

Quantitative Structure–Cytotoxicity Relationship of 3-Styrylchromones

CHIYAKO SHIMADA¹, YOSHIHIRO UESAWA², REIKO ISHII-NOZAWA²,
MARIKO ISHIHARA³, HAJIME KAGAYA², TAISEI KANAMOTO⁴, SHIGEMI TERAOKUBO⁴,
HIDEKI NAKASHIMA⁴, KOICHI TAKAO⁵, YOSHIAKI SUGITA⁵ and HIROSHI SAKAGAMI¹

¹Divisions of Pharmacology and ³Basic Chemistry, Meikai University School of Dentistry, Sakado, Saitama, Japan;

²Department of Clinical Pharmaceutics, Meiji Pharmaceutical University, Noshio, Kiyose, Tokyo, Japan;

⁴St. Marianna University School of Medicine, Kanagawa, Japan;

⁵Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama, Japan

Abstract. *Background:* Fifteen 3-styrylchromones were subjected to quantitative structure–activity relationship (QSAR) analysis based on their cytotoxicity, tumor selectivity and anti-HIV activity, in order to explore their biological activities. *Materials and Methods:* Cytotoxicity against four human oral squamous cell carcinoma (OSCC) cell lines and three human oral normal cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Tumor-selectivity was evaluated by the ratio of the mean CC_{50} (50% cytotoxic concentration) against normal human oral cells to that against OSCC cell lines. Anti-HIV activity was evaluated by the ratio of CC_{50} to EC_{50} (50% cytoprotective concentration from HIV infection). Physicochemical, structural and quantum-chemical parameters were calculated based on the conformations optimized by the LowModeMD method followed by the density functional theory (DFT) method. *Results:* All 3-styrylchromone derivatives showed moderate-to-high tumor selectivity. Especially, compounds that have a methoxy group at 6-position of the chromone ring and hydroxyl group at 4'-position of phenyl group in styryl moiety [**11**] showed the highest tumor-selectivity. On the other hand, their cytotoxicity against normal cells showed good correlation to the descriptors that reflect hydrophobic interaction and molecular shapes. *Conclusion:* Multivariate statistics with

chemical descriptors for the location of substituted group, molecular shape and electrostatic interaction may be useful for designing the most favorable compound with higher tumor selectivity.

4H-1-benzopyran-4-ones (chromones) are an important class of oxygenated heterocyclic compounds since the chromone core structure is found in flavones, isoflavones and 2-styrylchromones. As compared with flavones and isoflavones, 2-styrylchromones constitute a much smaller group of naturally occurring chromones. Synthetic 2-styrylchromones showed antioxidant (1), anti-allergic (2), anti-inflammatory (3), anti-tumor (4-6), anti-viral (7, 8) activities. In contrast, as far as we know there were only two papers of 3-styrylchromones that investigated the antiviral (9) and antibacterial activity (10).

In order to further explore novel biological activities of 3-styrylchromones, we recently synthesized a series of 3-styrylchromone derivatives and reported antioxidant and α -glucosidase inhibition activities (11). In the present study, we investigated the cytotoxicity and anti-HIV activity of fifteen 3-styrylchromones and then performed the quantitative structure–activity relationship (QSAR) analysis.

For the cytotoxicity assay, both human normal oral cells (gingival fibroblast, HGF; pulp cells, periodontal ligament fibroblast, HPLF; pulp cell, HPC) and human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) were used as target cells. The anti-tumor potential was evaluated by the tumor-selectivity index (TS), calculated by dividing the mean 50% cytotoxic concentration (CC_{50}) against normal oral cells by that against OSCC cell lines. We have already confirmed that the TS value determined by this method reflects the anti-tumor potential of test samples, although these normal oral cells and OSCC cell lines are classified as different types of cells (mesenchymal or epithelial) (12).

This article is freely accessible online.

Correspondence to: Hiroshi Sakagami, Division of Pharmacology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan. Tel: +81 492792758, Fax: +81 492855171, e-mail: sakagami@dent.meikai.ac.jp

Key Words: 3-styrylchromones, QSAR analysis, cytotoxicity, tumor selectivity, anti-HIV activity.

