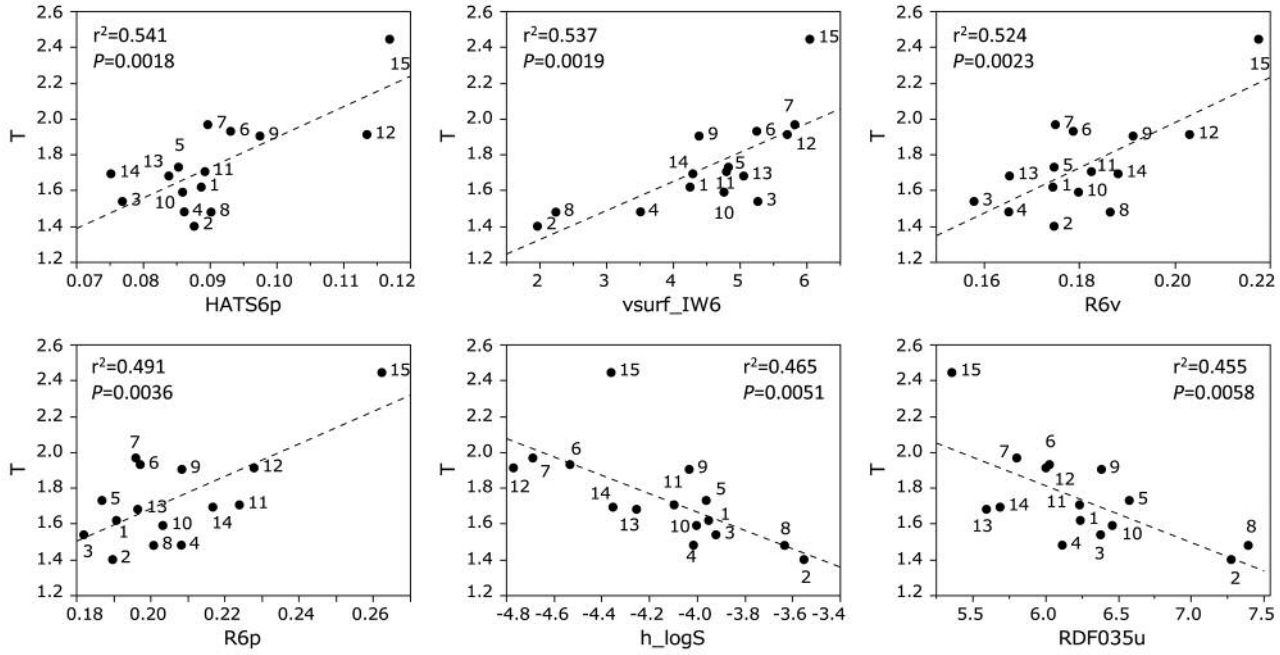


Figure 3. *Determination of correlation coefficient between chemical descriptors and cytotoxicity of chalcones against tumor cells. The mean values of pCC_{50} (i.e., the −log of the concentration causing 50% cytotoxicity) for tumor cell lines were defined as T.*

(aqueous solubility) (r^2=0.465, p=0.0051) and RDF035u (spherically averaged information on the atomic correlation, unweighted) (r^2=0.455, p=0.0058) (Figure 3).

Cytotoxicity of chalcones against human normal oral mesenchymal cells was correlated with CATS3D_10_DA (donor-acceptor BIN) (r^2=0.732, p<0.0001), RDF010s (atomic ionization state) (r^2=0.696, p=0.0001), Q_VSA_PNEG (total negative polar van der Waals surface area) (r^2=0.633, p=0.0004), PEOE_VSA-6 (atomic partial charges) (r^2=0.633, p=0.0004), B10[O-O] (presence/absence of O-O at topological distance 10) (r^2=0.620, p=0.0005) and F10[O-O] (frequency of O-O at topological distance 10) (r^2=0.620, p=0.0005) (Figure 4).

Tumor specificity of chalcones was correlated with R6p (polarizability) (r^2=0.601, p=0.0007), R5v+(van der Waal's volume) (r2=0.598, p=0.0007), L3m (mass) (r^2=0.581, p=0.0009), VE2_H2 (average coefficient of the last eigenvector from reciprocal squared distance matrix) (r^2=0.575, p=0.0010), L3s (atomic ionization state) (r^2=0.565, p=0.0012) and HATS6p (polarizability) (r^2=0.563, p=0.0013) (Figure 5).

