# Quantitative Structure–Cytotoxicity Relationship of Furo[2,3-*b*]chromones

YOSHIHIRO UESAWA[1], HIROSHI SAKAGAMI[2], HAIXIA SHI[2,3],
MARIA HIROSE[4], KOICHI TAKAO[4] and YOSHIAKI SUGITA[4]

[1]*Department of Medical Molecular Informatics, Meiji Pharmaceutical University, Tokyo, Japan;*
[2]*Meikai University Research Institute of Odontology (M-RIO),*
*Mekai University School of Dentistry, Saitama, Japan;*
[3]*Department of Traditional Chinese Medicine, Shanghai Ninth People's Hospital,*
*Shanghai Jiatong University School of Medicine, Shanghai, P.R. China;*
[4]*Department of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences,*
*Josai University, Saitama, Japan*

**Abstract.** *Background/Aim: The furo[2,3-b]chromone derivatives are natural products that have been used as folklore medicines for various diseases. Here, the cytotoxicity of 12 synthesized furo[2,3-b]chromone derivatives was investigated and subjected to quantitative structure–activity relationship (QSAR) analysis. Materials and Methods: Cytotoxicity against three human oral squamous cell carcinoma cell lines and three types of 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 as the ratio of the mean 50% cytotoxic concentration (CC$_{50}$) against normal oral cells to that against carcinoma cell lines. Potency-selectivity expression (PSE) was calculated by dividing the TS value by CC$_{50}$ against tumor cells. Apoptosis induction was evaluated by cell morphology and caspase-3 activation. Morphological changes were monitored under light microscopy. For QSAR analysis, 288 physicochemical, structural and quantum chemical features were calculated from the most stabilized structure optimized using Corina. Results: Four furo[2,3-b]chromone derivatives showed relatively strong tumor selectivity. In particular, the derivatives with phenylethenyl and methoxy groups showed the highest TS, equivalent to that of melphalan, although their PSE values did not reach those of*

*doxorubicin and 5-fluorouracil. Microscopical observation demonstrated that at cytotoxic concentrations, (2R,3aR,9aR)-rac-3a,9a-dihydro-7-methoxy-4-oxo-2-(2-phenylethenyl)-4H-furo[2,3-b][1]benzopyran-3,3(2H)-dicarboxylic acid 3,3-dimethyl ester and (2R,3aR,9aR)-rac-3a,9a-dihydro-7-methoxy-4-oxo-2-(1-propen-1-yl)-4H-furo[2,3-b][1]benzopyran-3,3(2H)-dicarboxylic acid 3,3-dimethyl ester did not produce a population of shrunken cells typical of apoptotic cells, in contrast to cells treated with actinomycin D. Tumor selectivity of furo[2,3-b]chromone derivatives strongly correlated with features related to the number of intramolecular unsaturated bonds, molecular flexibility, molecular density, lipophilicity, molecular size, and molecular shape. Conclusion: Chemical modification of the lead compound may be a potential choice for designing a new type of anticancer drug.*

Many furochromone derivatives have been isolated from *Ammi visnaga* (Umbelliferae), used in folk medicine for different ailments. However, there are very few documentations of their synthetic methodologies and biological activities (1). Sesquiterpene chromones isolated from *Ferula fukanensis* inhibited nitirc oxide (NO) production in lipopolysaccharide and interferon-γ-stimulated Raw264.7 mouse macrophage-like cells, through inhibition of inducible NO synthase gene expression (2). Sesquiterpenoids isolated from the roots of *Ferula mongolica* were identified as new class of α-glucosidase inhibitors (3). Four oxepinochromenones (conioxepinols A-D) and one furochromenone (coniofurol A), from an organic solvent extract of solid-substrate fermentation culture of fungus *Coniochaeta* sp. (*Coniochaetaceae*) (4), and three sesquiterpene coumarin derivatives and two sesquiterpene chromone derivatives (5) showed moderate cytotoxicity against several human tumor cell lines. However, these previous studies did not investigate cytotoxicity against normal control cells.

*This article is freely accessible online.*

*Correspondence to:* Yoshihiro Uesawa, Department of Medical Molecular Informatics, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-858, Japan. Tel/Fax: +81 424958983, e-mail: uesawa@my-pharm.ac.jp

*Key Words:* Furo[2,3-*b*]chromones, QSAR analysis, cytotoxicity, tumor selectivity, apoptosis induction.

We established a simple *in vitro* assay system for assessing antitumor potential using human oral squamous cell carcinoma (OSCC) cell lines and human normal oral cells (6, 7). Using this system, we have demonstrated that many anticancer drugs showed excellent tumor specificity (8). Among a total of 133 compounds, (*E*)-3-[2-(4-hydroxyphenyl)ethenyl]-6-methoxy-4*H*-1-benzopyran-4-one (classified as 3-styrylchromone) (9, 10), (*E*)-3-[2-(4-chlorophenyl)ethenyl]-7-methoxy-2*H*-1-benzo-pyran (classified as 3-styryl-2*H*-chromenes) (11) showed the highest tumor specificity with the least keratinocyte toxicity (7).

