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Quantitative Structure–Cytotoxicity Relationship of 2-Azolylchromones

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Abstract. *Background/Aim: Twenty-four 2-azolylchromones 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 two human oral squamous cell carcinoma cell lines and two human normal oral mesenchymal cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Tumor specificity (TS) was evaluated by the ratio of the mean 50% cytotoxic concentration (CC_{50}) against oral cells to that against 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: Three sets of 4H-1-benzopyran-4-ones with indole ring showed much higher TS values than those with pyrrole, pyrazole, imidazole, 1,2,4-triazole, 1,2,3-triazole, indazole and benzimidazole rings. Among those with an indole ring, the compound having a 6-methoxy group, that exhibited the highest cytotoxicity, yielded one to three-order higher PSE values to compared with other groups of compounds. Western blot analysis demonstrated that this compound stimulated the cleavage of caspase-3, suggesting the induction of apoptosis. QSAR analysis demonstrated that TS values were correlated with 3D shape, polarizability, ionization potential and lipophilicity. Conclusion: Chemical modification of the lead compound may be a potential choice for designing a new type of anticancer drug.*

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Key Words: 2-Azolylchromones, QSAR analysis, cytotoxicity, tumor selectivity, apoptosis induction.

4*H*-1-Benzopyran-4-ones (chromones) are an important class of oxygenated heterocyclic compounds, since the core structure is found ubiquitously in the plant kingdom in notable amounts. They show anti-inflammatory, antitumor and antimicrobial activities, and inhibitory activities towards cyclo-oxygenases, kinases, phosphatases, aromatases, acetylcholinesterases, and monoamine oxidases (1, 2).

Among a total of 133 compounds, (*E*)-3-[2-(4-hydroxyphenyl)ethenyl]-6-methoxy-4*H*-1-benzopyran-4-one (classified as 3-styrylchromones), (*E*)-3-[2-(4-chlorophenyl) ethenyl]-7-methoxy-2*H*-1-benzopyran (classified as 3-styryl-2*H*-chromenes) were found to have the highest tumor specificity with the least keratinocyte toxicity (3). This finding urged us to synthesize various compounds derived from chromone.

Recently we synthesized a series of 2-azolylchromone derivatives that showed potent and selective inhibition of monoamine oxidases A and B (4). The publication of data on the biological activity of azolylchroman derivatives, however, has been limited. A series of imidazolylchromanone oximes containing phenoxyethyl ether moiety showed marginal activity against filamentous fungi (*Aspergillus fumigatus* and *Exophiala dermatitidis*), while they exhibited potent activity against yeast (*Cryptococcus gattii*), possibly binding to the homology modeled active site of Cryptococcus CYP51-14α-demethylase (5). 7-Chloro-3-(1*H*-imidazol-1-yl)chroman-4-one more effectively delayed seizures, as well as decreasing seizure duration, in a model of epilepsy (6).

In continuation of discovering new biological activities of chromone derivatives, a total of 24 2-azolylchromone derivatives were investigated for their cytotoxicity against

