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Cytotoxic Activity Evaluation and QSAR Study of Chromene-based Chalcones

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Chalcone and chromene motifs are synthetic or naturally occurring scaffolds with significant cytotoxic profile. Two types of novel regioisomeric chromene-chalcone hybrids, namely 1-(6-chloro or 6-methoxy-2*H*-chromen-3-yl)-3-phenylprop-2-en-1-one (Type **A**) and 3-(6-chloro or 6-methoxy-2*H*-chromen-3-yl)-1-phenylprop-2-en-1-one (Type **B**), both with different substituents on the phenyl ring attached to propenone linkage, have been evaluated for their cytotoxic activity against breast cancer cell lines (MCF-7 and MDA-MB-231). The results indicate that type **A** of chromene-chalcones demonstrated better cytotoxic profile than type **B** especially in MDA-MB-231 cell line. In addition, the growth inhibitory activity of most of the target compounds is higher than Etoposide as a reference drug. QSAR analysis of these novel compounds demonstrated that topological and geometrical parameters are among the important descriptors that influence the cytotoxic activity profile of compounds.

Key words: Chromene, Chalcones, Cytotoxic activity, QSAR

INTRODUCTION

Chalcones are synthetic or naturally occurring substructures in many plants as a precursor of flavonoids and isoflavonoids. The chemical structure of chalcone consists of two aromatic rings joined by a three carbon, α,β-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-one) (Akihisa et al., 2006; Zhang et al., 2006; Nowakowska, 2007; Patil et al., 2009) (Fig. 1).

Chalcones have displayed widespread pharmacological activities, including anti-inflammatory (Rojas et al., 2002), antibacterial (Sivakumar et al., 2009), antileishmanial (Liu et al., 2003), antioxidant (Yayli et al., 2004), cytotoxic, antitumor and Pgp inhibitory properties (Bois et al., 1999; Go et al., 2005; Liu et al., 2008; Reddy et al., 2008; Sashidhara et al., 2010a). Recent studies demonstrated that chalcones inhibit cell proliferation in cancer cell lines and also induce apoptosis in different cancerous cell types with various proposed mechanisms such as depletion of tubulin assembly (Lawrence et al., 2000, 2005; Hadfield et al., 2003; Rozmer et al., 2006), tyrosine kinase inhibition (Nakatani et al., 2005), trigger of apoptosis signaling pathway and etc. (Dimmock et al., 1998, 1999; Nakatani et al., 2005; Reddy et al., 2011). The major advantage of chalcone derivatives as cytotoxic agents is their low propensity to interact with DNA, and such propensity

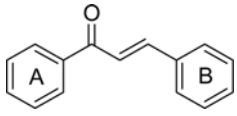

Fig. 1. General structure of the typical chalcone (1,3-diphenyl-2-propen-1-one).

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eliminates the risk of mutagenesity as the common side effect of current chemotherapeutic agents (Dimmock et al., 1999).

Recently, several researchers developed the potent anticancer hybrid molecules of the chalcone type through the introduction of some cytotoxic heterocyclic pharmacophores such as coumarin and stilbene derivatives (Belluti et al., 2010; Sashidhara et al., 2010a). These heterocycle-chalcone hybrids have found much attention and demonstrated promising antitumor effects (Reddy et al., 2008; Belluti et al., 2010; Sashidhara et al., 2010a). On the other hand, Chromene (2*H*-1-benzopyran) derivatives have been widely employed as important intermediates in the synthesis of many natural products and medicinal agents. This important class of compounds received profound attention as a potent anticancer and apoptosis-inducing scaffold with different proposed mechanisms (Zhou et al., 2007; Huang et al., 2009; Mayur et al., 2009; Heo et al., 2011). The chemical structures of some naturally occurring chromene derivatives such as acronicine (Hughes et al., 1948) and phaseollidin (Gunatilaka et al., 1994) are demonstrated in Fig. 2.

According to these findings and in continuation of our work on chalcone derivatives (Foroumadi et al., 2010; Nazarian et al., 2010), herein we focused our attention on two types of regioisomeric chromene-chalcones hybrids (type **A** and type **B**) as novel cytotoxic agents. We have previously reported the antileishmanial activity of some of these compounds (Foroumadi et al., 2010; Nazarian et al., 2010); however, according to our interest in the design of potent cytotoxic agents (Alizadeh et al., 2010; Mahmoodi et al., 2010; Akbarzadeh et al., 2012) and the cytotoxic activity potential of chromene and chalcone derivatives, we have further investigated the cytotoxic activity of these derivatives. As breast cancer is one of the most commonly diagnosed cancer and the leading cause of cancer deaths in women worldwide today (Sharma et al., 2010), many molecules based on the chromene ring system including coumarins have been synthesized and found to be useful in antiproliferative activity against breast cancer (Mao et al., 2009; Sashidhara et al., 2010b). Accordingly, we also expect to incorporate a chromene ring instead of aryl moieties of chalcones to investigate their primary biological activity against MCF-7 (estrogen receptor-positive) and MDA-MB-231 (estrogen receptor-negative) breast cancer cell lines.

