ANTICANCER RESEARCH *38*: 817-823 (2018)
doi:10.21873/anticanres.12289

Quantitative Structure–Cytotoxicity Relationship of Cinnamic Acid Phenetyl Esters

YOSHIHIRO UESAWA[1], HIROSHI SAKAGAMI[2], NORIYUKI OKUDAIRA[3], KAZUHIRO TODA[4], KOICHI TAKAO[4], HAJIME KAGAYA[1] and YOSHIAKI SUGITA[4]

[1]*Department of Clinical Pharmaceutics, Meiji Pharmaceutical University, Tokyo, Japan;*
[2]*Meikai University Research Institute of Odontology (M-RIO) and*
[3]*Division of Pharmacology, Mekai University School of Dentistry, Tokyo, Japan;*
[4]*Department of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, Sakado, Japan*

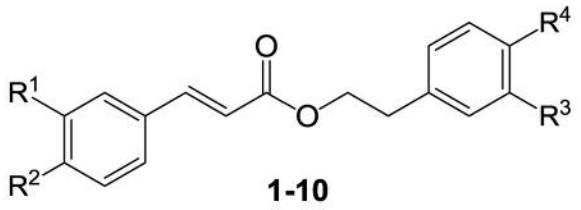
Abstract. *Background/Aim: Many phenolic acid phenethyl esters possess diverse biological effects including antioxidant, cytoprotective, anti-inflammation and anti-tumor activities. However, most previous antitumor studies have not considered the cytotoxicity against normal cells. Ten cinnamic acid phenetyl esters 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 normal 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 oral cells to that against human oral squamous cell carcinoma cell lines. Potency-selectivity expression (PSE) value was calculated by dividing the TS value 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: Western blot analysis demonstrated that [**9**] stimulated the cleavage of caspase-3, suggesting the induction of apoptosis. QSAR analysis demonstrated that TS values were correlated with shape, size and ionization potential. Conclusion: Chemical modification of the lead compound may be a potential choice for designing a new type of anticancer drugs.*

Correspondence to: Yoshihiro Uesawa, Department of Clinical Pharmaceutics, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-858, Japan. Tel/Fax: +81 424958892, e-mail: uesawa@my-pharm.ac.jp

Key Words: Cinnamic acid phenetyl esters, QSAR analysis, cytotoxicity, tumor selectivity, apoptosis induction.

Many phenolic acid phenethyl esters possess diverse biological effects including antioxidant activity (evaluated by radical scavenging activity) (1-4), cytoprotective activity (against oxidative stress) (5-7), anti-inflammation activity (evaluated by inhibition of 5-lipoxygenase and leukotriene biosynthesis) (8) and anti-tumor activity against implanted tumors (9) in mice and various tumor cell lines (10-12). However, most previous antitumor studies *in vitro* have not assayed the cytotoxicity against control normal cells. We have established the simple *in vitro* assay system of antitumor potential, 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 cell, HPC) (13, 14). Using this system, we have demonstrated that many anticancer drugs (camptothecin, SN-38, etoposide, doxorubicin, daunorubicin, mitomycin C, methotrexate, 5-fluorouracil, docetaxel, melphalan, gefitinib) showed excellent tumor-specificity (15). Among a total of 133 compounds, (*E*)-3-[2-(4-hydroxyphenyl)ethenyl]-6-methoxy-4*H*-1-benzopyran-4-one (classified as 3-styrylchromone) (16, 17), (*E*)-3-[2-(4-chlorophenyl)ethenyl]-7-methoxy-2*H*-1-benzopyran (classified as 3-styryl-2*H*-chromenes) (18) showed the highest tumor specificity with the least keratinocyte toxicity (14).

In continuation of discovering new biological activities of phenylpropanoid derivatives, a total of ten cinnamic acid phenetyl esters (Figure 1) (4) were investigated for their cytotoxicity against four human OSCC cell lines and four human normal oral cells, and then subjected to quantitative structure–activity relationship (QSAR) analysis.



1-10

Compound	R^1	R^2	R^3	R^4
1	OMe	OH	OH	OH
2	H	OH	OH	OH
3	OH	OH	OH	OH
4	OMe	OH	H	OH
5	H	OH	H	OH
6	OH	OH	H	OH
7	H	H	H	OH
8	H	OH	H	H
9	OH	OH	H	H
10	H	H	H	H

Figure 1. *Structure of ten cinnamic acid esters.*

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 from Sigma-Aldrich Inc., St. Louis, MO, USA; dimethyl sulfoxide (DMSO). Culture plastic dishes and plates (96-well) were purchased from Becton Dickinson (Franklin Lakes, NJ, USA).

