Relation between structures of naphthalenylchalcone derivatives and their cytotoxic effects on HCT116 human colon cancer cells

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Abstract To find potent chemotherapeutic agents, cytotoxic effects of 42 synthetic chalcone derivatives bearing naphthyl groups on HCT116 human colon cancer cell lines were tested using the clonogenic long-term survival assay. The relationships between their half-maximal cell growth inhibitory concentrations (GI₅₀) and structural properties were obtained using comparative molecular field analysis and comparative molecular similarity indices analysis. The structural conditions that showed maximum cytotoxic effects on the colon cancer cells were determined. In addition, a derivative, (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one, showing the best GI₅₀ value, was assessed for stimulating reactive oxygen species (ROS) production. While its treatment on non-tumorigenic epithelial MCF-12A cell line did not affect the intracellular ROS levels, its treatment on MDA-MB-231 human breast cancer cell line showed ROS accumulation. These findings demonstrate that naphthalenylchalcones can be developed as potent chemotherapeutic agents.

Keywords Clonogenicity · Naphthalenylchalcone · Reactive oxygen species generation · Quantitatively structure–activity relationships

Introduction

Flavonoids share common features with chalcone, 1,3-diphenylprop-2-en-1-one, such as a C6–C3–C6 skeleton (Supplementary Materials Fig. 1A) [1, 2]. However, unlike flavonoids, which consist of a closed ring of C3, chalcone bears an α,β -unsaturated carbonyl group. Because various substituents can be attached to both phenyl rings, many chalcone derivatives exist, and they show diverse biological activities: anticancer, antimicrobial, antifungal, anti-inflammatory, anti-tuberculosis, and anti-malarial effects [3–8]. Even though there are many chemotherapeutic agents, more potent and safe agents are being developed. An additional benzene ring in chalcone increases its cell permeability. Accordingly, we designed and synthesized the following chalcone derivatives: 3-naphthyl-1-phenylprop-2-en-1-one (Supplementary Materials Fig. 1B) and 1-naphthyl-3-phenylprop-2-en-1-one (Supplementary Materials Fig. 1C). There are many methods to measure cytotoxic effects of drugs on cancer cell lines [9–11]. Among them, the clonogenic long-term survival assay requires long experimental times, but it can distinguish between cytotoxic effects of compounds with similar structures [12]. Therefore, we tested the synthesized naphthalenylchalcone derivatives with this assay. Prop-2-en-1-one moiety contained in chalcone can act as a Michael acceptor [13]. Our previous experiments demonstrated that a compound with Michael acceptor causes reactive oxygen species (ROS) generation [14]. Therefore,

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we evaluated whether (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one, showing maximum cytotoxicity among the derivatives tested, causes ROS generation. In addition, the relationships between the physicochemical properties of naphthalenylchalcone derivatives and their cytotoxic effects on HCT116 human colon cancer cells were elucidated using comparative molecular field analysis and comparative molecular similarity indices analysis. The findings obtained from these relationships will give us information regarding the optimum structural conditions to develop potent chemotherapeutic agents containing naphthalenylchalcone moiety.

Materials and methods

The synthetic procedures to obtain naphthalenylchalcone derivatives have been described in the methods reported previously [15, 16]. Their cytotoxic effects on HCT116 human colon cancer cells were measured using a clonogenic long-term survival assay [12]. Detailed procedure was followed from the methods reported previously [17]. The clonogenic assay data of 42 naphthalenylchalcone derivatives were obtained at five different concentrations (0, 5, 10, 20, and 40 μM ; Fig. 1). The clonogenic densities were measured using a densitometer (MultiGauge, Fuji-film, Japan), and their half-maximal cell growth inhibitory concentrations (GI_{50}) were calculated using SigmaPlot (SYSTAT, Chicago, IL) [18]. The relationships between the physicochemical properties of naphthalenylchalcone derivatives and their cytotoxic effects were obtained from comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA), using the Sybyl 7.3 program (Tripos, St. Louis, MO) [19]. Intracellular ROS levels were detected after incubation with 10 μM 2',7'-dichlorofluorescein diacetate (DCF-DA; Sigma-Aldrich), using a FACSCalibur flow cytometer (Becton–Dickinson Immunocytometry Systems, San Jose, CA), as described previously [14]. Statistical analyses were carried out using a one-way analysis of variance (ANOVA), followed by Kruskal–Wallis test using Graph-Pad InStat software (La Jolla, CA). All experiments were performed in triplicates [20]. The structures and names of 42 naphthalenylchalcone derivatives are listed in Supplementary Materials Table 1. While derivatives 1–30 contain 3-naphthyl-1-phenyl-prop-2-en-1-one, derivatives 31–42 contain 1-naphthyl-3-phenyl-prop-2-en-1-one. Their structures are summarized in Supplementary Materials Fig. 2. The three-dimensional (3D) structures of derivatives 1–30 were based on the crystallographic structure of derivative 20 [21], and the 3D of derivatives 31–42 were based on the crystallographic structure of derivative 34 [22]. They were

subjected to energy minimization using the Sybyl program, as reported previously [18, 19].