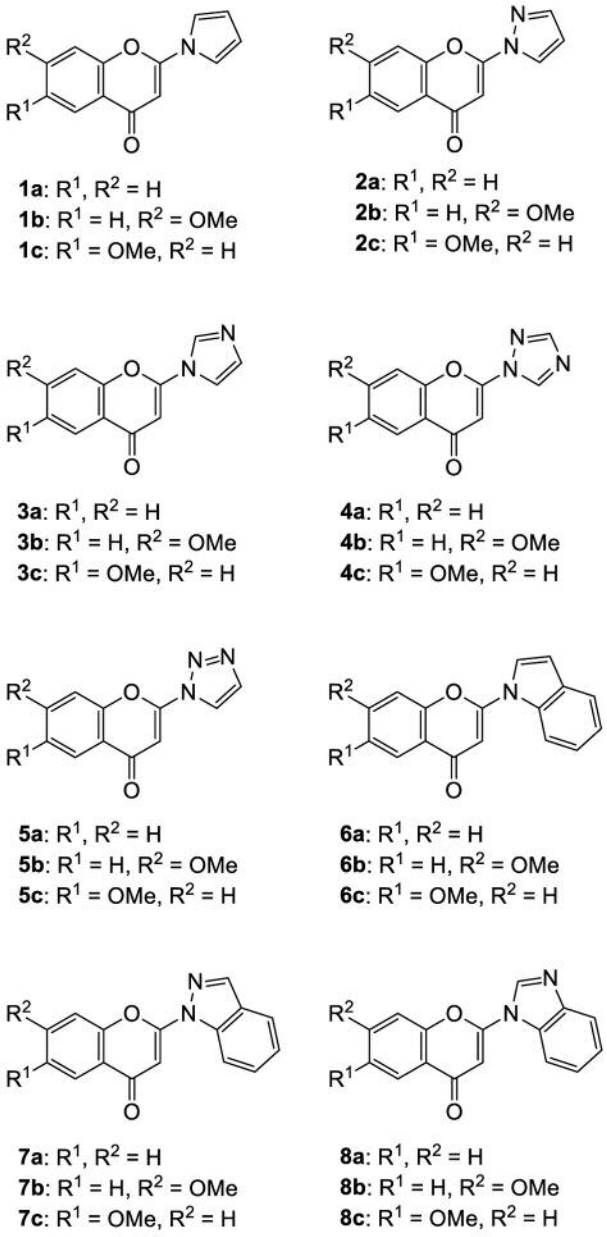

Figure 1. *Structure of 24 2-azolylchromones of this study.*

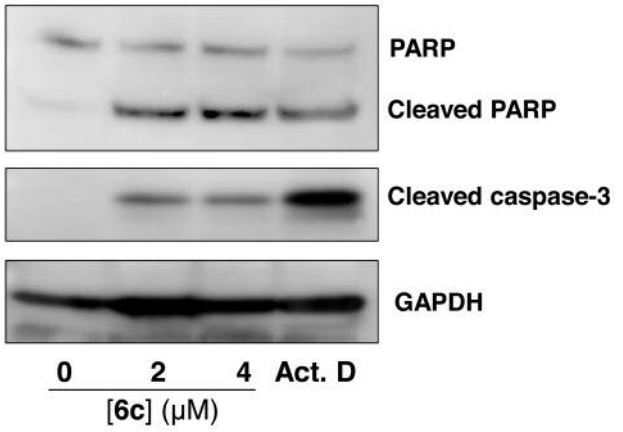

Figure 2. *Induction of apoptosis by [**6c**] in HSC-2 human oral squamous cell carcinoma cell line. PARP: Poly (ADP-ribose) polymerase; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; Act D: actinomycin D.*

two human oral squamous cell carcinoma (OSCC) and two 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. 2-(1*H*-Pyrrol-1-yl)-4*H*-1-benzopyran-4-one [**1a**], 7-methoxy-2-(1*H*-pyrrol-1-yl)-4*H*-1-benzopyran-4-one [**1b**], 6-methoxy-2-(1*H*-pyrrol-1-yl)-4*H*-1-benzopyran-4-one [**1c**], 2-(1*H*-pyrazol-1-yl)-4*H*-1-benzopyran-4-one [**2a**], 7-methoxy-2-(1*H*-pyrazol-1-yl)-4*H*-1-benzopyran-4-one [**2b**], 6-methoxy-2-(1*H*-pyrazol-1-yl)-4*H*-1-benzopyran-4-one [**2c**], 2-(1*H*-imidazol-1-yl)-4*H*-1-benzopyran-4-one [**3a**], 2-(1*H*-imidazol-1-yl)-7-methoxy-4*H*-1-benzopyran-4-one [**3b**], 2-(1*H*-imidazol-1-yl)-6-methoxy-4*H*-1-benzopyran-4-one [**3c**], 2-(1*H*-1,2,4-triazol-1-yl)-4*H*-1-benzopyran-4-one [**4a**], 7-methoxy-2-(1*H*-1,2,4-triazol-1-yl)-4*H*-1-benzopyran-4-one [**4b**], 6-methoxy-2-(1*H*-1,2,4-triazol-1-yl)-4*H*-1-benzopyran-4-one [**4c**], 2-(1*H*-1,2,3-triazol-1-yl)-4*H*-1-benzopyran-4-one [**5a**], 7-methoxy-2-(1*H*-1,2,3-triazol-1-yl)-4*H*-1-benzopyran-4-one [**5b**], 6-methoxy-2-(1*H*-1,2,3-triazol-1-yl)-4*H*-1-benzopyran-4-one [**5c**], 2-(1*H*-indol-1-yl)-4*H*-1-benzopyran-4-one [**6a**], 2-(1*H*-indol-1-yl)-7-methoxy-4*H*-1-benzopyran-4-one [**6b**], 2-(1*H*-indol-1-yl)-6-methoxy-4*H*-1-benzopyran-4-one [**6c**], 2-(1*H*-indazol-1-yl)-4*H*-1-benzopyran-4-one [**7a**], 2-(1*H*-indazol-1-yl)-7-methoxy-4*H*-1-benzopyran-4-one [**7b**], 2-(1*H*-indazol-1-yl)-6-methoxy-4*H*-1-benzopyran-4-one [**7c**], 2-(1*H*-benzimidazol-1-yl)-4*H*-1-benzopyran-4-one [**8a**], 2-(1*H*-benzimidazol-1-yl)-7-methoxy-4*H*-1-benzopyran-4-one [**8b**], 2-(1*H*-benzimidazol-1-yl)-6-methoxy-4*H*-1-benzopyran-4-one [**8c**] were synthesized by the conjugated addition reactions of 3-iodochromone derivatives with selected azoles, 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) were established from the first premolar tooth extracted from the lower jaw of a 12-year-old girl (7), and cells at 10-18 population-doubling levels