Synthesis of test compounds. (2*E*)-3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid 2-(3,4-dihydroxyphenyl)ethyl ester [**1**], (2*E*)-3-(4-hydroxyphenyl)-2-propenoic acid 2-(3,4-dihydroxyphenyl)ethyl ester [**2**], (2*E*)-3-(3,4-dihydroxyphenyl)-2-propenoic acid 2-(3,4-dihydroxyphenyl)ethyl ester [**3**], (2*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid 2-(4-hydroxyphenyl)ethyl ester [**4**], (2*E*)-3-(4-hydroxyphenyl)-2-propenoic acid 2-(4-hydroxyphenyl)ethyl ester [**5**], (2*E*)-3-(3,4-dihydroxyphenyl)-2-propenoic acid 2-(4-hydroxyphenyl)ethyl ester [**6**], (2*E*)-3-phenyl-2-propenoic acid 2-(4-hydroxyphenyl)ethyl ester [**7**], (2*E*)-3-(4-hydroxyphenyl)-2-propenoic acid 2-phenylethyl ester [**8**], (2*E*)-3-(3,4-dihydroxyphenyl)-2-propenoic acid 2-phenylethyl ester [**9**], (2*E*)-3-phenyl-2-propenoic acid 2-phenylethyl ester [**10**] were synthesized by the condensations of cinnamic acid derivatives with selected phenethylalcohol derivatives, according to previous methods (4). All compounds were dissolved in DMSO at 40 mM and stored at –20˚C before use.

Cell culture. Human normal oral mesenchymal cells (human gingival fibroblast, HGF; human periodontal ligament fibroblast, HPLF; human pulp cells, HPC) were established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl (19), and cells at 10-18 population doubling levels were used in this study. Human oral squamous cell carcinoma (OSCC) cell lines [Ca9-22 (derived from gingival tissue); HSC-2, HSC-3, HSC-4 (derived from tongue)] were purchased from Riken Cell Bank (Tsukuba, Japan). All of these cells 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.

Assay for cytotoxic activity. Cells were inoculated at 2.5×10^3 cells/0.1 ml in a 96-microwell plate. 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 (15, 17, 18). The relative viable cell number was determined by the absorbance of the cell lysate at 560 nm, using a microplate reader (Infinite F 50 R, TECAN, Kawasaki, Japan). 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 triplicate assays.

Calculation of tumor-selectivity index (TS). TS was calculated using the following equation: TS = mean CC_{50} against three normal cells/mean CC_{50} against for four OSCC cell lines [(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 did not use human normal oral keratinocytes as controls, since many anticancer drugs showed potent cytotoxicity against normal keratinocytes by inducing apoptosis (15).

Calculation of potency-selectivity expression (PSE). PSE was calculated using the following equation: PSE=TS/CC_{50} against tumor cells ×100 (10) [that is, $(D/B^2)\times100$ (HGF, HPLF, HPC *vs.* Ca9-22, HSC-2, HSC-3, HSC-4) and $(C/A^2)\times100$ (HGF *vs.* Ca9-22 in Table I).

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 (18).

Calculation of chemical descriptors. The 3D-structure of each chemical structure (drawn by Marvin Sketch ver 16, ChemAxon, Budapest, Hungary, http://www.chemaxon.com) was optimized by CORINA Classic (Molecular Networks GmbH, Germany) and force-field calculations (amber-10: EHT) in Molecular Operating Environment (MOE) version 2015.1001 (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 284 and 2750, respectively.

Table I. *Cytotoxic activity of ten cinnamic acid esters against oral malignant and non-malignant cells. Each value represents the mean of triplicate determinations.*