For the anti-HIV assay, mock- and HIV-infected-human T-cell lymphotropic virus-I (HTLV-I) carrying human T-cell line MT4 was used. The selectivity index (SI) was calculated by dividing the CC_{50} by the 50% cytoprotective concentration from HIV infection (EC_{50}).

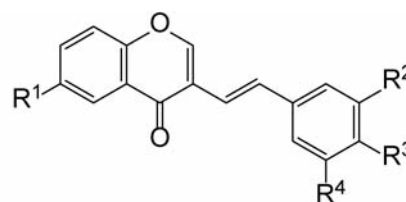
Materials and Methods

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), azidothymidine and 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; 5-fluorouracil (5-FU) from Kyowa, Tokyo, 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. (*E*)-3-(4-Methoxystyryl)-4*H*-chromen-4-one [1], (*E*)-3-(3,4-Dimethoxystyryl)-4*H*-chromen-4-one [2], (*E*)-3-(3,4,5-Trimethoxystyryl)-4*H*-chromen-4-one [3], (*E*)-6-Methoxy-3-(4-methoxystyryl)-4*H*-chromen-4-one [4], (*E*)-6-Methoxy-3-(3,4-dimethoxystyryl)-4*H*-chromen-4-one [5], (*E*)-6-Methoxy-3-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one [6], (*E*)-3-(4-Fluorostyryl)-6-methoxy-4*H*-chromen-4-one [7], (*E*)-3-(4-Chlorostyryl)-6-methoxy-4*H*-chromen-4-one [8], (*E*)-3-(4-Hydroxystyryl)-4*H*-chromen-4-one [9], (*E*)-3-(3,4-Dihydroxystyryl)-4*H*-chromen-4-one [10], (*E*)-3-(4-Hydroxystyryl)-6-methoxy-4*H*-chromen-4-one [11], (*E*)-3-(3,4-Dihydroxystyryl)-6-methoxy-4*H*-chromen-4-one [12], (*E*)-6-Hydroxy-3-(4-methoxystyryl)-4*H*-chromen-4-one [13], (*E*)-6-Hydroxy-3-(4-hydroxystyryl)-4*H*-chromen-4-one [14] and (*E*)-6-Hydroxy-3-(3,4-dihydroxystyryl)-4*H*-chromen-4-one [15] (Figure 1) were synthesized by Knoevenagel condensation of the appropriate 3-formylchromone with selected phenylacetic acid derivatives, according to previous methods (11). All compounds were dissolved in DMSO at 80 mM and stored at -20°C before use.

Cell culture. HGF, HPLF and HPC cells, established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl (13), and OSCC cell lines (Ca9-22, HSC-2, HSC-3, HSC-4), 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 $\mu\text{g}/\text{ml}$ streptomycin sulfate under a humidified 5% CO_2 atmosphere. Cells were then harvested by treatment with 0.25% trypsin-0.025% EDTA-2Na in PBS(-) and either subcultured or used for experiments.

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 hours, the medium was removed by suction with an aspirator and replaced with 0.1 ml of fresh medium containing different concentrations of single test compounds. Control cells were treated with the same amounts of DMSO present in each diluent solution. Cells were incubated for 48 hours and the relative viable cell number was then determined by the MTT method. In brief, the treated cells were incubated for another three hours in fresh culture medium containing 0.2 mg/ml MTT. Cells were then lysed



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

Figure 1. Structure of 3-styrylchromones.

with 0.1 ml of DMSO and the absorbance at 540 nm of the cell lysate was determined using a microplate reader (Biochromatic Labsystem, Helsinki, Finland). The CC_{50} was determined from the dose-response curve and the mean value of CC_{50} for each cell type was calculated from three independent experiments.

Calculation of TS. The tumor-selectivity index (TS) was calculated by the following equation: $TS = \text{mean } CC_{50} \text{ against normal cells} / \text{mean } CC_{50} \text{ against tumor cells}$ [(D/B) in Table I]. Since Ca9-22 cells were derived from gingival tissue (14), the relative sensitivity of Ca9-22 and HGF was also compared [(C/A) in Table I].