Table I. *Cytotoxic activity of 24 2-azolylchromone derivatives against oral malignant and non-malignant cells. Each value represents the mean of triplicate determinations.*

	CC_{50} (μM)														
	Human oral squamous cell carcinoma cell lines					Human normal oral cells									
Compound	Ca9-22 (A)	SD	HSC-2	SD	Mean (B)	HGF (C)	SD	HPLF	SD	Mean (D)	TS		PSE		Ref.
											(D/B)	(C/A)	$(D/B^2)\times100$	$(C/A^2)\times100$	
1a	167.0	9.6	352.0	42.2	259.5	274.0	5.2	228.3	18.9	251.2	1.0	1.6	0.4	1.0	15
1b	135.7	17.2	183.7	14.4	159.7	354.3	38.0	223.0	7.0	288.7	1.8	2.6	1.1	1.9	4
1c	190.7	21.1	>400	0.0	>295	276.0	3.5	230.7	9.2	253.3	<0.9	1.4	<0.3	0.8	4
2a	>400	0.0	>400	0.0	>400	>400	0.0	>400	0.0	>400	><1.0	><1.0	><0.3	><0.3	15
2b	185.0	12.8	>400	0.0	>293	318.7	6.1	281.7	8.6	300.2	<1.0	1.7	<0.4	0.9	4
2c	>360	69.3	>400	0.0	>380	>400	0.0	>364	52.1	>382	><1.0	><1.1	><0.3	><0.3	4
3a	63.7	0.9	78.2	1.1	71.0	>400	0.0	>373	29.8	>387	>5.4	>6.3	>7.7	>9.8	15
3b	>400	0.0	>400	0.0	>400	>400	0.0	>400	0.0	>400	><1.0	><1.0	><0.3	><0.3	4
3c	>400	0.0	>400	0.0	>400	>400	0.0	129.3	13.2	>265	><0.7	><1.0	><0.2	><0.3	4
4a	130.0	3.6	177.0	7.5	153.5	>367	29.0	142.0	3.6	>254	>1.7	>2.8	>1.1	>2.2	15
4b	80.7	7.9	130.7	6.0	105.7	>397	5.8	74.9	0.7	>236	>2.2	>4.9	>2.1	>6.1	15
4c	88.5	28.2	>400	0.0	>244	>400	0.0	46.7	3.1	>223	><0.9	>4.5	><0.4	>5.1	4
5a	31.5	1.7	68.6	5.1	50.0	103.8	44.4	>400	0.0	>252	>5.0	3.3	>10.1	10.5	4
5b	29.2	4.1	80.2	8.5	54.7	103.8	17.6	>396	6.9	>250	>4.6	3.6	>8.4	12.2	4
5c	23.3	0.7	69.9	3.3	46.6	74.1	2.3	>360	69.3	>217	>4.7	3.2	>10.0	13.7	4
6a	**6.9**	**0.6**	**5.3**	**0.5**	**6.1**	**57.1**	**10.2**	**>400**	**0.0**	**>229**	**>37.5**	**8.3**	**>614.3**	**121.2**	15
6b	**6.5**	**0.1**	**6.1**	**0.6**	**6.3**	**265.0**	**15.7**	**40.3**	**16.9**	**152.6**	**24.2**	**40.8**	**384.6**	**627.2**	4
6c	**1.6**	**0.1**	**1.3**	**0.3**	**1.5**	**35.9**	**12.8**	**35.5**	**3.3**	**35.7**	**24.1**	**22.0**	**1622.5**	**1346.9**	4
7a	33.2	13.8	68.8	16.6	51.0	>400	0.0	>400	0.0	>400	>7.8	>12.0	>15.4	>36.2	15
7b	76.3	12.0	68.1	18.8	72.2	>400	0.0	>369	53.1	>385	>5.3	>5.2	>7.4	>6.9	4
7c	>400	0.0	28.1	7.0	>214	>400	0.0	63.6	31.6	>232	><1.1	><1.0	><0.5	><0.3	4
8a	85.6	9.3	64.1	3.1	74.9	362.7	64.7	164.6	61.3	263.6	3.5	4.2	4.7	5.0	15
8b	23.0	0.6	22.3	0.8	22.7	>400	0.0	28.9	9.8	>214	>9.5	>17.4	>41.7	>75.4	4
8c	6.0	0.9	3.5	0.7	4.7	75.0	22.3	47.7	7.8	61.3	13.0	12.6	273.8	210.7	4
DXR	0.16	0.02	0.08	0.00	0.12	8.45	1.35	>10	0.00	>9.3	>78.1	53.4	>66077.7	33719.7	