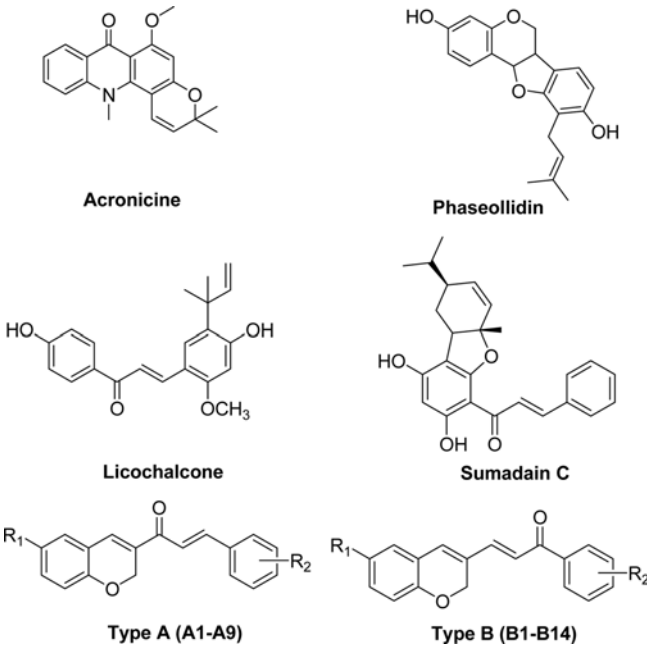


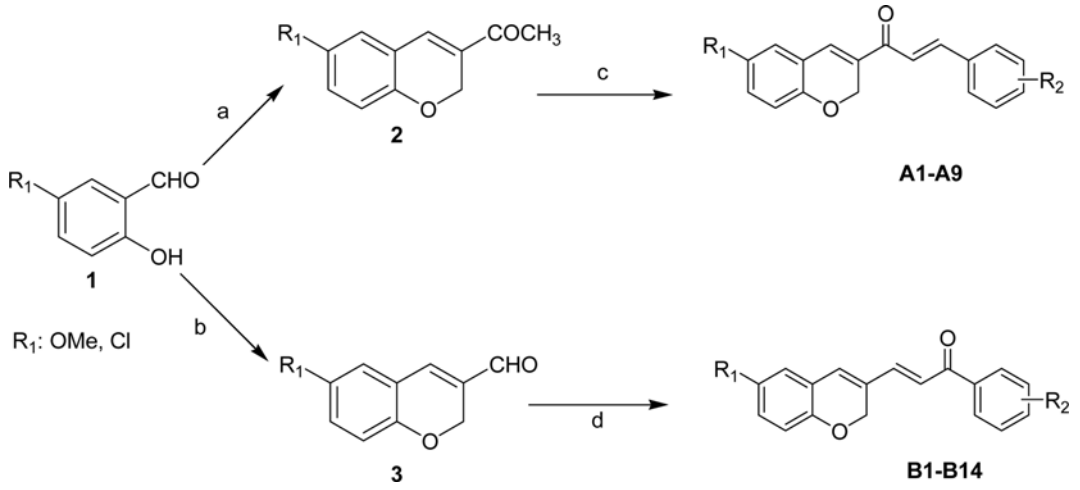
Fig. 2. Chemical structures of some naturally occurring chromenes (Acronicine and phaseollidin) and chalcones [Licochalcone (Park et al., 1998) and Sumadain C (Liu et al., 2009)] with potent anticancer activity and general structure of two types of designed regioisomeric chromene-chalcones Type **A** and Type **B**.

Thus, the synthesis of some new compounds and *in vitro* cytotoxic activity of both regioisomeric chromene-based chalcones are investigated in this article. We also examined the effects of structural parameters on the cell proliferation inhibitory activity of designed compounds in each cell line by means of linear quantitative structure-activity relationship (QSAR) models.

MATERIALS AND METHODS

Chemistry

All chemicals and solvents used in this study were purchased from Merck Chemical. The general procedures for synthesis of type **A** and type **B** of chromene-chalcone hybrids are illustrated in Scheme 1. Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The IR spectra were obtained on a Shimadzu 470 spectrophotometer (potassium bromide disks). ^{1}H-NMR spectra were recorded using a Bruker 500 spectrometer and chemical shifts are reported in parts per million (ppm) relative to TMS as the internal standard. Elemental analyses were carried out on CHN–O rapid elemental analyzer (GmbH) for C, H and N, and the results are within ± 0.4% of the theoretical values. Merck silica gel F254 plates were used for analytical TLC. Column chromatography was performed on Merck silica gel (70–230 mesh). Yields were calculated for purified products and were not optimized. The intermediates **2** and **3** were prepared according to the literature methods (Foroumadi et al., 2010; Nazarian et al., 2010). The physicochemical pro-