Assay for HIV activity. HTLV-I-carrying human T-cell line MT4 cells, highly sensitive to human immunodeficiency virus-1 (HIV-1), were infected with HIV-1_{IIIIB} at a multiplicity of infection (m.o.i.) of 0.01. HIV- and mock-infected (control) MT-4 cells were incubated for five days with different concentrations of samples and the relative viable cell number was determined by the MTT assay. The CC_{50} and EC_{50} were determined from the dose-response curve for mock-infected and HIV-infected cells, respectively (15). All data represent the mean values of triplicate measurements. The anti-HIV activity was evaluated by $SI (=CC_{50}/EC_{50})$.

Estimation of CC_{50} values. Original data contain the sign of inequality such as ">". For the convenience of analysis, these values were changed into forms suitable for arithmetic calculation. Since

Table I. Cytotoxic activity of fifteen 3-styrylchromones. Each value represents the mean of triplicate determinations.

3-Styrylchromones	CC ₅₀ (μM)										TS	
	Human oral squamous cell carcinoma cell lines					Human normal oral cells					(D)/(B)	(C)/(A)
	Ca9-22 (A)	HSC-2	HSC-3	HSC-4	Mean±S.D. (B)	HGF (C)	HPLF	HPC	Mean±SD			
1	183	94	200	80	139±61	643	533	475	550±85	4.0	3.5	
2	41	82	41	77	60±22	64	68	156	96±52	1.6	1.6	
3	263	300	278	310	288±21	560	518	715	598±104	2.1	2.1	
4	4.4	4.4	7.7	9.2	6.4±2.4	229	150	396	258±126	40.3	52.0	
5	33	92	36	61	56±27	98	83	171	117±47	2.1	3.0	
6	10	6.8	21	13	13±6.1	544	190	284	339±183	26.1	54.4	
7	43	57	58	38	49±10	140	126	97	121±22	2.5	3.3	
8	13	48	83	204	87±83	554	657	733	648±90	7.4	42.6	
9	23	25	14	28	23±6.0	457	520	600	526±72	22.9	19.9	
10	33	6.3	24	6.3	17±13	154	141	241	179±54	10.5	4.7	
11	2.1	1.0	3.6	1.2	2.0±1.2	67	74	272	138±116	69.0	31.9	
12	25	2.4	32	3.3	16±15	161	187	267	205±55	12.8	6.4	
13	>800	>800	240	>800	>660±280	>800	>800	>800	>800	><1.2	><1.0	
14	19	11	11	15	14±3.8	19	31	80	43±32	3.1	1.0	
15	33	9.1	22	7.5	18±12	247	222	507	325±158	18.1	7.4	
Positive control												
Doxorubicin	0.26	0.12	0.11	0.094	0.15±0.077	0.87	0.87	>10	>3.9±5.3	>26.0	3.3	
5-FU	29	13	16	13	18±7.6	>1000	>1000	>1000	>1000	>55.6	>34.5	

HGF, Human gingival fibroblast; HPC, pulp cells; HPLF, periodontal ligament fibroblast; Ca9-22, HSC-2, HSC-3 and HSC-4, oral squamous cell carcinoma cell lines; TS, tumor selectivity index; CC₅₀, 50% cytotoxic concentration; 5-FU, 5-fluorouracil.

">400" is equal to "from 400 to ∞", we calculated the harmonic mean as follows: $1/[\text{average}(1/400, 1/\infty)]=800$. Since the CC₅₀ values had a distribution pattern close to a logarithmic normal distribution, we used the pCC₅₀ (*i.e.*, the $-\log \text{CC}_{50}$) for the comparison of the cytotoxicity between the compounds. The mean pCC₅₀ values for normal cells and tumor cell lines were defined as N and T, respectively (16).

Calculation of the representative value for tumor selectivity. Tumor selectivity is defined by the balance between pCC₅₀ values for normal (N) and tumor (T) cells. The difference (T–N) was used as a tumor-selectivity index in the following analyses.