	CC_{50} (μM)														
	Human oral squampous cell carcinoma cell lines						Human normal oral cells					TS		PSE	
	Ca9-22	HSC-2	HSC-3	HSC-4	mean	SD	HGF	HPLF	HPC	mean	SD				
Compd.	(A)				(B)		(C)			(D)		(D/B)	(C/A)	$(D/B^2)\times100$	$(C/A^2)\times100$
1	84.1	50.1	80.6	51.7	66.6	18.3	159.0	268.7	285.0	237.6	68.5	3.6	1.9	5.4	2.2
2	98.0	33.3	73.8	41.6	61.7	29.9	284.0	307.0	321.7	304.2	19.0	4.9	2.9	8.0	3.0
3	**55.5**	**12.9**	**9.1**	**16.5**	**23.5**	**21.5**	**313.3**	**373.3**	**332.0**	**339.6**	**30.7**	**14.4**	**5.6**	**61.3**	**10.2**
4	91.8	114.3	104.1	108.9	104.8	9.6	130.3	150.3	263.0	181.2	71.5	1.7	1.4	1.7	1.5
5	162.0	63.8	160.3	157.7	136.0	48.1	288.0	332.0	355.3	325.1	34.2	2.4	1.8	1.8	1.1
6	**32.0**	**18.1**	**24.8**	**25.6**	**25.1**	**5.7**	**270.7**	**247.3**	**236.3**	**251.4**	**17.5**	**10.0**	**8.5**	**39.8**	**26.4**
7	157.7	42.1	76.1	133.7	102.4	52.8	218.0	185.0	280.0	227.7	48.2	2.2	1.4	2.2	0.9
8	54.4	26.1	105.9	45.1	57.9	34.1	139.0	147.3	152.3	146.2	6.7	2.5	2.6	4.4	4.7
9	**13.4**	**10.7**	**4.3**	**5.6**	**8.5**	**4.3**	**153.0**	**173.3**	**269.7**	**198.7**	**62.3**	**23.4**	**11.4**	**275.1**	**85.2**
10	176.0	105.4	265.0	240.0	196.6	71.4	>398	>400	>400	>399.3	1.2	>2.0	>2.3	>1.0	>1.3
DXR	0.29	0.10	0.08	0.07	0.13	0.10	2.34	10.00	10.00	7.45	4.42	56.8	8.2	43392.3	2847.1

HGF: Human gingival fibroblast; HPLF: human periodontal ligament fibroblast; HPC: human pulp cell; 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; TS: tumor-selectivity index; PSE: potency-selectivity expression.

The following 14 Dragon descriptors (21) and 2 MOE descriptors (22) listed in Table II were significantly correlated with T, N and T-N.

Western blot analysis. The cells were washed with PBS and processed for western blot analysis, as described previously (15). Antibodies against cleaved caspase-3 (Cell Signaling Technology Inc., Beverly, MP, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Trevigen, Gaithersburg, MD, USA) were used as primary antibodies. As secondary antibodies, we used α-rabbit IgG (DAKO, Tokyo, Japan) antibodies which were conjugated with horseradish peroxidase.

Statistical treatment. The relation among cytotoxicity, tumor specificity index 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. We have synthesized a total of ten cinnamic acid phenetyl esters, without or with different numbers of substituted methoxy or hydroxyl group (Figure 1). Backbone structure is (2*E*)-3-phenyl-2-propenoic acid 2-phenylethyl ester [**10**], that showed the lowest cytotoxicity against both OSCC (Ca9-22, HSC-2, HSC-3, HSC-4) and normal oral cells (HGF, HPLF, HPC) (mean of CC_{50}=196.6 and >399.3 μM) (Table I). Introduction of hydroxy group caused increased their cytotoxicity to varying degrees. Compounds [**3, 6, 9**], that have two hydroxy group at R^1 and R^2 positions of benzene ring, showed the highest cytotoxicity against OSCC cell lines (CC_{50}=23.5, 25.1 and 8.5 μM) (Table I).

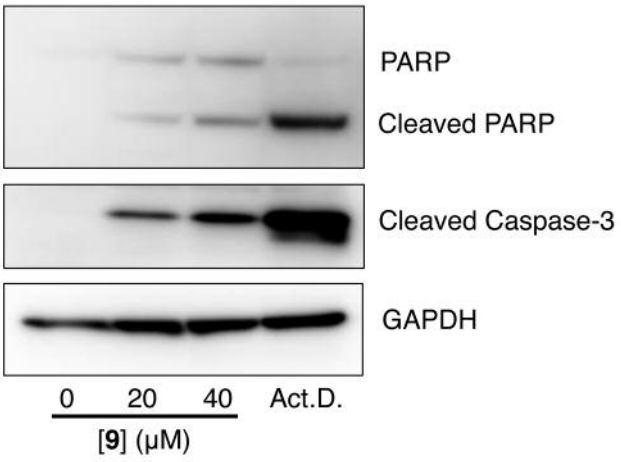


Figure 2. *Apoptosis induction by [9] in HSC-2 human oral squamous cell carcinoma cell line. Act.D: Actinomycin D (1 μM).*

Tumor-specificity. Tumor-specificity (TS) were calculated by dividing the mean CC_{50} value towards three normal cells by the mean CC_{50} value towards four OSCC cell lines (D/B, Table I). Since compounds [**3, 6, 9**] showed one order less cytotoxicity against human oral normal cells (CC_{50}=339.6, 254.1 and 198.7 μM, respectively), they showed the highest tumor specificity (TS=14.4, 10, 23.4) (Table I).