In continuation of the discovery of new antitumor compounds, here 12 furo[2,3-*b*]chromones (Figure 1) (12) were investigated for their cytotoxicity against three OSCC cell lines and three types of human normal oral cells, and then subjected to quantitative structure–activity relationship (QSAR) analysis.

## 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.* (3a*R*,9a*R*)-Rac-3a,9a-dihydro-2,2-dimethyl-4-oxo-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**1a**), (3a*R*,9a*R*)-rac-3a,9a-dihydro-7-methoxy-2,2-dimethyl-4-oxo-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**1b**), (3a*R*,9a*R*)-rac-3'a,9'a-dihydro-4'-oxo-spiro[cyclopentane-1,2'(3'*H*)-[4*H*]furo[2,3-*b*][1]benzopyran]-3',3'-dicarboxylic acid 3',3'-dimethyl ester (**2a**), (3a*R*,9a*R*)-rac-3'a,9'a-dihydro-7-methoxy-4'-oxo-spiro[cyclopentane-1,2'(3'*H*)-[4*H*]furo[2,3-*b*][1]benzopyran]-3',3'-dicarboxylic acid 3',3'-dimethyl ester (**2b**), (3a*R*,9a*R*)-rac-3'a,9'a-dihydro-4'-oxo-spiro[cyclohexane-1,2'(3'*H*)-[4*H*]furo[2,3-*b*][1]benzopyran]-3',3'-dicarboxylic acid 3',3'-dimethyl ester (**3a**), (3a*R*,9a*R*)-rac-3'a,9'a-dihydro-7-methoxy-4'-oxo-spiro[cyclohexane-1,2'(3'*H*)-[4*H*]furo[2,3-*b*][1]benzopyran]-3',3'-dicarboxylic acid 3',3'-dimethyl ester (**3b**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-4-oxo-2-phenyl-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**4a**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-7-methoxy-4-oxo-2-phenyl-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**4b**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-4-oxo-2-(2-phenylethenyl)-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**5a**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-7-methoxy-4-oxo-2-(2-phenylethenyl)-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**5b**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-4-oxo-2-(1-propen-1-yl)-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**6a**), (2*R*,3a*R*,9a*R*)-rac-3a,9a-dihydro-7-methoxy-4-oxo-2-(1-propen-1-yl)-4*H*-furo[2,3-*b*][1]benzopyran-3,3(2*H*)-dicarboxylic acid 3,3-dimethyl ester (**6b**) were synthesized by the ring-expansion reactions of methanochromanones with selected aldehydes or ketones, according to previous methods (12). All compounds were dissolved in DMSO at 40 mM and stored at –20˚C before use.
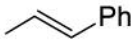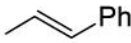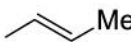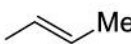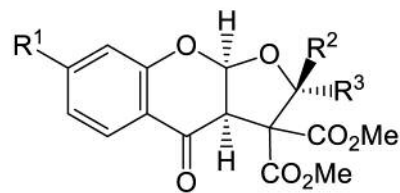


| Compound | R$^1$ | R$^2$ | R$^3$ |
|---|---|---|---|
| **1a** | H | Me | Me |
| **1b** | OMe | Me | Me |
| **2a** | H | —(CH$_2$)$_4$— | |
| **2b** | OMe | —(CH$_2$)$_4$— | |
| **3a** | H | —(CH$_2$)$_5$— | |
| **3b** | OMe | —(CH$_2$)$_5$— | |
| **4a** | H | H | Ph |
| **4b** | OMe | H | Ph |
| **5a** | H | H | Ph |
| **5b** | OMe | H | Ph |
| **6a** | H | H | Me |
| **6b** | OMe | H | Me |

Figure 1. *Structure of 12 furo[2,3-b]chromones.*

*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 (13), and cells at 10-18 population-doubling levels were used in this study. Human OSCC cell lines [Ca9-22 (derived from gingival tissue); HSC-2, 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. Cell morphology was checked periodically under the light microscope (EVOSfl; ThermoFisher Scientific, Waltham, MA, USA).

*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 (8-11). The relative viable cell number was determined by the absorbance of the cell lysate at 560 nm using a microplate reader (Infinite F50R; TECAN, Kawasaki,

Table I. *Cytotoxic activity of 12 furo[2,3-b]chromones against oral malignant and non-malignant cells. Each value represents the mean of triplicate determinations. Two sets of tumor-specificity (TS) and potency-selectivity expression (PSE) values were determined using all malignant and non-malignant cells, or the pair of the cells derived from the same (gingival) tissue.*