Results and discussion

The GI_{50} values of 42 naphthalenylchalcone derivatives ranged from 1.02 (derivative 4, (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one) to 96.6 μM (derivative 17, (*E*)-1-(2-hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxynaphthalen-1-yl)prop-2-en-1-one). The negative logarithmic scale of GI_{50} values (pGI_{50}) was used as the biological data for the quantitative structure–activity relationships (QSAR) calculation (Supplementary Materials Table 1). Forty-two derivatives were separated into a training set to build the QSAR model, and a test set to validate the QSAR model. Nine derivatives (8, 12, 15, 19, 21, 27, 29, 30, and 40) were chosen for the test set arbitrarily and were validated using hierarchical clustering analysis. As shown in Supplementary Materials Fig. 3, they belong to separate structural groups. Thirty-three derivatives of the training set were aligned to identify interactions between probe atoms and remainder of the derivatives, using the Sybyl/DATABASE Alignment module (Supplementary Materials Fig. 4). The QSAR was analyzed using a CoMFA model, which provides information about steric and electrostatic field descriptors, as described in a previous report [23]. Of many CoMFA models generated using partial least-squares regression and region focusing method, the model showing the best cross-validation correlation coefficient (q^2) of 0.529 was chosen. According to this model, the non-cross-validated coefficient (r^2), the optimal number of components, the standard error of estimate, and the *F* value were determined to be 0.978, 6, 0.068, and 194.814, respectively. The pGI_{50} values, predicted using this model, were compared to the experimental data. As listed in Supplementary Materials Table 2, the residuals between the two values ranged from 0.34 to 7.55%. Because the test set was prepared to validate the CoMFA model, the pGI_{50} values of the test set were calculated using this model, and the predicted values were compared to the experimental data. Their residuals ranged between 2.65 and 23.32%. As a result, this CoMFA model can be used to explore relationships between the physicochemical properties of 42 naphthalenylchalcone derivatives and their cytotoxic effects on HCT116 human colon cancer cells. The plot of experimental data versus predicted values is shown in Supplementary Materials Fig. 5.

The same procedures were performed to generate CoMSIA models, which provide information about the steric and electrostatic field descriptors, as well as the hydrophobic, hydrogen bond (H-bond) donor and acceptor fields. Out of many models, the CoMSIA model, which shows the best q^2 of 0.504, was selected, where r^2 , the

Fig. 1 Clonogenic long-term survival assays of 42 naphthalenylchalcone derivatives at five different concentrations: 0, 5, 10, 20 and 40 μM

optimal number of components, the standard error of estimate, and F value were determined to be 0.937, 6, 0.116, and 64.272, respectively. This CoMSIA model includes steric and hydrophobic fields. The residuals between experimental values of pGI_{50} of the training set and values predicted using this model ranged from 0.12 to 12.74%. Likewise, the residuals for the test set ranged between 6.35 and 25.08%. They are listed in Supplementary Materials Table 2. Therefore, this CoMSIA model is reliable. The plot of experimental data versus predicted values is shown in Supplementary Materials Fig. 6.

To visualize the results obtained from the CoMFA model, its contour maps were generated using the Sybyl program, where steric and electrostatic field descriptors contributed to 51.3 and 48.7%, respectively. The steric field descriptors could be divided into a bulky favored region (80%) and a bulky disfavored region (20%), as shown in Supplementary Materials Fig. 7, where derivative

4 was inserted as a template. While the existence of an additional benzene ring at the 3-phenyl ring increases the cytotoxic effects, its existence at 1-phenyl ring decreases the cytotoxic effect. The activities of derivatives **1–30** with 3-naphthyl-1-phenyl-prop-2-en-1-one are better than those of derivatives **31–42** with 1-naphthyl-3-phenyl-prop-2-en-1-one. Because substituents of all naphthalenylchalcone derivatives, except derivative **42**, vary in the number or position of methoxy groups, the electrostatic field descriptors generated by the CoMFA model do not discriminate between the pharmacophores. Like the CoMFA model, the contour maps by the CoMSIA model were generated using Sybyl. As mentioned above, the CoMSIA model includes the steric and hydrophobic fields. The contour maps of the steric field descriptors are same as those from the CoMFA model. The hydrophobic field descriptors could be divided into a favored region (88%) and a disfavored region (12%), as shown in Supplementary