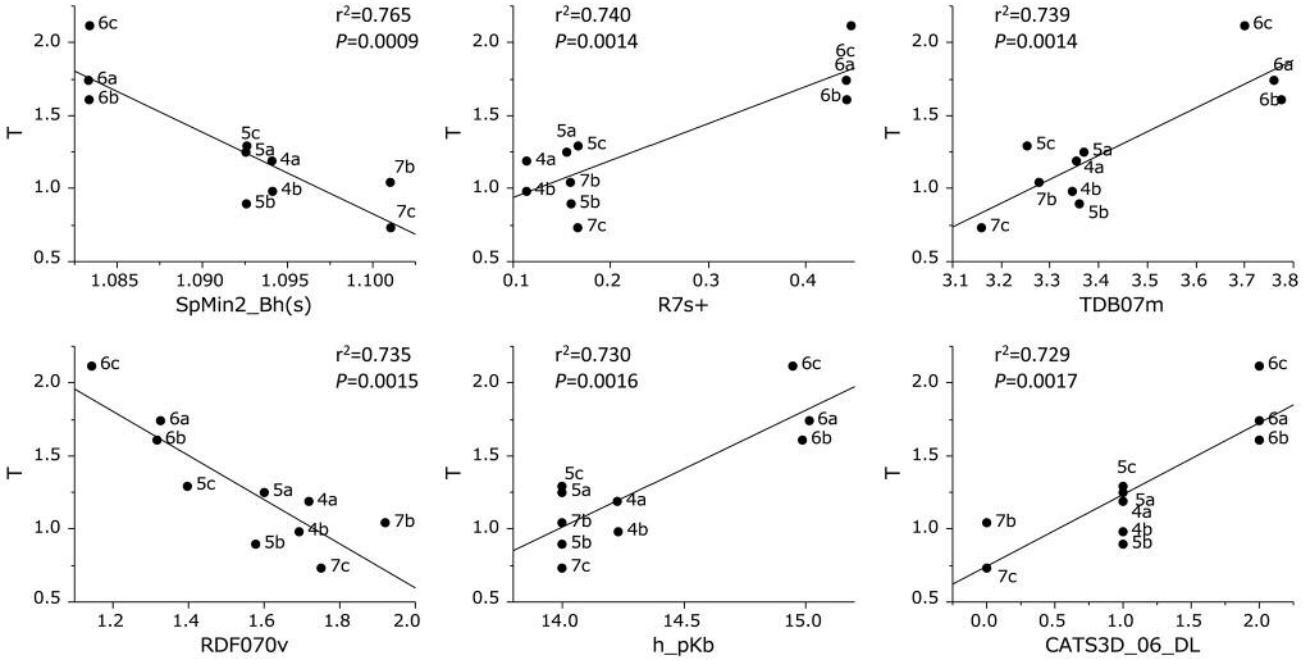


Figure 3. *Determination of coefficient between chemical descriptors and cytotoxicity of ten cinnamic acid esters against tumor cells (defined as T). The mean (pCC_{50} i.e., the $-log\ CC_{50}$) values for tumor cell lines were defined as T.*

Table II. *Source and explanation of chemical descriptors that correlate with cytotoxicity to tumor cells, normal cells and tumor-specificity.*

Descriptor	Source	Meaning	Explanation
BIC3	Dragon	Symmetry	Bond Information Content index (neighborhood symmetry of 3-order)
CATS3D_06_DL	Dragon	H bond & lipophilicity	CATS3D Donor-Lipophilic BIN 06 (6.000-7.000 Å)
CIC2	Dragon	Symmetry	Complementary Information Content index (neighborhood symmetry of 2-order)
CIC3	Dragon	Symmetry	Complementary Information Content index (neighborhood symmetry of 3-order)
E2m	Dragon	Shape & size	2nd component accessibility directional WHIM index/weighted by mass
h_pKb	MOE	pKb	The pKb of the reaction that adds a proton from the ensemble of states with a hydrogen count equal to the input structure
H7s	Dragon	Shape & electoric state	H autocorrelation of lag 7/weighted by I-state
PEOE_VSA+1	MOE	Size & partial charge	Sum of vi where qi is in the range from 0.05 to 0.10. (qi: the partial charge of atom i. vi: the van der Waals surface area (Å2) of atom i)
R7s+	Dragon	Shape & electoric state	R maximal autocorrelation of lag 7/weighted by I-state
RDF070v	Dragon	Shape & Size	Radial Distribution Function - 070/weighted by van der Waals volume
SIC3	Dragon	Symmetry	Structural Information Content index (neighborhood symmetry of 3-order)
SpMin2_Bh(s)	Dragon	Electronic state	smallest eigenvalue n. 2 of Burden matrix weighted by I-state
TDB07m	Dragon	Shape & size	3D Topological distance based descriptors - lag 7 weighted by mass
TDB10i	Dragon	Shape & ionization potential	3D Topological distance based descriptors - lag 10 weighted by ionization potential
VE1sign_Dt	Dragon	Shape	coefficient sum of the last eigenvector from detour matrix
VE2_B(s)	Dragon	Shape & electoric state	average coefficient of the last eigenvector (absolute values) from Burden matrix weighted by I-State