| | CC$_{50}$ (μM) | | | | | | | | | | TS | | PSE | |
| | Human oral squamous cell carcinoma | | | | | Human normal oral cells | | | | | | | | |
| Compound | Ca9-22 (A) | HSC-3 | HSC-4 | Mean (B) | SD | HGF (C) | HPLF | HPC | Mean (D) | SD | (D/B) | (C/A) | (D/B$^2$)×100 | (C/A$^2$)×100 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1a** | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 1.0 | 1.0 | 0.3 | 0.3 |
| **1b** | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 1.0 | 1.0 | 0.3 | 0.3 |
| **2a** | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 1.0 | 1.0 | 0.3 | 0.3 |
| **2b** | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 1.0 | 1.0 | 0.3 | 0.3 |
| **3a** | 369.0 | 400.0 | 400.0 | 389.7 | 17.9 | 393.7 | 400.0 | 400.0 | 397.9 | 3.7 | 1.0 | 1.1 | 0.3 | 0.3 |
| **3b** | 307.7 | 303.0 | 264.7 | 291.8 | 23.6 | 347.7 | 400.0 | 400.0 | 382.6 | 30.2 | 1.3 | 1.1 | 0.4 | 0.4 |
| **4a** | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 398.0 | 400.0 | 400.0 | 399.3 | 1.2 | 1.0 | 1.0 | 0.2 | 0.2 |
| **4b** | 363.3 | 400.0 | 400.0 | 387.8 | 21.2 | 400.0 | 400.0 | 400.0 | 400.0 | 0.0 | 1.0 | 1.1 | 0.3 | 0.3 |
| **5a** | 14.1 | 27.1 | 81.6 | 40.9 | 35.8 | 141.3 | 155.7 | 147.3 | 148.1 | 7.2 | 3.6 | 10.0 | 8.8 | 70.8 |
| **5b** | 13.8 | 27.7 | 70.1 | 37.2 | 29.3 | 185.3 | 273.3 | 324.0 | 260.9 | 70.2 | 7.0 | 13.5 | 18.9 | 98.0 |
| **6a** | 19.7 | 62.1 | 66.1 | 49.3 | 25.7 | 142.7 | 282.7 | 294.7 | 240.0 | 84.5 | 4.9 | 7.2 | 9.9 | 36.8 |
| **6b** | 16.3 | 50.1 | 54.5 | 40.3 | 20.9 | 141.0 | 269.3 | 329.7 | 246.7 | 96.4 | 6.1 | 8.7 | 15.2 | 53.1 |
| 5-FU | 28.1 | 12.5 | 7.8 | 16.1 | 10.6 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 0.0 | 61.9 | 35.6 | 383.7 | 126.6 |
| Melphalan | 22.8 | 10.1 | 11.2 | 14.7 | 7.1 | 130.3 | 196.0 | 153.3 | 159.9 | 33.3 | 10.9 | 5.7 | 74.0 | 25.0 |
| DXR | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 10.0 | 9.9 | 9.0 | 9.6 | 0.5 | 66.0 | 44.8 | 45107.7 | 20049.0 |

HGF: Human gingival fibroblast; HPLF: human periodontal ligament fibroblast; Ca9-22 (derived from gingival tissue), HSC-3, HSC-4 (derived from tongue): oral squamous cell carcinoma (OSCC) cell lines; CC$_{50}$: 50% cytotoxic concentration; DXR: doxorubicin; 5-FU: 5-fluorouracil; TS: tumor-selectivity index; PSE: potency-selectivity expression; CC$_{50}$: 50% cytotoxic concentration.

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 types of normal oral cells/mean CC$_{50}$ against for OSCC cell lines [(D/B) in Table I]. Since both Ca9-22 and HGF cells were derived from the gingival tissue (14), 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 (8).

*Calculation of potency-selectivity expression (PSE).* PSE was calculated by the following equation: PSE=TS/CC$_{50}$ against tumor cells ×100 [that is, (D/B$^2$) ×100 (HGF, HPLF, HSC *vs*. Ca9-22, HSC-3, HSC-4) using all non-malignant and malignant cells, and (C/A$^2$) ×100 (HGF *vs*. Ca9-22) using the pair of the cells from the same tissue (gingiva) (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 (11).

*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, Nürnberg, Germany) with partial charge 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 288 and 2532, respectively.

Seven MOE descriptors listed in Table II were significantly correlated with T, N and T-N. Since the use of Dragon descriptors produced heavy overlapping of similar descriptors that showed higher correlation with T, N and T−N, and therefore correlation diagrams were plotted using MOE descriptors.

*Statistical treatment.* The relationships among cytotoxicity. TS 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 12 furo[2,3-*b*]chromones were synthesized, without (a series) or with (b series) introduction of methoxy group at C-7 of benzopyran ring (Figure 1). Introduction of dimethyl (**1a, 1b**), spirocyclopentyl (**2a, 2b**), spirocyclohexyl (**3a, 3b**) or phenyl group (**4a, 4b**) at C-2

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

| Descriptor | Explanation |
|---|---|
| b_double | Number of double bonds. Aromatic bonds are not considered to be double bonds. |
| b_rotR | Fraction of rotatable bonds. |
| dens | Mass density: molecular weight divided by van der Waal's volume. |
| E_str | Bond stretch potential energy. |
| rsynth | The synthetic reasonableness, or feasibility, of the chemical structure. |
| SlogP_VSA2 | Sum of approximate accessible van der Waal's surface area i such that logP for atom i is from – 0.2 to 0. |
| std_dim3 | Standard dimension 3: the square root of the third largest eigenvalue of the covariance matrix of the atomic coordinates. |

position showed essentially no cytotoxic activity against human oral carcinoma cell lines (mean $CC_{50}$=291.8-400 μM) and normal oral cells mean $CC_{50}$=382.6-400 μM). On the other hand, addition of phenylethenyl (**5a, 5b**) or propenyl (**6a, 6b**) group significantly enhanced the cytotoxicity for both oral carcinoma cell lines (mean $CC_{50}$=37.2-49.3 μM) and normal oral cells (mean $CC_{50}$=148.1-260.9 μM) (Table I). Representative dose-response curves of compounds (**5b, 6b**) are shown in Figure 2A, which demonstrated that these compounds were cytotoxic rather than cytostatic.