Calculation of chemical descriptors. Each chemical structure was optimized by the LowModeMD method (17), a suitable search method for minimum energy conformers of flexible molecules, with Merck Molecular Force Field (MMFF94x) in Molecular Operating Environment (MOE) 2013.08 (Chemical Computing Group Inc., Quebec, Canada). Each structure was refined with density functional theory (DFT-B3LYP/6-31G**) by using Spartan10 for Windows (Wavefunction, Inc., Irvine, CA, USA) (18). The descriptors used were: R1 OMe (methoxy substitution at the 6-position on the chromone ring group), R3 OH (4'-hydroxy substitution in the phenyl group of styryl moiety), vsurf_DD23 (the interaction with hydrophobic probe assumed surrounding the molecule), G1u (the first component symmetry directional WHIM index/unweighted encoding molecular symmetry that extracts the global symmetry

information), G2u (the second component symmetry directional WHIM index/unweighted encoding molecular symmetry that extracts the global symmetry information) (19), BCUT_SMR_3 (BCUT descriptor using atomic contribution to molar refractivity), rgvr (radius of gyration), diameter (largest value in the distance matrix defined by the elements D_{ij} , where D_{ij} is the length of the shortest path from atoms i to j). The BCUT descriptors are calculated from the eigenvalues of a modified adjacency matrix (20).

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 10.0.2 (SAS Institute Inc., Cary, NC, USA). The significance level was set at $p<0.05$.

Results

Cytotoxicity. Among fifteen 3-styrylchromone derivatives, compound [11] showed the highest cytotoxicity against four human OSCC cell lines (mean CC₅₀=2.0±1.2 μM) followed by [4] (6.4±2.4 μM) and [6] (13±6.1 μM) (Table I). Since these compounds showed much lower cytotoxicity against three human oral normal cells (mean CC₅₀=138±116, 258±126 and 339±183 μM, respectively), they showed the highest tumor-selectivity [TS (D/B)=69.0, 40.3 and 26.1, respectively], comparable with that of doxorubicin (TS=>26)

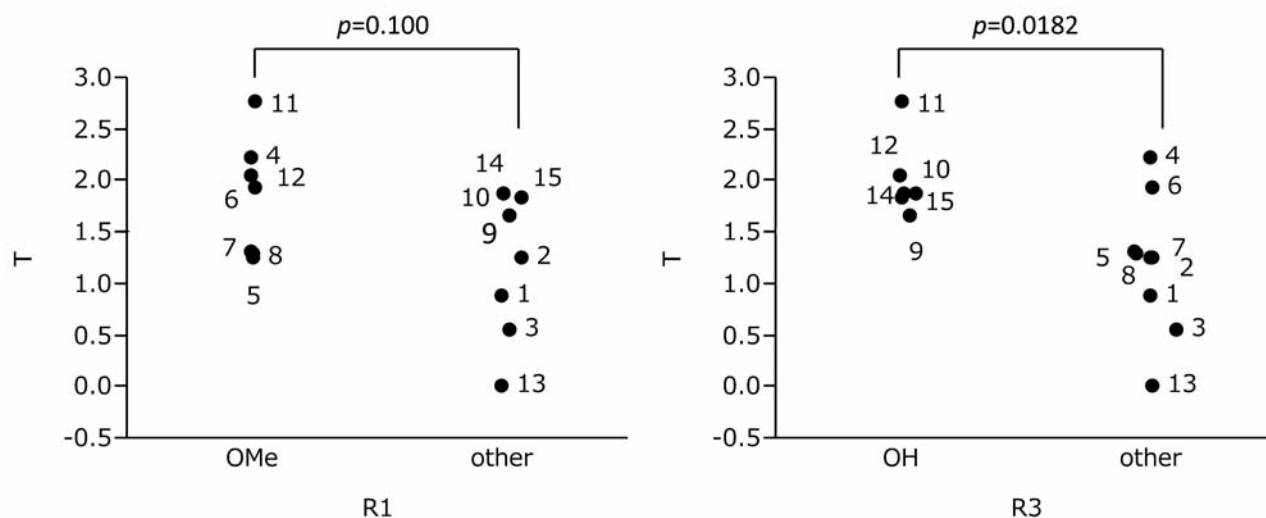


Figure 2. Effects of functional groups on cytotoxicity of 3-styrylchromones against tumor cells (defined as T). The mean (pCC_{50} i.e., the $-\log CC_{50}$) values for tumor cell lines were defined as T.

and 5-FU (TS=>55.6) (Table I). When tumor-selectivity was calculated by different equation using Ca9-22 and HGF cells, both derived from gingival tissues ([TS(C/A)], these three compounds again showed very high tumor-selectivity (31.9, 52.0 and 54.4, respectively) (Table I).