Considering that HGF is the normal cell corresponds to cancer cell Ca9-22 (both derived from gingival tissues), TS values were also generated by dividing the average CC_{50} value towards HGF cells by the CC_{50} value towards Ca9-22 cells (C/A, Table I). Compounds [**3, 6, 9**] showed TS value of 5.6, 8.5 and 11.4, respectively, comparable with doxorubicin, anthracycline-type anticancer drug (Table I).

PSE value. In order to identify the most promising compounds in terms of both good potencies and selective cytotoxicity, the

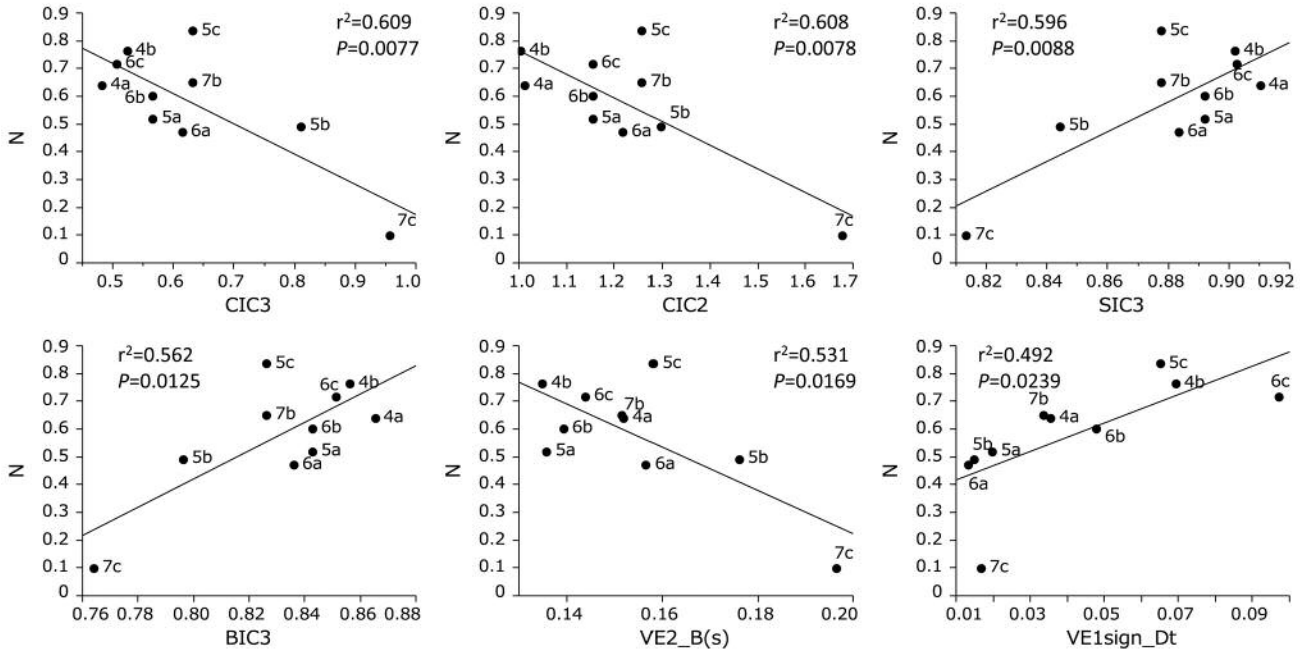


Figure 4. *Determination of coefficient between chemical descriptors and cytotoxicity of ten cinnamic acid esters against normal cells (defined as N). The mean (pCC_{50} i.e., the $-log\ CC_{50}$) values for normal cells were defined as N.*

potency-selectivity expression (PSE) values were calculated. Among [**3, 6, 9**], [**9**] having two hydroxyl groups in R^1 and R^2, but not in R^3 and R^4, showed the highest cytotoxicity (CC_{50}=8.5 μM). As expected, [**9**] yielded the greatest PSE values: 275.1 and 85.2 (calculated as D/B^2 ×100 and C/A^2 ×100, respectively, in Table I), one or two orders higher than that of other compounds [**1, 2, 4, 5, 7, 8, 10**] (Table I).