*Tumor specificity.* TS vales were calculated by dividing the mean $CC_{50}$ value for three types of normal cells by that for four OSCC cell lines (D/B, Table I). Compounds **1a, 1b, 2a, 2b, 3a, 3b, 4a** and **4b** showed very low tumor specificity (TS=1.0-1.3). On the other hand, compounds **5a, 5b, 6a** and **6b** showed much higher tumor specificity (TS=3.6-7.0). In particular, compound **5b** (TS=7.0) showed comparable tumor specificity with melphalan (TS=10.9) (Table I).

Considering that HGF is the normal cell type corresponding to cancer cell Ca9-22 (both derived from gingival tissues), TS values were also generated by dividing the average $CC_{50}$ value for HGF cells by that for Ca9-22 cells (C/A, Table I). Compounds **5a, 5b, 6a** and **6b** (TS=7.2-13.5) showed slightly higher tumor specificity than melphalan (TS=5.7). Compound **5b** had the highest TS value (13.5) (Table I).

*PSE value.* In order to identify the most promising compounds in terms of both good potency and selective cytotoxicity, PSE values were calculated. Compounds **5a, 5b, 6a** and **6b** had much higher PSE values than compounds **1a, 1b, 2a, 2b, 3a, 3b, 4a** and **4b**, while that for **5b** gave comparable PSE value with melphalan (Table I).

Compounds **5b** and **6b** at the cytotoxic concentration (30 and 60 μM) produced spread, round cells, but failed to produce a population of shrunken cells, in contrast to actinomycin D-treated apoptotic cells (Figure 2B). Western blot analysis failed to detect cleaved products of poly (ADP-ribose) polymerase (PARP) and caspase-3, in contrast to actinomycin D (data not shown).

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

| | Descriptor | Meaning | $R^2$ | $p$ (Prob>F) |
|---|---|---|---|---|
| T | b_double | Double bond | 0.989 | <0.0001 |
| T | SlogP_VSA2 | Size and lipophilicity | 0.989 | <0.0001 |
| T | rsynth | Synthetic feasibility | 0.930 | <0.0001 |
| T | std_dim3 | Shape | 0.866 | <0.0001 |
| T | E_str | Bond stretch | 0.633 | 0.002 |
| T | dens | Density | 0.622 | 0.0023 |
| N | rsynth | Synthetic feasibility | 0.895 | <0.0001 |
| N | b_double | Double bond | 0.857 | <0.0001 |
| N | SlogP_VSA2 | Size and lipophilicity | 0.857 | <0.0001 |
| N | std_dim3 | Shape | 0.792 | 0.0001 |
| N | E_str | Bond stretch | 0.626 | 0.0022 |
| N | dens | Density | 0.622 | 0.0023 |
| T-N | b_double | Double bond | 0.969 | <0.0001 |
| T-N | SlogP_VSA2 | Size and lipophilicity | 0.969 | <0.0001 |
| T-N | rsynth | Synthetic feasibility | 0.876 | <0.0001 |
| T-N | std_dim3 | Shape | 0.832 | <0.0001 |
| T-N | b_rotR | Flexibility | 0.606 | 0.0029 |
| T-N | E_str | Bond stretch | 0.590 | 0.0035 |

*Computational analysis.* We next performed the QSAR analysis of the 12 furo[2,3-*b*]chromones in regards to their cytotoxicity against tumor cells and normal cells. Among a total of 288 MOE descriptors, seven descriptors correlated well with cytotoxicity and tumor specificity (Table III).

Cytotoxicity of the 12 furo[2,3-*b*]chromones against human OSCC cell lines was correlated positively with double bond (b_double; $r^2$=0.989, $p$<0.0001), size and lipophilicity (SlogP_VSA2; $r^2$=0.989, $p$<0.0001) and bond stretch (E_str; $r^2$=0.633, $p$=0.002), while negatively with synthetic feasibility (r synth; $r^2$=0.930, $p$<0.0001), shape (std_dim3; $r^2$=0.866, $p$<0.0001) and density (dens; $r^2$=0.622, $p$=0.0023) (Figure 3).