HGF: Human gingival fibroblast; HPLF: human periodontal ligament fibroblast; Ca9-22 (derived from gingival tissue), HSC-2 (derived from tongue): oral squamous cell carcinoma (OSCC) cell lines; CC_{50}: 50% cytotoxic concentration; DXR: doxorubicin; TS: tumor-selectivity index; PSE: potency-selectivity expression; CC_{50}: 50% cytotoxic concentration.

were used in this study. Human OSCC cell lines [Ca9-22 (derived from gingival tissue); HSC-2 (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 (8). 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 human normal cells/mean CC_{50} against for human OSCC cell lines [(D/B) in Table I]. Since both Ca9-22 and HGF cells were derived from the gingival tissue (9), 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 using the following equation: PSE=TS/CC_{50} against tumor cells ×100 (10) [that is, $(D/B_2)\times100$ (HGF, HPLF *vs.* Ca9-22, HSC-2) and $(C/A^2)\times100$ (HGF *vs.* Ca9-22 in Table I).

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

Descriptor	Source*	Explanation
CATS2D_02_LL	Dragon	CATS2D lipophilic-lipophilic at lag 02
G3e	Dragon	3rd Component symmetry directional WHIM index/weighted by Sanderson electronegativity
G3m	Dragon	3rd Component symmetry directional WHIM index/weighted by mass
G3p	Dragon	3rd Component symmetry directional WHIM index/weighted by polarizability
G3s	Dragon	3rd Component symmetry directional WHIM index/weighted by I-state
G3v	Dragon	3rd Component symmetry directional WHIM index/weighted by van der Waals volume
Gm	Dragon	total symmetry index/weighted by mass
Kp	Dragon	K global shape index/weighted by polarizability
Mor32i	Dragon	signal 32/weighted by ionization potential in 3D-MoRSE descriptors
Mor32u	Dragon	signal 32/unweighted in 3D-MoRSE descriptors
P1p	Dragon	1st Component shape directional WHIM index/weighted by polarizability
P2p	Dragon	2nd Component shape directional WHIM index/weighted by polarizability
Q_RPC-	MOE	Relative negative partial charge: the smallest negative partial charge atom i divided by the sum of the negative partial charge atom i.
SpMin8_Bh(s)	Dragon	Smallest eigenvalue n. 8 of Burden matrix weighted by I-state

*Dragon (11), MOE (12).

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 (10).

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 291 and 2771, respectively. The 13 Dragon descriptors (11) and 1 MOE descriptor (12) 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 (8). 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 synthesized a total of 24 2-azolylchromones, classified into the following eight sets of 1*H*-pyrrol-1-yl [**1a-c**], 1*H*-pyrazol-1-yl [**2a-c**], 1*H*-imidazol-1-yl [**3a-c**], 1*H*-1,2,4-triazol-1-yl [**4a-c**], 1*H*-1,2,3-triazol-1-yl [**5a-c**], 1*H*-indol-1-yl [**6a-c**], 1*H*-indazol-1-yl [**7a-c**] and 1*H*-benzimidazol-1-yl [**8a-c**] compounds. Among these eight groups, [**6a-c**] showed the highest cytotoxicity against human OSCC cell lines (Ca9-22, HGF) (mean CC_{50}=1.5-6.3 μM), followed by [**8a-c**] (4.7-74.9 μM), [**5a-c**] (46.6-50.0 μM), [**7a-c**] (51.0->214 μM), [**4a-c**] (105.7->244 μM), [**1a-c**] (159.7->295 μM), [**3a-c**] (71.0->400μM) and [**2a-c**] (>293 μM).