Western blot analysis demonstrated that [**9**] stimulated the cleavage of caspase-3, suggesting the induction of apoptosis (Figure 2).

Computational analysis. We next performed the QSAR analysis of ten cinnamic acid phenetyl esters in regards to their cytotoxicity against tumor cells and normal cells. Among a total of 3,034 descriptors (dragon, 2,750 descriptors; MOE, 284 descriptors), 14 dragon descriptors and 2 MOE descriptors correlated well with cytotoxicity and tumor specificity (Table III).

Cytotoxicity of ten cinnamic acid phenetyl esters against OSCC cell lines was correlated with SpMin2_Bh(s) (Electronic state) (r^2=0.765, p=0.0009), R7s+ (Shape & electoric state (r^2=0.740, p=0.0014), TDB07m (Shape & size) (r^2=0.739, p=0.0014), RDF070v (Shape & Size) (r^2=0.735, p=0.0015), h_pKb (pKb) (r^2=0.730, p=0.0016) and CATS3D_06_DL (H bond & lipophilicity) (r^2=0.729, p=0.0017) (Figure 3).

Table III. *Properties of descriptors that significantly affects the cytotoxicity against tumor cells (T), normal cells (N) and tumor-specificity (T-N).*

	Descriptor	r^2	*p*-Value	Meaning
T	SpMin2_Bh(s)	0.765	0.0009	Electronic state
T	R7s+	0.740	0.0014	Shape & electoric state
T	TDB07m	0.739	0.0014	Shape & size
T	RDF070v	0.735	0.0015	Shape & Size
T	h_pKb	0.730	0.0016	pKb
T	CATS3D_06_DL	0.729	0.0017	H bond & lipophilicity
N	CIC3	0.609	0.0077	Symmetry
N	CIC2	0.608	0.0078	Symmetry
N	SIC3	0.596	0.0088	Symmetry
N	BIC3	0.562	0.0125	Symmetry
N	VE2_B(s)	0.531	0.0169	Shape & electoric state
N	VE1sign_Dt	0.492	0.0239	Shape
T-N	R7s+	0.840	0.0002	Shape & electoric state
T-N	PEOE_VSA+1	0.784	0.0007	Size & partial charge
T-N	TDB10i	0.774	0.0008	Shape & ionization potential
T-N	h_pKb	0.729	0.0017	pKb
T-N	E2m	0.719	0.0019	Shape & size
T-N	H7s	0.716	0.002	Shape & electoric state

Cytotoxicity of ten cinnamic acid phenetyl esters against normal oral mesenchymal cells was correlated with CIC3 (Symmetry) (r^2=0.609, p=0.0077), CIC2 (Symmetry) (r^2=0.608,