Fig. 2 MDA-MB-231 human breast cancer cells and non-tumorigenic epithelial MCF-12A cell lines were incubated with 10 mmol/L DCF-DA for 60 min, followed by the addition of 10 μ M derivative **4** or 10 μ M DPP23 for 1 h. Fluorescence was then measured using a FACSCalibur

Materials Fig. 8, where derivative **4** was inserted as a template. The hydrophobic groups at R₂, R₅, and R₈, shown in Supplementary Materials Fig. 2, decrease the activities. Derivatives **8**, **14**, and **26** with methoxy groups at R₂ position show better activities than derivatives **7**, **13**, and **25**, respectively. Derivatives **19**, **21**, **22**, **23**, **24**, and **40** with methoxy groups at R₅ position show better activities than derivatives **7**, **9**, **10**, **11**, **12**, and **31**, respectively. Derivative **37** with methoxy group at R₈ position shows better activity than derivative **38**. Derivative **42** with hydroxy group at R₈ position shows better activity than derivative **36**.

All derivatives include the Michael acceptor, as marked in Supplementary Materials Fig. 2. Because the compounds containing the Michael acceptor could generate ROS, derivative **4** showing the best GI₅₀ value was assessed for stimulating ROS production. A chalcone, (*E*)-3-(3,5-dimethoxyphenyl)-1-(2-methoxyphenyl)prop-2-en-1-one (named as DPP23), was proved to generate ROS in our previous experiments [14]. Therefore, DPP23 was used as a reference compound. The intracellular ROS levels were measured using DCF-DA fluorescence, which indicates ROS accumulation. The treatment of DPP23 on non-tumorigenic epithelial MCF-12A cell line did not affect the intracellular ROS levels, as shown in Fig. 2 (left top). However, its treatment on MDA-MB-231 human breast cancer cell line showed ROS accumulation, as shown in Fig. 2 (left bottom). Similarly, derivative **4** was added to both non-tumorigenic epithelial MCF-12A cell line and MDA-MB-231 human breast cancer cell line. Like DPP23, while the treatment of derivative **4** on non-tumorigenic epithelial MCF-12A cell line did not affect the intracellular ROS levels, as shown in Fig. 2 (right top), its treatment on MDA-MB-231 human breast cancer cell line showed ROS accumulation, as shown in Fig. 2 (right down). As a result, we confirm that derivative **4**, (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one, is a ROS generating naphthalenylchalcone.

2'-Hydroxy-2,3,5'-trimethoxychalcone induces apoptosis in breast cancer cell lines [24]. 2-Hydroxy-3',5,5'-trimethoxychalcone inhibits nuclear factor-kappaB (NF-κB)-mediated GROα expression and prevents invasion of MDA-MB-231 human breast cancer cells [25]. 2'-Hydroxy-2,4,6-trimethoxy-5',6'-naphthochalcone disturbs the microtubular network of colon cancer cells, which results in inducing G2/M cell cycle arrest and apoptosis [26]. 2-Hydroxy-4-methoxy-2',3'-benzochalcone inhibits tubulin polymerization [27]. They all belong to the group of synthetic chalcones. As listed above, various chalcones with methoxy or naphthyl groups have been known to show anticancer effects [28–31]. However, anticancer effects of chalcones with naphthyl group have rarely been studied, and the relationships between their structural properties and cytotoxic effects have not been reported.

In this research, the clonogenic long-term survival effects of 42 naphthalenylchalcone derivatives on HCT116 human colon cancer cells were measured. The relationships between their cytotoxic effects and physicochemical properties, obtained using comparative molecular field analysis and comparative molecular similarity indices analysis, demonstrated the optimal structural conditions that show maximum cytotoxic effects on colon cancer cells. In addition, ROS generation was measured for the title compound of the current research, (*E*)-1-(2-hydroxy-4,5-dimethoxyphenyl)-3-(naphthalen-1-yl)prop-2-en-1-one, which showed the maximum half-maximal cell growth inhibitory effect. As expected from previous studies, the compound showed ROS generation. Thus, these findings demonstrated that naphthalenylchalcones can be developed as potent chemotherapeutic agents.

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