On the other hand, all these groups of compounds generally showed much lower cytotoxicity against human normal oral cells (HGF and HPLF). [**6a-c**] showed slightly higher cytotoxicity (mean CC_{50}=35.7->229 μM) than other groups: [**1a-c**] (251.2-288.7 μM), [**2a-c**] (300.2->400 μM), [**3a-c**] (>265->400 μM), [**4a-c**] (>223->254 μM), [**5a-c**] (>217->252 μM), [**7a-c**] (>232->400 μM) and [**8a-c**] (61.3->214 μM).

Tumor specificity. TS values were calculated for the compounds by dividing the average CC_{50} value towards normal cells into the average CC_{50} value towards cancer cell lines. When two human OSCC cell lines (Ca9-22, HGF) and two normal oral cells (HGF, HPLF) were used, [**6a-c**] had much higher TS values (24.1->37.5) than [**1a-c**] (<0.9-1.8), [**2a-c**] (><1.0), [**3a-c**] (><1.0->5.4), [**4a-c**] (><0.9->2.2), [**5a-c**] (>4.6->5.0), [**7a-c**] (><1.1->7.8) and [**8a-c**] (3.5-13.3). (D/B in Table I)

When Ca9-22 and HGF cells, both derived from gingival tissue, were used, [**6a-c**] again had higher TS valueS (8.3-40.8) than [**1a-c**] (1.4-2.6), [**2a-c**] (><1.0-1.7), [**3a-c**] (><1.0->6.3), [**4a-c**] (>2.8->4.9), [**5a-c**] (3.2-3.6), [**7a-c**] (><1.0->12.0) and [**8a-c**] (4.2->17.4) (C/A in Table I).