Cytotoxicity of the 12 furo[2,3-*b*]chromones against human normal oral mesenchymal cells was correlated positively with b_double ($r^2$=0.857, $p$<0.0001), SlogP_VSA2 ($r^2$=0.857, $p$<0.0001) and E_str ($r^2$=0.626, $p$=0.0022), while negatively
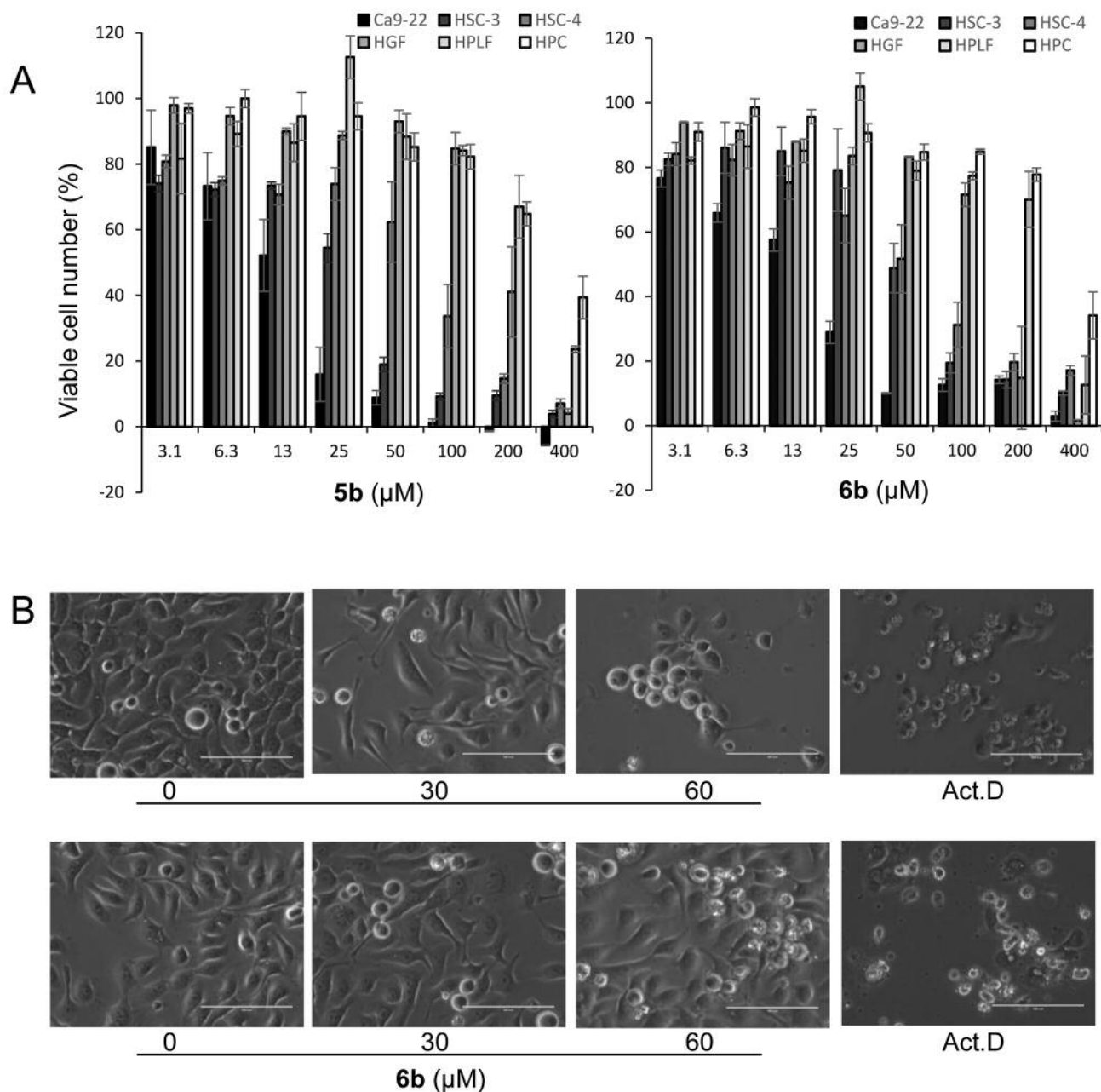
Figure 2. *Dose–response for loss of cellular viability (A) and morphological changes (B) induced by compounds **5b** and **6b**. A: Ca9-22, HSC-3, HSC-4, human gingival fibroblast (HGF), human periodontal ligament fibroblast (HPLF) and human pulp cell (HPC) were incubated for 48 h with the indicated concentrations of compound **5b** or **6b**, and cell viability was determined by the MTT method. Each value represents the mean±S.D. of triplicate assays. B: Ca9-22 cells were incubated for 24 h without or with compound **5b** or **6b** (30 or 60 μM ) or 1 μM actinomycin D (Act. D, positive control), and then assessed for morphology under light microscopy (EVOSFL; ThermoFisher Scientific, Waltham, Mass, USA). Bar=100 μm.*

with rsynth ($r^2$=0.895, $p$<0.0001), std_dim3 ($r^2$=0.792, $p$=0.0001) and density ($r^2$=0.622, $p$=0.0023) (Figure 4).