Anti-HIV activity. In contrast to popular anti-HIV agents (dextran sulfate, curdlan sulfate, azidothymidine, 2',3'-dideoxycytidine) (SI=2445-20421), none of 3-styrylchromones **1-15** protected the cells from the cytopathic effect of HIV infection (SI<1) (Table II). Based on these data, the following QASR analysis was focused on the cytotoxicity of 3-styrylchromones.

Computational analysis. Cytotoxicity of 3-styrylchromones against tumor cells (defined by T) correlated with descriptors based on the substituent group. R¹ OMe (methoxy substitution at the 6-position on the chromone ring) ($p=0.100$) and R³ OH (4'-hydroxy substitution in the phenyl group of styryl moiety) ($p=0.0182$) are crucial for the enhancement of cytotoxicity against OSCC cell lines (Figure 2).

On the other hand, cytotoxicity of 3-styrylchromones against normal cells (defined by N) was correlated with vsurf_DD23 (the interaction with hydrophobic probe assumed surrounding the molecule) ($r^2=0.413$, $p=0.0078$) and G1u (the first component symmetry directional WHIM index/unweighted encoding molecular symmetry that extracts the global symmetry information) ($r^2=0.445$, $p=0.0066$) (Figure 3).

Tumor selectivity of 3-styrylchromones (defined by T-N) correlated with R³ OH ($p=0.0616$), vsurf_DD23 ($r^2=0.280$, $p=0.0426$) and G2u (the second component symmetry directional WHIM index/unweighted encoding molecular

Table II. Anti-HIV activity of 3-styrylchromones and chemotherapeutic agents. Each value represents the mean of triplicate determinations.

3-Styrylchromones	CC ₅₀ (μM)	EC ₅₀ (μM)	SI
1	528.45	>800	<1
2	463.38	>800	<1
3	629.23	>800	<1
4	5.87	>800	<1
5	>800	>800	>>1
6	3.39	>800	<1
7	435.71	>800	<1
8	270.56	>800	<1
9	13.76	>800	<1
10	12.92	>800	<1
11	3.26	>800	<1
12	59.91	>800	<1
13	223.28	>800	<1
14	3.59	>800	<1
15	4.76	>800	<1
Positive controls			
Dextran sulfate (μg/ml)	985.6	0.06	17125
Curdlan sulfate (μg/ml)	>1000	0.14	>7002
Azidothymidine	222.2	0.011	20421
2',3'-Dideoxycytidine	2511.59	1.03	2445

CC₅₀, 50% Cytotoxic concentration; EC₅₀, 50% effective concentration; SI: selectivity index (CC₅₀/EC₅₀).

symmetry that extracts the global symmetry information) ($r^2=0.406$, $p=0.0106$) (Figure 4).

A model for the estimation of T, N and T-N values was constructed by multiple regression analysis using the descriptors calculated in this study. T can be estimated by

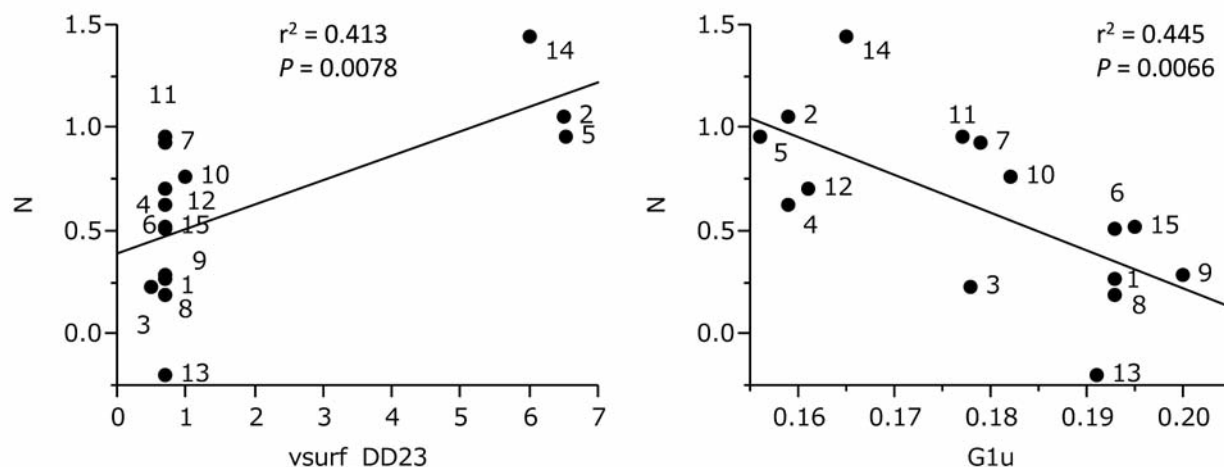


Figure 3. Determination coefficient of chemical descriptors and cytotoxicity of 3-styrylchromones against normal cells (defined as *N*). The mean (pCC_{50} i.e., the $-\log CC_{50}$) values for normal cells were defined as *N*.