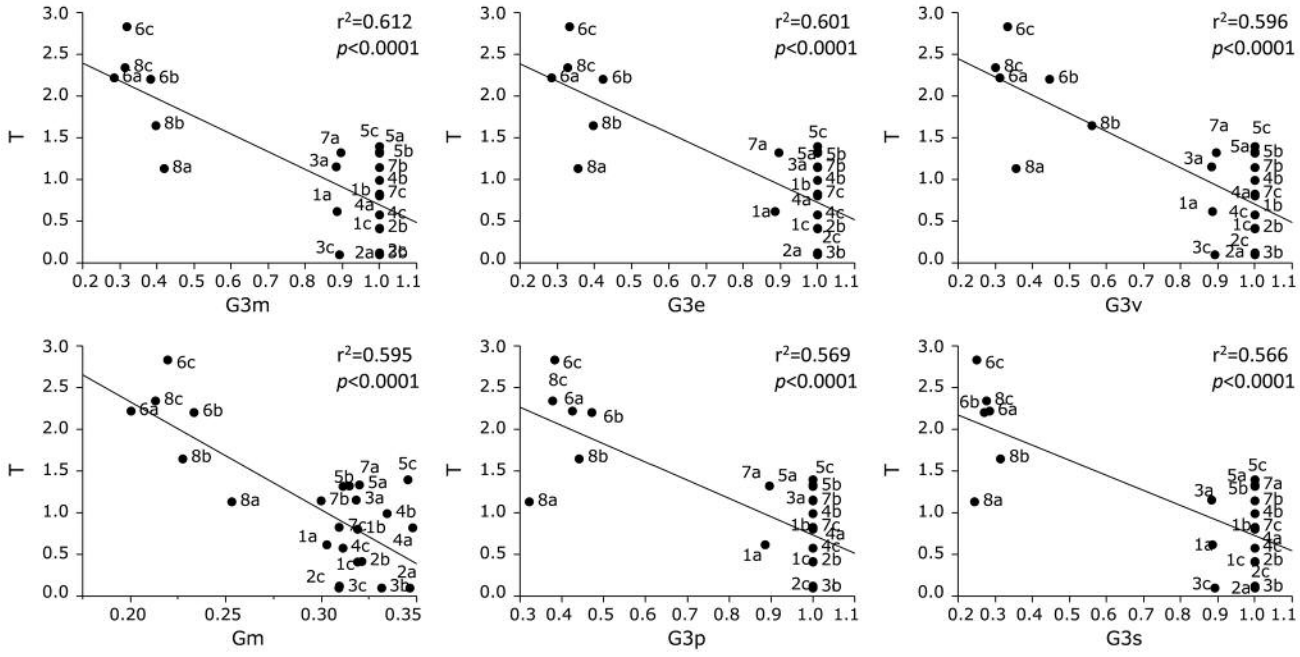

Figure 3. *Determination of correlation coefficient between chemical descriptors and cytotoxicity [50% cytotoxic concentration (CC_{50})] of 24 2-azolylchromones against tumor cells (defined as T). The mean pCC_{50}, i.e. the −log CC_{50} values for tumor cell lines, were defined as T.*

PSE value. In order to identify the most promising compounds in terms of both good potencies and selective cytotoxicity, the PSE values were calculated. Among [**6a-c**], [**6c**] having a 2-(1*H*-indol-1-yl)-6-methoxy group, exhibited the highest cytotoxicity (CC_{50}=1.5 μM). As expected, [**6c**] yielded the greatest PSE values:1622.5 and 1346.9, followed by [**6b**] (384.6 and 627.2) and [**6a**] (>614.3 and 121.2). On the other hand, PSE values of other groups were two-orders less in most cases (Table I).

Western blot analysis demonstrated that [**6c**] stimulated the cleavage of caspase-3 (Figure 2), suggesting the induction of apoptosis.

Computational analysis. We next performed the QSAR analysis of 2-azolylchromones in regards to their cytotoxicity against tumor cells and normal cells. Among a total of 3062 descriptors, 14 descriptors described below correlated well with cytotoxicity and TS (Table III).

Cytotoxicity of 2-azolylchromones against human OSCC cell lines was correlated with G3m (3D-shape and molecular weight) (r^2=0.612, p<0.0001), G3e (3D-shape and electronegativity) (r^2=0.601, p<0.0001), G3v (3D-shape and molecular volume) (r^2=0.596, p<0.0001). Gm (symmetry and molecular weight) (r^2=0.595, p<0.0001). G3p (3D-shape and polarizability) (r^2=0.569, p<0.0001) and G3s (3D-shape and intrinsic state) (r^2=0.566, p<0.0001) (Figure 3).

Cytotoxicity of 2-azolylchromones against human normal oral mesenchymal cells was correlated with SpMin8_Bh(s) (topological shape and intrinsic state) (r^2=0.536, p<0.0001), Q_RPC-(negative partial charge) (r^2=0.485, p=0.0002), G3s (3D-shape and intrinsic state) (r^2=0.462, p=0.0003), G3e

Table III. *Properties of descriptors that significantly affect the cytotoxicity of compounds towards tumor cells (T), and normal cells (N), and their tumor specificity (T-N).*

Descriptor	r^2	p-Value	Meaning
G3m	0.612	<0.0001	3D-shape & molecular weight
G3e	0.601	<0.0001	3D-shape & electronegativity
G3v	0.596	<0.0001	3D-shape & molecular volume
Gm	0.595	<0.0001	Symmetry & molecular weight
G3p	0.569	<0.0001	3D-shape & polarizability
G3s	0.566	<0.0001	3D-shape & intrinsic state
SpMin8_Bh(s)	0.536	<0.0001	Topological shape & intrinsic state
Q_RPC-	0.485	0.0002	Negative partial charge
G3s	0.462	0.0003	3D-shape & intrinsic state
G3e	0.453	0.0003	3D-shape & electronegativity
G3m	0.451	0.0003	3D-shape & molecular weight
Gm	0.448	0.0003	Symmetry & molecular weight
Kp	0.577	<0.0001	3D-shape & polarizability
P1p	0.577	<0.0001	3D-shape & polarizability
Mor32i	0.570	<0.0001	3D-shape & ionization potential
P2p	0.564	<0.0001	3D-shape & polarizability
Mor32u	0.560	<0.0001	3D-shape
CATS2D_02_LL	0.555	<0.0001	Topological shape & lipophilicity