TS of the 12 furo[2,3-*b*]chromones was correlated positively with b_double ($r^2$=0.969, $p$<0.0001), SlogP_VSA2 ($r^2$=0.969, $p$<0.0001), flexibility (b_rotR; $r^2$=0.606, $p$=0.0029) and E_str ($r^2$=0.590, $p$=0.0035), while negatively with rsynth ($r^2$=0.876, $p$<0.0001) and std_dim3 ($r^2$=0.832, $p$<0.0001) (Figure 5).
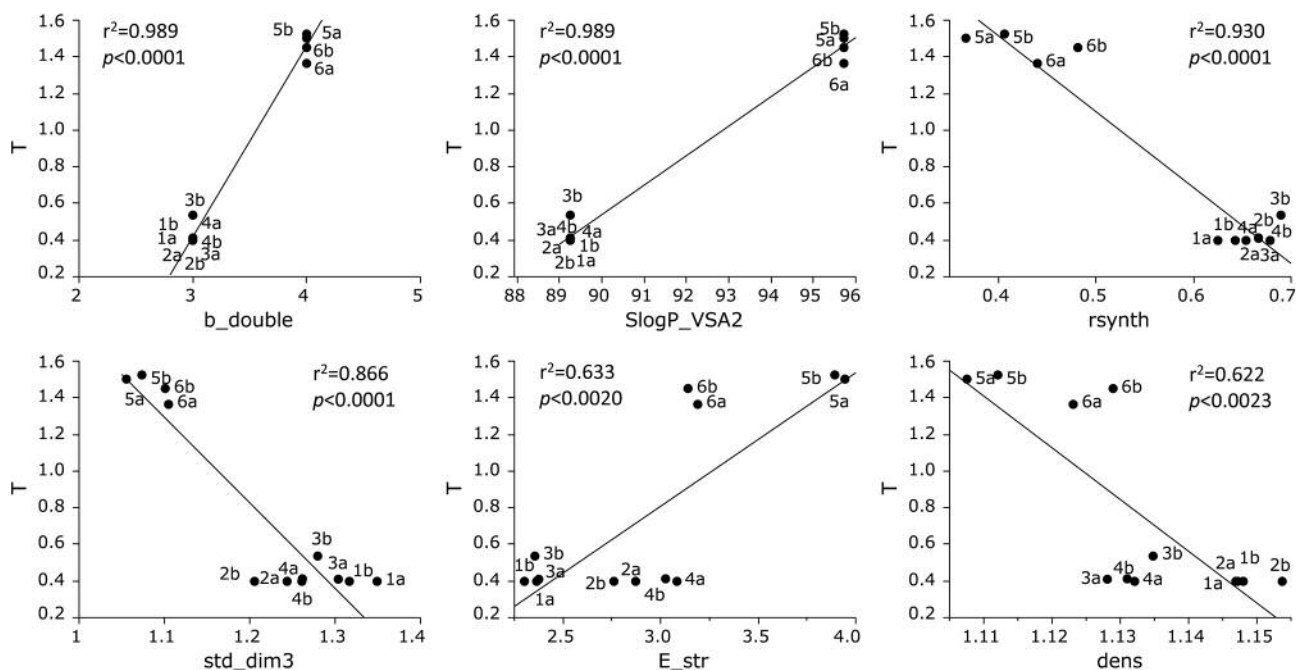
Figure 3. *Determination of correlation coefficient between chemical descriptors and cytotoxicity of 12 furo[2,3-b]chromones against tumor cells (defined as T). The mean values of the negative log of the concentration of compound that reduced the viable tumor cell number by 50% (pCC$_{50}$) were defined as T. See Table II for explanation of chemical descriptors.*
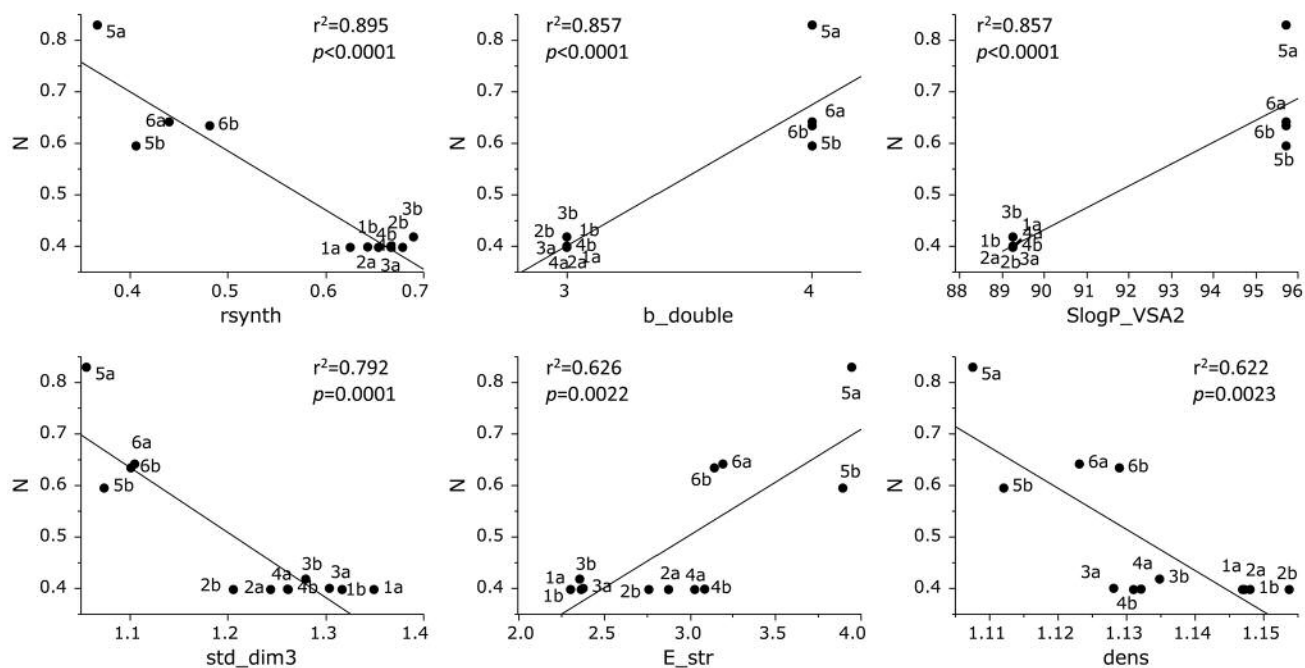