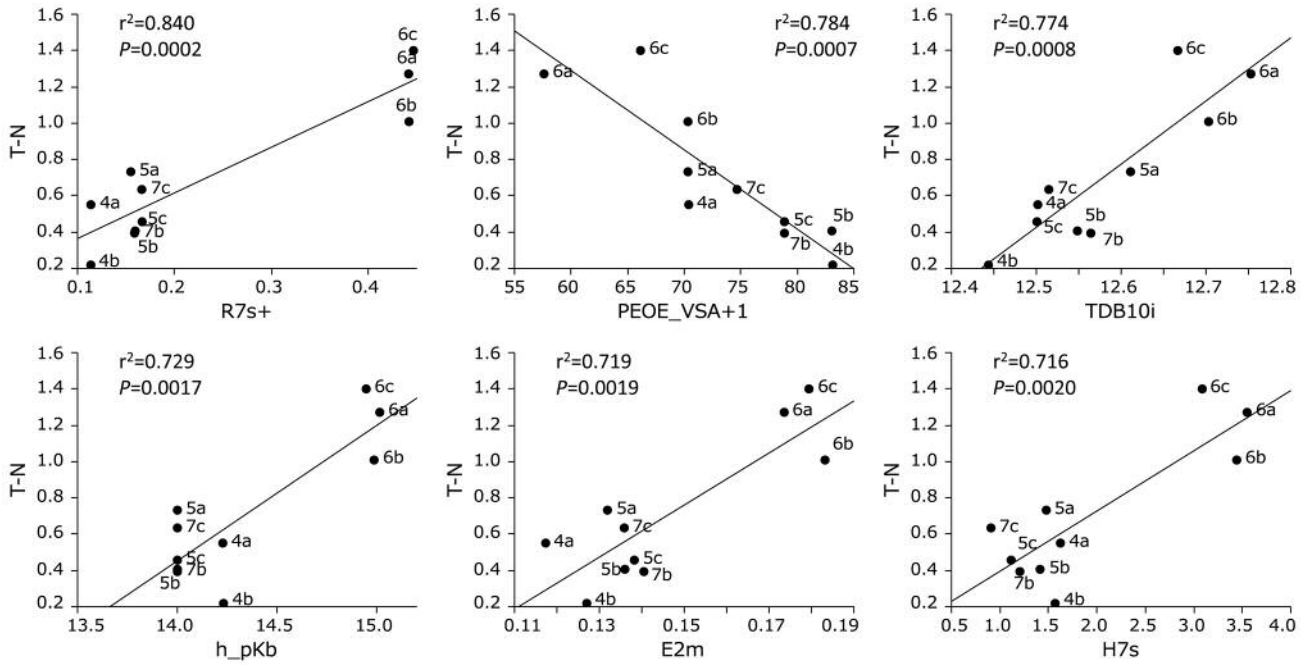


Figure 5. *Determination of coefficient between chemical descriptors and tumor specificity of ten cinnamic acid esters (defined as T-N).*

p=0.0078), SIC3 (Symmetry) (r^2=0.596, p=0.0088), BIC3 (Symmetry) (r^2=0.562, p=0.0125), VE2_B(s) (Shape & electoric state) (r^2=0.531, p=0.0169) and VE1sign_Dt (Shape) (r^2=0.492, p=0.0239) (Figure 4).

Tumor specificity of ten cinnamic acid phenetyl esters was correlated with R7s+ (Shape & electoric state) (r^2=0.840, p=0.0002), PEOE_VSA+1 (Size & partial charge) (r^2=0.784, p=0.0007), TDB10i (Shape & ionization potential) (r^2=0.774, p=0.0008), h_pKb (pKb) (r^2=0.729, p=0.0017), E2m (Shape & size) (r^2=0.719, p=0.0019) and H7s (Shape & electoric state) (r^2=0.716, p=0.002) (Figure 5).

Discussion

The present study demonstrated that among ten cinnamic acid phenetyl esters, compounds [**3, 6, 9**], that have two hydroxy group at R^1 and R^2 positions of benzene ring, showed the highest cytotoxicity against OSCC cell lines (CC_{50}=23.5, 25.1 and 8.5 μM) (Table I). We found that when hydroxyl group in R^1 of these compounds was replaced with methoxy group, their cytotoxic activity was reduced by 2.8-fold (66.6/23.5), 4.2-fold (104.8/25.1) and 12.0-fold (102.4/8.5), respectively (Table I). This suggests the importance of hydroxyl group at R^1 of benzene in expressing higher cytotoxicity induction against OSCC cell lines.

Especially, [**9**], having two hydroxyl groups in R^1 and R^2, but not in R^3 and R^4, showed the highest tumor-specific cytotoxicity, as evidenced by the greatest TS and PSE values (Table I), and also apoptosis-inducing activity against OSCC cell line (Figure 2). Since a wide range of anticancer drugs (camptothecin, SN-38, etoposide, doxorubicin, daunorubicin, mitomycin C, methotrexate, 5-fluorouracil, docetaxel, melphalan, gefitinib) induced potent keratinocyte toxicity, albeit their much lower cytotoxicity against human normal oral mesenchymal cells (15), it is urgent to investigate whether [**9**] shows such keratinocyte toxicity before going into further mechanistic study.

QSAR analysis demonstrated that tumor-specificity of ten cinnamic acid phenetyl esters was correlated with shape, size and ionization potential. Chemical modification of [**9**] may be a potential choice for designing a new type of anticancer drugs.

Conflicts of Interest

The Authors 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.

Acknowledgements

This work was partially supported by KAKENHI from the Japan Society for the Promotion of Science (JSPS) (15K08111, 16K11519).

References

1 Pagoni A and Vassiliou S: A novel and efficient synthesis of 3,4-dihydroxyphenylacetic ester and amide derivatives/conjugates and assessment of their antioxidant activity. Nat Prod Res *18*: 1-7, 2017.

2 Lee IK, Han MS, Kim DW and Yun BS: Phenylpropanoid acid esters from Korean propolis and their antioxidant activities. Bioorg Med Chem Lett *24(15)*: 3503-3505, 2014.

3 Lee YT, Don MJ, Liao CH, Chiou HW, Chen CF and Ho LK: Effects of phenolic acid esters and amides on stimulus-induced reactive oxygen species production in human neutrophils. Clin Chim Acta *352(1-2)*: 135-141, 2005.

4 Takao K, Toda K, Saito T and Sugita Y: Synthesis of amide and ester derivatives of cinnamic acid and its analogs: evaluation of their free radical scavenging and monoamine oxidase and cholinesterase inhibitory activities. Chem Pharm Bull (Tokyo) *65(11)*: 1020-1027, 2017.