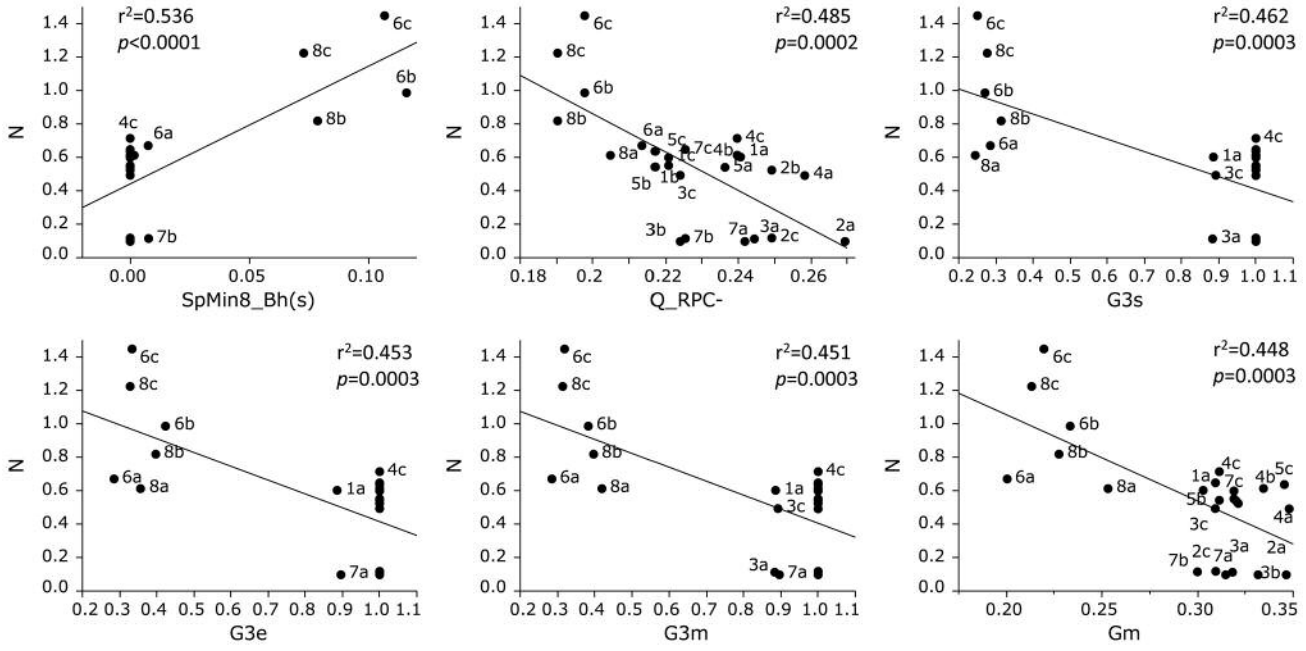

Figure 4. *Determination of correlation coefficient between chemical descriptors and cytotoxicity [50% cytotoxic concentration (CC_{50})] of 24 2-azolylchromones against normal cells (defined as N). The mean pCC_{50}, i.e. the −log CC_{50} values for normal cells, were defined as N.*

(3D-shape and electronegativity) (r^2=0.453, p=0.0003), G3m (3D-shape and molecular weight) (r^2=0.451, p=0.0003) and Gm (symmetry and molecular weight) (r^2=0.448, p=0.0003) (Figure 4)

Tumor specificity of 2-azolylchromones was correlated with Kp (3D-shape and polarizability) (r^2=0.577, p<0.0001), P1p (3D-shape and polarizability) (r^2=0.577, p<0.0001), Mor32i (3D-shape and ionization potential) (r^2=0.570, p<0.0001), P2p (3D-shape and polarizability) (r^2=0.564, p<0.0001), Mor32u (3D-shape) (r^2=0.560, p<0.0001) and CATS2D_02_LL (topological shape and lipophilicity) (r2=0.555, p<0.0001) (Figure 5).

Discussion

The present study demonstrated that the introduction of indole ring to 4*H*-1-benzopyran-4-ones [**6a-c**] produced much higher tumor-specific compounds, as compared with those induced with pyrrole [**1a-c**], pyrazole [**2a-c**], imidazole [**3a-c**], 1,2,4-triazole [**4a-c**], 1,2,3-triazole [**5a-c**], indazole [**7a-c**] and benzimidazole [**8a-c**] rings. Especially, [**6c**] with methoxy group attached at C-6 position showed the highest PSE value (1622.5 and 1346.9) and induced apoptosis in HSC-2 cell OSCC cell line. This PSE value was about three times higher than that for (*E*)-3-(4-hydroxystyryl)-6-methoxy-4*H*-chromen-4-one (PSE=630) that showed high TS and lower keratinocyte specificity (13), and slightly higher than that of (*E*)-3-(4-chlorostyryl)-7-methoxy-2*H*-chromene (PSE=1277.5)(14). The cytotoxicity of [**6c**] against human oral keratinocytes should be investigated.