Figure 4. *Determination of coefficient between chemical descriptors and cytotoxicity of 12 furo[2,3-b]chromones against normal cells (defined as N). The mean values of the negative log of the concentration of compound that reduced the viable normal cell number by 50% (pCC$_{50}$) were defined as N. See Table II for explanation of chemical descriptors.*
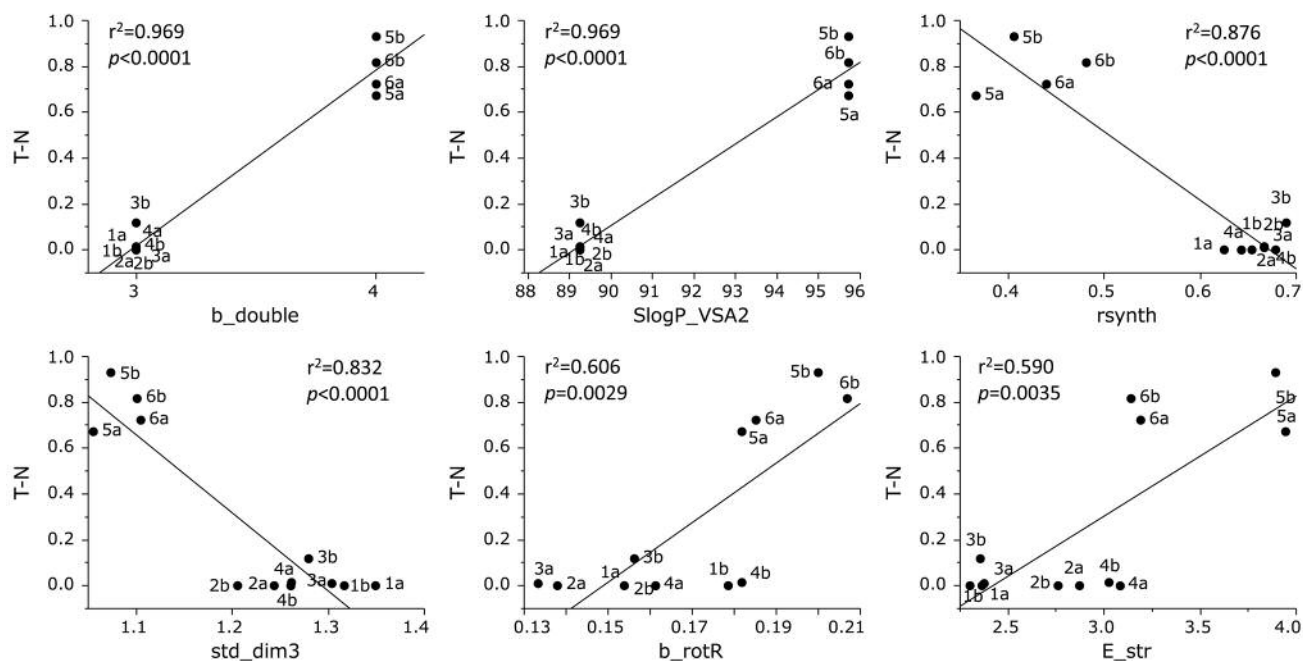
Figure 5. *Determination of coefficient between chemical descriptors and tumor specificity of 12 furo[2,3-b]chromones (defined as T-N, that is the difference in mean values for tumor and normal cells of the negative log of the concentration of compound that reduced the viable cell number by 50% (pCC$_{50}$). See Table II for explanation of chemical descriptors.*