5 Yang J, Marriner GA, Wang X, Bowman PD, Kerwin SM and Stavchansky S: Synthesis of a series of caffeic acid phenethyl amide (CAPA) fluorinated derivatives: comparison of cytoprotective effects to caffeic acid phenethyl ester (CAPE). Bioorg Med Chem *18(14)*: 5032-5038, 2010.

6 Shi H, Xie D, Yang R and Cheng Y: Synthesis of caffeic acid phenethyl ester derivatives, and their cytoprotective and neuritogenic activities in PC12 cells. J Agric Food Chem *62(22)*: 5046-5053, 2014.

7 Silva T, Oliveira C and Borges F: Caffeic acid derivatives, analogs and applications: a patent review (2009-2013). Expert Opin Ther Pat *24(11)*: 1257-1270, 2014.

8 Boudreau LH, Maillet J, LeBlanc LM, Jean-François J, Touaibia M, Flamand N and Surette ME: Caffeic acid phenethyl ester and its amide analogue are potent inhibitors of leukotriene biosynthesis in human polymorphonuclear leukocytes. PLoS One *7(2)*: e31833, 2012.

9 Guo X, Shen L, Tong Y, Zhang J, Wu G, He Q, Yu S, Ye X, Zou L, Zhang Z and Lian XY: Antitumor activity of caffeic acid 3,4-dihydroxyphenethyl ester and its pharmacokinetic and metabolic properties. Phytomedicine *20(10)*: 904-912, 2013.

10 Cárdenas M, Marder M, Blank VC and Roguin LP: Antitumor activity of some natural flavonoids and synthetic derivatives on various human and murine cancer cell lines. Bioorg Med Chem *14(9)*: 2966-2971, 2006.

11 Lee YT: Structure activity relationship analysis of phenolic acid phenethyl esters on oral and human breast cancers: the grey GM(0, N) approach. Comput Biol Med *41(7)*: 506-511, 2011.

12 Lee YT, Don MJ, Hung PS, Shen YC, Lo YS, Chang KW, Chen CF and Ho LK: Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells. Cancer Lett *223(1)*: 19-25, 2005.

13 Sakagami H: Biological activities and possible dental application of three major groups of polyphenols. J Pharmacol Sci *126*: 92-106, 2014.

14 Sugita Y, Takao K, Uesawa Y and Sakagami H: Search for new type of anticancer drugs with high tumor-specificity and less keratinocyte toxicity (Review). Anticancer Res *37(11)*: 5919-5924, 2017.

15 Sakagami H, Okudaira N, Masuda Y, Amano O, Yokose S, Kanda Y, Suguro M, Natori T, Oizumi H and Oizumi T: Induction of apoptosis in human oral keratinocyte by doxorubicin. Anticancer Res *37(3)*: 1023-1029, 2017.

16 Shimada C, Uesawa Y, Ishii-Nozawa R, Ishihara M, Kagaya H, Kanamto T, Terakubo S, Nakashima H, Takao K, Sugita Y and Sakagami H: Quantitative structure–cytotoxicity relationship of 3-styrylchromones. Anticancer Res *34*: 5405-5412, 2014.

17 Sakagami H, Shimada C, Kanda Y, Amano O, Sugimoto M, Ota S, Soga T, Tomita M, Sato A, Tanuma S, Takao K and Sugita Y: Effects of 3-styrylchromones on metabolic profiles and cell death in oral squamous cell carcinoma cells. Toxocol Rep *2*: 1281-1290, 2015.

18 Uesawa Y, Sakagami H, Ishihara M, Kagaya H, Kanamoto T, Terakubo S, Nakashima H, Yahagi H, Takao K and Sugita Y: Quantitative structure–cytotoxicity relationship of 3-styryl-2*H*-chromenes. Anticancer Res *35*: 5299-5308, 2015.

19 Kantoh K, Ono M, Nakamura Y, Nakamura Y, Hashimoto K, Sakagami H and Wakabayashi H: Hormetic and anti-radiation effects of tropolone-related compounds. In Vivo *24*: 843-852, 2010.

20 Horikoshi M, Kimura Y, Nagura H, Ono T and Ito H: A new human cell line derived from human carcinoma of the gingiva. I. Its establishment and morphological studies. Jpn J Oral Maxillofac Surg *20*: 100-106, 1974.

21 https://chm.kode-solutions.net/products_dragon_descriptors.php

22 Calculate Descriptors, MOE2015.10 on-line help manual, Chemical Computing Group.

Received November 20, 2017
Revised December 4, 2017
Accepted December 5, 2017