We also found that three sets of compounds with benzimidazol rings [**8a-c**] showed some TS (TS=3.5-13.0; PSE=4.7-273.8). It is interesting to state that cytotoxicity and PSE value of [**8c**] were 4.8-times (22.7/4.7) and 6.6-times (273.8/41.7) higher than those of [**8b**], although these two molecules have the same chemical formula with the only difference being in where the methoxy group attached. Similarly, cytotoxicity and PSE value of [**6c**] were 4.2-times (6.3/1.5) and 4.2-times (1,622.5/384.6) higher than those of [**6b**], both having the same chemical formula with the only difference being in the position of the methoxy group (Table I). This indicates that the chemical structure is not the sole determinant of TS. QSAR analysis demonstrated that TS of 2-azolylchromones was correlated with 3D-shape, polarizability, ionization potential and lipophilicity.

Anticancer drugs in clinical use have been reported to induce many side-effects in patients due to the lack of selectivity of the drugs to cancer tissues. Cytotoxicity of a compound is more valuable when it is selective. Although the chemical structures of all 24 compounds used in this study have already been reported (Table I) (4, 15), the present study is the first that investigated the relative potency of TS of all

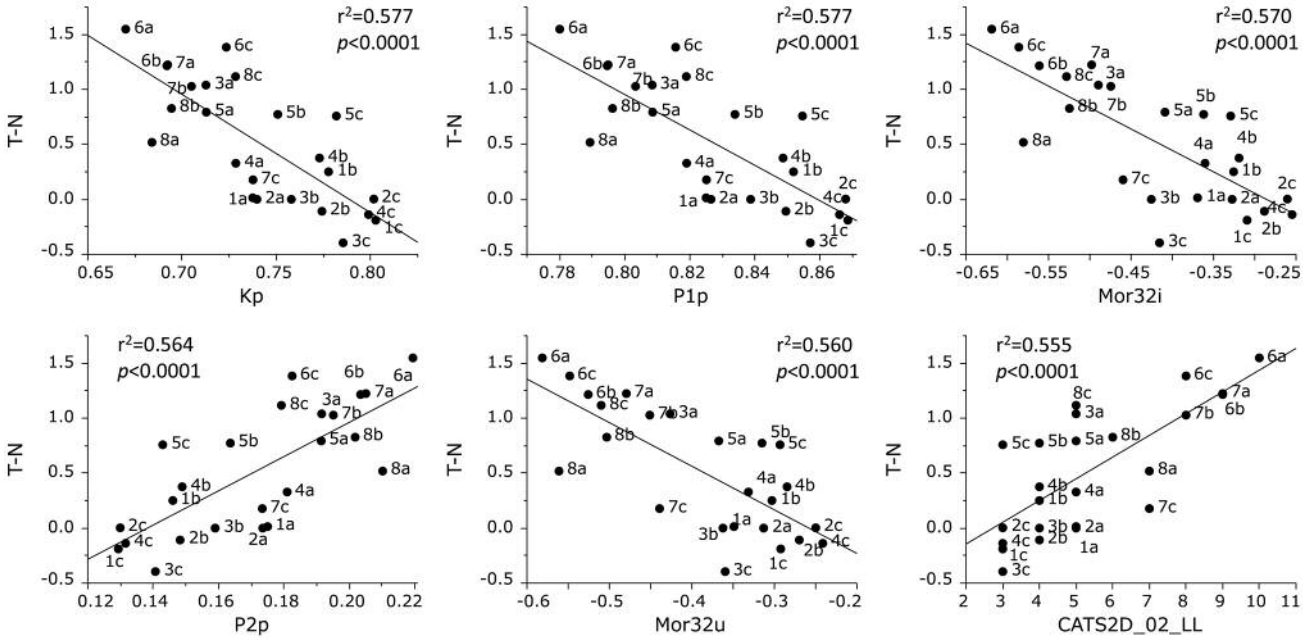

Figure 5. *Determination of correlation coefficient between chemical descriptors and tumor specificity of 24 2-azolylchromones (defined as T-N).*

of these compounds. Chemical modification of [**6c**] 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 has been no significant financial support for this work that could have influenced its outcome.

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