Table IV. *Effect of the introduction of a methoxy group (OMe) at C-7 of benzopyran ring on tumor-specificity (TS) and potency-selectivity expression (PSE) values for six sets of furo[2,3-b]chromone derivatives.*

| Compound | TS (D/B) | Effect of OMe substitution | TS (C/A) | Effect of OMe substitution | PSE (D/B$^2$)×100 | Effect of OMe substitution | PSE (C/A$^2$)×100 | Effect of OMe substitution |
|---|---|---|---|---|---|---|---|---|
| **1a** | 1.0 | 1.0/1.0=1.0 | 1.0 | 1.0/1.0=1.0 | 0.3 | 0.3/0.3=1.0 | 0.3 | 0.3/0.3=1.0 |
| **1b** | 1.0 | | 1.0 | | 0.3 | | 0.3 | |
| **2a** | 1.0 | 1.0/1.0=1.0 | 1.0 | 1.0/1.0=1.0 | 0.3 | 0.3/0.3=1.0 | 0.3 | 0.3/0.3=1.0 |
| **2b** | 1.0 | | 1.0 | | 0.3 | | 0.3 | |
| **3a** | 1.0 | 1.3/1.0=1.3 | 1.1 | 1.1/1.1=1.0 | 0.3 | 0.4/0.3=1.3 | 0.3 | 0.4/0.3=1.3 |
| **3b** | 1.3 | | 1.1 | | 0.4 | | 0.4 | |
| **4a** | 1.0 | 1.0/1.0=1.0 | 1.0 | 1.1/1.0=1.1 | 0.2 | 0.3/0.2=1.5 | 0.2 | 0.3/0.2=1.5 |
| **4b** | 1.0 | | 1.1 | | 0.3 | | 0.3 | |
| **5a** | 3.6 | 7.0/3.6=1.9 | 10.0 | 13.5/10.0=1.4 | 8.8 | 18.9/8.8=2.1 | 70.8 | 98.0/70.8=1.4 |
| **5b** | 7.0 | | 13.5 | | 18.9 | | 98.0 | |
| **6a** | 4.9 | 6.1/4.9=1.2 | 7.2 | 8.7/7.2=1.2 | 9.9 | 15.2/9.9=1.5 | 36.8 | 53.1/36.8=1.4 |
| **6b** | 6.1 | | 8.7 | | 15.2 | | 53.1 | |

## Discussion

The present study demonstrated that among 12 furo[2,3-*b*]chromones, compounds introduced with dimethyl (**1a, 1b**), spirocyclopentyl (**2a, 2b**), spirocyclohexyl (**3a, 3b**) or phenyl group (**4a, 4b**) at C-2 position showed essentially no cytotoxic activity nor tumor specificity, whereas compounds with phenylethenyl (**5a, 5b**) or propenyl (**6a, 6b**) group significantly enhanced the cytotoxicity and tumor specificity. Among the latter group, (**5b**) showed the greatest tumor specificity (evaluated by TS and PSE value), comparable with that of melphalan, although much weaker than that of 5-fluorouracil (5-FU) and doxorubicin (Table I). We also found that when a methoxy group was introduced at C-7 of

benzopyran ring, both TS and PSE values of most of the compounds increased, especially of (**5b**) (Table IV). It was unexpected that both (**5b**) and (**6b**) at cytotoxic concentration would not induce apoptotic cells characterized by cell shrinkage. It is difficult to demonstrate that these compounds actually lack the ability to induce apoptosis. In order to elucidate the mechanism of cell death induction by these compounds, the possibility of the induction of other types of cells and growth arrest in OSCC cells should be tested.

QSAR analysis demonstrated that tumor selectivity of furo[2,3-*b*]chromone derivatives strongly correlated with intramolecular unsaturated bond number, molecular flexibility, molecular density, lipophilicity, molecular size, and molecular shape. Chemical modification of the lead compound may be a potential choice for designing a new type of anticancer drug.

## Conflicts of Interest

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

## Acknowledgements

## References

1 Abu-Hashem AA and El-Shazly M. Synthesis, reactions and biological activities of furochromones: a review. Eur J Med Chem *90*: 633-665, 2015.

2 Motai T and Kitanaka S: Sesquiterpene chromones from *Ferula fukanensis* and their nitric oxide production inhibitory effects. J Nat Prod *68*: 1732-1735, 2005.

3 Choudharya MI, Baig I, Nur-e-Alam M, Shahzad-ul-Hussan S, Öndognii P, Bunderya M, Oyun Z and Atta-ur-Rahman. New α-glucosidase inhibitors from the Mongolian medicinal plant *Ferula mongolica*. Helvetica Chimica Acta *84*: 2409-2416, 2001.

4 Wang Y, Zheng Z, Liu S, Zhang H, Li E, Guo L and Che Y: Oxepinochromenones, furochromenone, and their putative precursors from the endolichenic fungus *Coniochaeta* sp. J Nat Prod *73*: 920-924, 2010.

5 Meng H, Li G, Huang J, Zhang K, Wang H and Wang J: Sesquiterpene coumarin and sesquiterpene chromone derivatives from *Ferula ferulaeoides* (Steud.) Korov. Fitoterapia *86*: 70-70, 2013.

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

7 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.

8 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.

9 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.

10 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.

11 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.

12 Sugita Y, Kawai K and Yokoe I: Diastereoselective ring-expansion reaction of methanochromanone with aldehydes: formation of trans-fused tetrahydrofuro[2,3-b][1]benzopyranones and their isomerization. Heterocycles 55: 135-144, 2001.

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.