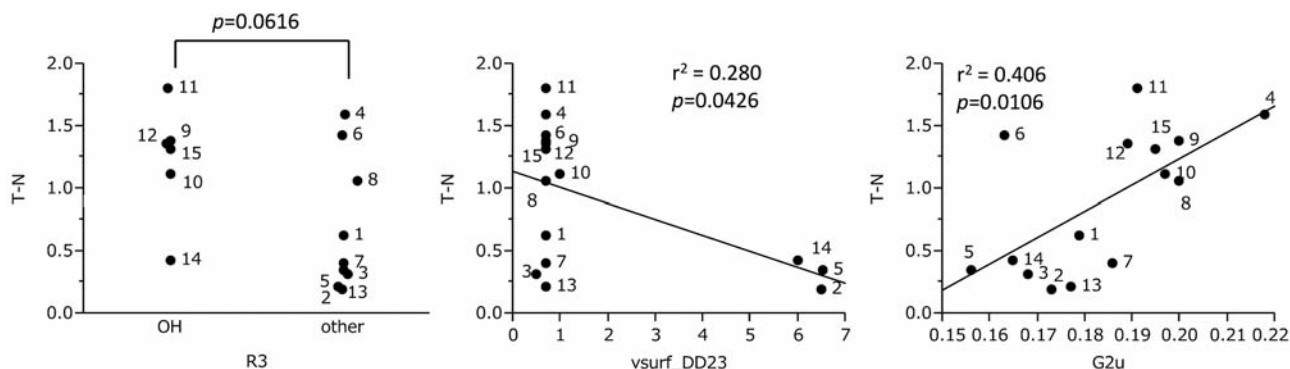
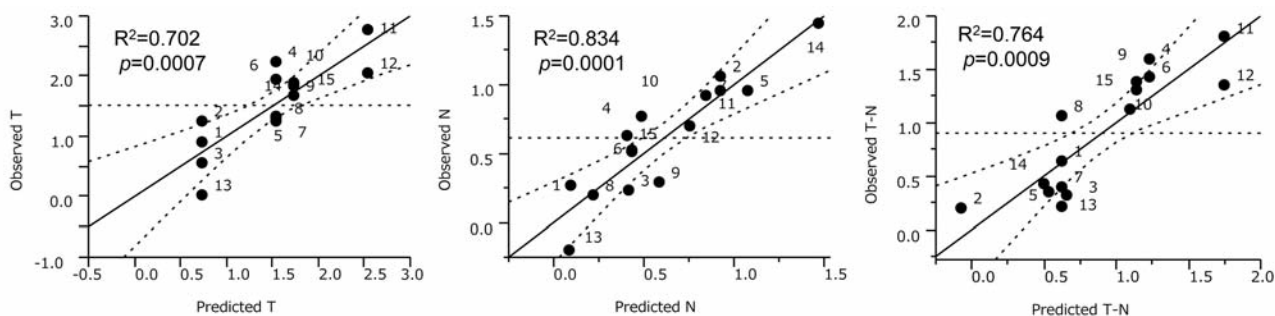


Figure 4. Determination coefficient of chemical descriptors and tumor specificity of 3-styrylchromones (defined as *T-N*).



$$T = 0.806(\pm 0.182)R1Ome + 0.999(\pm 0.221)R3OH + 0.739(\pm 0.182), n=15, R^2=0.702, Q^2=0.532, s=0.409$$

$$N = 120(\pm 24)BCUT_SMR_3 - 2.49(\pm 0.49)rgyr + 0.166(\pm 0.024)vsurf_DD23 - 304(\pm 62), n=15, R^2=0.834, Q^2=0.695, s=0.194$$

$$T - N = 0.607(\pm 0.169)diameter - 0.121(\pm 0.035)vsurf_DD23 + 1.11(\pm 0.235)R3OH - 7.17(\pm 2.26), n=15, R^2=0.764, Q^2=0.570, s=0.308$$

Figure 5. Multiple regression models for the estimation of *T*, *N* and *T-N*.