Discussion

The present study demonstrated that 15 chalcones showed relatively higher cytotoxicity against four OSCC cell lines compared to that against human normal oral mesenchymal oral cells; among them, compound **15** had the highest TS and PSE values, although this is not a new compound. It should be noted that the TS value of **15** was comparable with that of doxorubicin, and the PSE value of **15** was comparable with that of methotrexate (Table I). It is ideal to use human epithelial cells as control normal cells in comparison with OSCC cell lines. However, we recently found that doxorubicin induced apoptosis in human oral keratinocytes (*i.e.* loss of cell surface microvilli, chromatin condensation, nuclear fragmentation, caspase-3 activation) at the concentration that affected the viability of OSCC cell lines (31). Until the mechanism of keratinocyte toxicity is clarified and a preventive method is explored, the use of human oral mesenchymal cells rather than normal epithelial cells may be the only choice for us to use in comparison with tumor cells. We calculated the possible contribution of substituted groups to the expression of cytotoxicity against OSCC cell lines and normal oral mesenchymal cells and tumor-specificity (Table III). Most of the substituents listed did not affect these activities (p=0.1067-0.9465) except for hydroxyl group (p=0.0167) or oxygen (p=0.0248) at R5 in determining cytotoxicity against normal cells. These data, suggest that tumor specificity of chalcones was rather correlated with molecular structure and polarization (Figure 5). We also found that correlated parameters differed between tumor cells and normal cells. For example, R6P (which represents polarizability) is correlated with cytotoxicity against tumor cells (Figure 3) and with tumor selectivity (Figure 5), but not

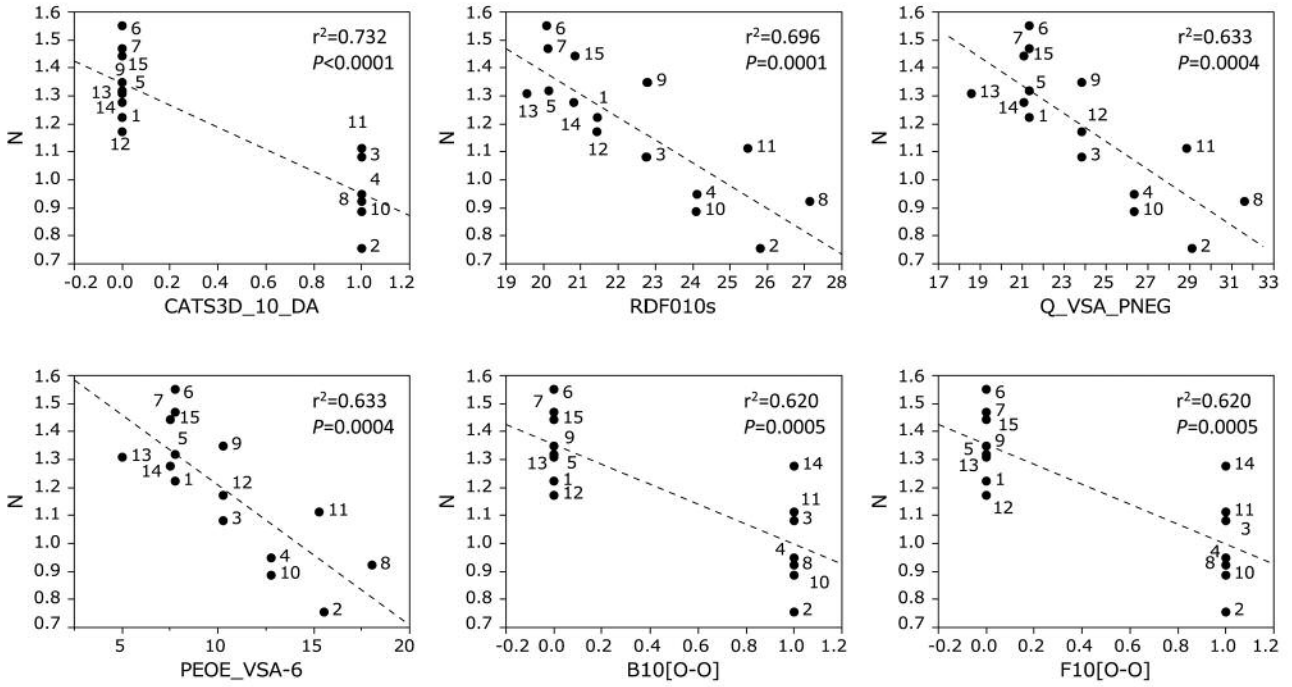


Figure 4. *Determination of correlation coefficient between chemical descriptors and cytotoxicity of chalcones against normal cells. The mean values of pCC_{50} (i.e., the −log of the concentration causing 50% cytotoxicity) for normal cells were defined as N.*