R^1 OMe and R^3 OH ($r^2=0.702$, $Q^2=0.532$, $s=0.409$) (left). N can be estimated by BCUT_SMR_3, rgyr (radius of gyration) and vsurf_DD23 ($R^2=0.834$, $Q^2=0.695$, $s=0.194$) (left). T-N can be estimated by diameter (largest value in the distance matrix defined by the elements D_{ij}), vsurf_DD23 and R^3 OH ($R^2=0.764$, $Q^2=0.570$, $s=0.308$) (right) (Figure 5).

Discussion

The present study demonstrated for the first time that fifteen 3-styrylchromones showed moderate-to-potent tumor-specificity and no detectable anti-HIV activity (Table I). Three compounds [4, 6, 11] showed the highest tumor-selectivity, comparable to doxorubicin and 5-FU (Table I). The lack of anti-HIV activity is not in agreement with a previous study where 4*H*-chromen-4-one and 2*H*-chromene derivatives inhibited the replication of picornavirus in vitro (9). The discrepancy between our and their results may be due to the fact that they did not produce the chemotherapeutic index (safety margin).

The present study is the first report of QSAR analysis of 3-styrylchromones. We used the following soft Spartan (34 quantum chemistry-related descriptors), MOE (330 quantum chemistry, structure and physical chemistry-related descriptors), dragon (114 three-dimensional molecular shape-related descriptors) and 9 descriptors (that relate the location of substituent groups and types). The variables used are T, N and T-N. Descriptors that showed significant difference or significant trends ($p \leq 0.1$) were R^1 OMe (methoxy substitution at the 6-position on the chromone ring), R^3 OH (4'-hydroxy substitution in the phenyl group of styryl moiety), vsurf_DD23, G1u and G2u.

Van der Waals interaction through the methoxy substitution at the 6-position and the hydrogen bonding through the 4'-hydroxy substitution may be important for the interaction with key proteins engaged in the cytotoxicity against OSCC cells (Figure 2). Hydrophobic interaction and molecular shape may be important for the cytotoxicity against normal cells (Figure 3). Molecular shape and 4'-hydroxy substitution in the phenyl group of styryl moiety affected the tumor-selectivity (Figure 4).

For the multiple regression analysis model, the following descriptors were used: BCUT_SMR_3 (that relates to the topological shape of molecule and the interaction between the low molecular weight molecule and protein interaction), rgyr (that relates to the molecular size and shape) and diameter (that reflects the topological size of molecule). Leave-one-out cross verification method confirmed that cytotoxicity against OSCC cells (T), oral normal cells (N) and tumor-specificity (T-N) can be estimated by the combination of descriptors for substituent groups, molecular shape and hydrophobic interaction (Figure 5).

We have previously reported that 2-styrylchromone derivatives having methoxy group in the phenyl group of styryl moiety showed relatively higher tumor-selectivity and induced apoptosis in the human promyelocytic leukemia HL-60 cell line and non-apoptosis in the human oral squamous cell carcinoma HSC-2 cell line (4). In contrast, tumor-selectivity of 3-styrylchromones was significantly enhanced by the introduction of methoxy group on the chromone ring and hydroxyl group in the phenyl group of styryl moiety. It remains to be investigated which type of cell death is induced by 3-styrylchromones in OSCC cell lines.

In conclusion, the present study demonstrated that 3-styrylchromones substituted with methoxy group on the chromone ring and hydroxy group in the phenyl group of styryl moiety are promising for the exploration of new anti-tumor agents. Multivariate statistics with chemical descriptors for the location of substituted group, molecular shape and electrostatic interaction may be useful for designing the most favorable compound with higher tumor selectivity.

References

- Gomes A, Fernandes E, Silva AMS, Santos CMM, Pinto DCGA, Cavaleiro JAS and Lima JLFC: 2-Styrylchromones: novel strong scavengers of reactive oxygen and nitrogen species. *Bioorg Med Chem* 15: 6027-6036, 2007.
- Doria G, Romeo C, Forgione A, Sberze P, Tibolla N, Corno ML, Cruzzola G and Cadelli G: Antiallergic agents. III. Substituted trans-2-ethenyl-4-oxo-4*H*-benzopyran-6-carboxylic acids. *Eur J Med Chem* 14: 347-351, 1979.
- Gomes A, Fernandes E, Silva AMS, Pinto DCGA, Santos CMM, Cavaleiro JAS and Lima JLFC: Anti-inflammatory potential of 2-styrylchromones regarding their interference with arachidonic acid metabolic pathways. *Biochem Pharmacol* 78: 171-177, 2009.
- Momoi K, Sugita Y, Ishihara M, Satoh K, Kikuchi H, Hashimoto K, Yokoe I, Nishikawa H, Fujisawa S and Sakagami H: Cytotoxic activity of styrylchromones against human tumor cell lines. *In Vivo* 19: 157-163, 2005.
- Marinho J, Pedro M, Pinto DCGA, Silva AMS, Cavaleiro JAS, Sunkel CE and Nascimento MSJ: 4'-Methoxy-2-styrylchromone a novel microtubule-stabilizing antimitotic agent. *Biochem Pharmacol* 75: 826-835, 2008.
- Shaw AY, Chang CY, Liao HH, Lu PJ, Chen HL, Yang CN and Li HY: Synthesis of 2-styrylchromones as a novel class of antiproliferative agents targeting carcinoma cells. *Eur J Med Chem* 44: 2552-2562, 2009.
- Desideri N, Mastromarino P and Conti C: Synthesis and evaluation of antirhinovirus activity of 3-hydroxy and 3-methoxy 2-styrylchromones. *Antivir Chem Chemother* 14: 195-203, 2003.
- Conti C, Mastromarino P, Goldoni P, Portalone G and Desideri N: Synthesis and anti-rhinovirus properties of fluoro-substituted flavonoids. *Antivir Chem Chemother* 16: 267-276, 2005.
- Conti C and Desideri N: New 4*H*-Chromen-4-one and 2*H*-chromene derivatives as anti-picornavirus capsid-binders. *Bioorg Med Chem* 18: 6480-6488, 2010.

- 10 Silva VLM, Silva AMS, Pinto DCGA, Cavaleiro JAS, Vasas A and Patonay T: Synthesis of (E)- and (Z)-3-styrylchromones. *Monatsh Chem* 139: 1307-1315, 2008.
- 11 Takao K, Ishikawa R and Sugita Y: Synthesis and biological evaluation of 3-styrylchromone derivatives as free radical scavengers and α -glucosidase inhibitors. *Chem Pharm Bul* 62: 810-815, 2014.
- 12 Suzuki R, Matsuo S, Sakagami H, Okada Y and Shirataki Y: Search of new cytotoxic crude materials against human oral squamous cell carcinoma using ¹H NMR-based metabolomics. *Anticancer Res* 34: 4117-4120, 2014.
- 13 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.
- 14 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 (in Japanese).
- 15 Nakashima H, Murakami T, Yamamoto N, Sakagami H, Tanuma S, Hatano T, Yoshida T and Okuda T: Inhibition of human immunodeficiency viral replication by tannins and related compounds. *Antiviral Res* 18: 91-103, 1992.
- 16 Ohno H, Araho D, Uesawa Y, Kagaya H, Ishihara M, Sakagami H and Yamamoto M: Evaluation of cytotoxicity and tumor specificity of licorice flavonoids based on chemical structures. *Anticancer Res* 33: 3061-3068, 2013.
- 17 Labute P: LowModeMD—implicit low-mode velocity filtering applied to conformational search of macrocycles and protein loops. *J Chem Inf Model* 50: 792-800, 2010.
- 18 <http://www.computational-chemistry.co.uk/spartan10.html>
- 19 Todeschini R and Gramatica P: The WHIM THEORY: New 3D-molecular descriptors for QSAR in environmental modeling. *SAR QSAR Environ Res* 7: 89-115, 1997.
- 20 Pearlman RS and Smith KM: Metric validation and the receptor-relevant subspace concept. *J Chem Inf Comput Sci* 39: 28-35, 1999.

Received June 9, 2014

Revised July 16, 2014

Accepted July 17, 2014