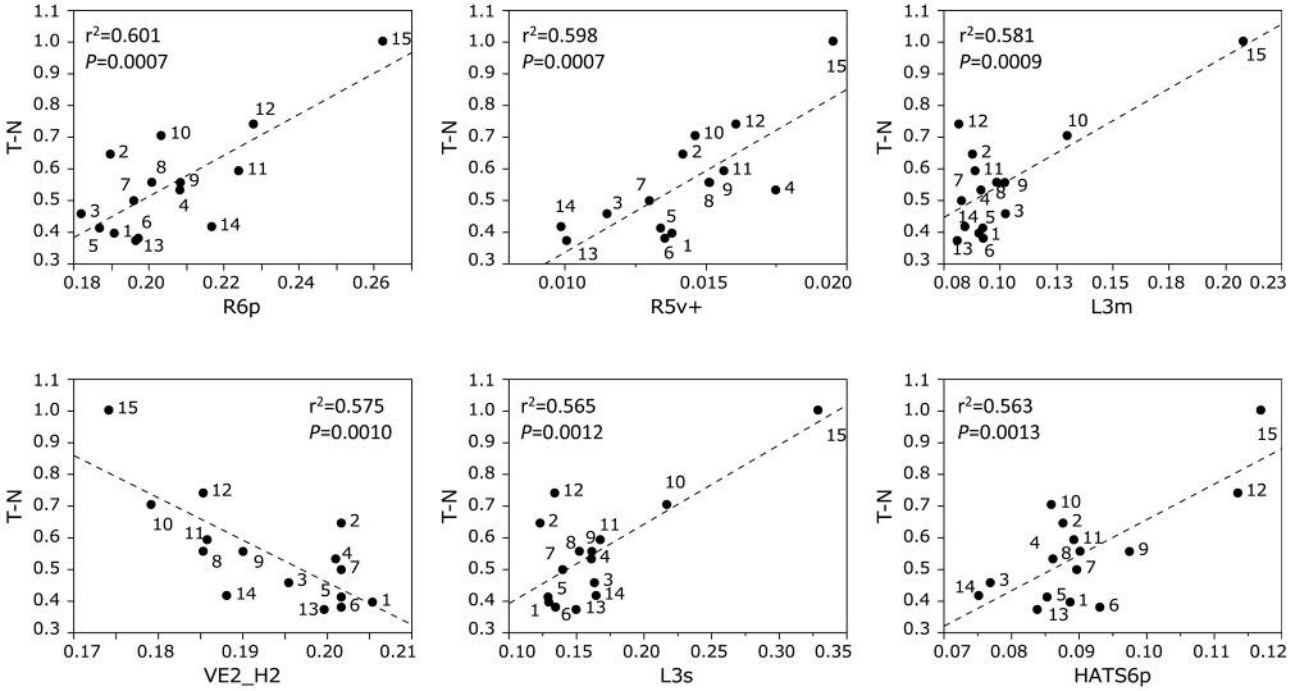


Figure 5. *Determination of correlation coefficient between chemical descriptors and tumor specificity of chalcones (defined as the difference between the −log of the concentration causing 50% cytotoxicity in tumor cells and that for normal cells (T-N).*

Table III. *Substituted groups that affect the cytotoxicity against OSCC cell lines (T) and normal oral mesenchymal cells (N) and tumor specificity (T-N).*

	Factor	*p*-Value
T	R1	0.3433
T	R2Sbst	0.7795
T	R2OH	0.146
T	R4	0.4231
T	R5Sbst	0.9008
T	R5OH	0.0851
T	R5O	0.2186
T	R5X	0.2054
N	R1	0.9465
N	R2Sbst	0.5165
N	R2OH	0.2111
N	R4	0.3247
N	R5Sbst	0.5449
N	R5OH	0.0167
N	R5O	0.0248
N	R5X	0.0538
T-N	R1	0.1067
T-N	R2Sbst	0.1729
T-N	R2OH	0.6177
T-N	R4	0.9208
T-N	R5Sbst	0.5252
T-N	R5OH	0.6722
T-N	R5O	0.3378
T-N	R5X	0.5744

so with cytotoxicity against normal cells (Figure 4). These data indicate that an increase of polarizability of chalcones may increase their antitumor potential.

The present study demonstrated that 15 chalcones did not have any anti-HIV activity. This finding is not contradictory with recent reports that chalcones exert anti-HIV activity partially or in a very narrow range of concentrations (32, 33).

In conclusion, compound **15** is a potential lead compound for synthesizing more potent compounds targeted to OSCC cells.

Conflicts of Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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