Scheme 1. General pathways for synthesis of two regioisomers of chromene-chalcone derivatives. *Reagents and conditions:* (**a**) Methyl vinylketone, 1,4-dioxane, K_2CO_3, reflux; (**b**) Acrolein, 1,4-dioxane, K_2CO_3, reflux; (**c**) Appropriate aldehyde derivatives, NaOH, EtOH; (**d**) Acetophenone derivatives, NaOH, EtOH.

perties, ^{1}H-NMR and IR spectra of compounds **A1**, **A3**-**A9**, **B2**-**B4** and **B9**-**B14** have been previously reported (Foroumadi et al., 2010; Nazarian et al., 2010).

Typical procedure for synthesis of type A compounds

A solution of NaOH (3.5 M, 2 mL) was added to the mixture of compound **2** (1 mmol) and substituted benzaldehyde (1 mmol) in absolute ethanol (5 mL). The mixture was stirred in an ice bath overnight. The reaction mixture was diluted with water; the precipitate was filtered and crystallized from ethanol to give corresponding derivatives type **A**.

(2*E*)-1-(6-Chloro-2*H*-chromen-3-yl)-3-(4-(methylthio)prop-2-en-1-one (A2)

Yeild 74%; mp 128-129°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.70 (d, 1H, J = 15.5 Hz, H_3 propenone), 7.54 (d, 1H, J = 9 and 2.5 Hz, H_2 and H_6 phenyl), 7.35 (brs, 1H, H_4 chromene), 7.16 (d, 1H, J = 15.5 Hz, H_2 propenone), 7.25 (d, 2H, J = 9 and 2.5 Hz, H_3 and H_5 phenyl), 7.21 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.18 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.82 (d, 1H, J = 8.5 Hz, H_8 chromene), 5.12 (s, 2H, OCH_2), 2.53 (s, 3H, SMe). MS (m/z, %): 342 (M^+, 100), 232 (25), 177 (33), 165 (42), 149 (51), 134 (82), 116 (30), 102 (70), 89 (23), 75 (25), 54 (24). IR (KBr, cm^{-1}) ν_{max}: 1647 (C=O), 1175 (C-O). Anal. Calcd for $C_{19}H_{15}ClO_2S$: C, 66.56; H, 4.41. Found: C, 66.40; H, 4.4.50.

General procedure for synthesis of type B compounds

The solution of compound **3** (1 mmol) and appropriate acetophenone (1 mmol) in absolute ethanol (5 mL) was treated with 3.5 M NaOH solution (2 mL) and stirred overnight in an ice bath. The reaction mixture was diluted with water and the precipitate was filtered and crystallized from ethanol to give the corresponding type **B** compounds.

(2*E*)-3-(6-Chloro-2*H*-chromen-3-yl)-1-phenylprop-2-en-1-one (B1)

Yeild: 83%; mp 72-73°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.87 (dt, 2H, J = 7 and 1 Hz, H_2 and H_6 phenyl), 7.58 (dt, 2H, J = 7 and 1 Hz, H_3 and H_5 phenyl), 7.12 (d, 1H, J = 15.5 Hz, H_3 propenone), 7.34 (t, 1H, J = 7 Hz, H_4 phenyl), 7.14 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.08 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.87 (d, 1H, J = 15.5 Hz, H_2 propenone), 6.80 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.78 (brs, 1H, H_4 chromene), 5.09 (s, 2H, OCH_2). MS (m/z, %): 298 (M+2, 14), 296 (M^+, 55), 202 (14), 190 (35), 165 (14), 128 (20), 105 (100), 77 (41). IR (KBr, cm^{-1}) ν_{max}: 1657 (C=O), 1217 (C-O). Anal. Calcd for $C_{18}H_{13}ClO_2$: C, 72.85; H, 4.42. Found: C, 72.95; H, 4.55.

(2*E*)-3-(6-Chloro-2*H*-chromen-3-yl)-1-(1,2,3,4-tetrahydronaphthalen-6-yl)prop-2-en-1-one (B5)

Yeild 38%, mp 192-193°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.70-7.67 (m, 1H, H_5 tetrahydronaphthalene), 7.61-7.57 (m, 1H, H_7 tetrahydronaphthalene), 7.49 (d, 1H, J = 16 Hz, H_3 propenone), 7.18 (d, 1H, J = 8.5 Hz, H_8 tetrahydronaphthalene), 7.13 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.08 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.86 (d, 1H, J = 16 Hz, H_2 propenone), 6.79 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.75 (brs, 1H, H_4 chromene), 5.09 (s, 2H, OCH_2), 2.90-2.80 (m, 4H, H_1 and H_4 tetrahydronaphthalene), 1.59-1.58 (m, 4H, H_2 and H_3 tetra-

hydronaphthalene). MS (m/z, %): 353 (M+2, 30), 351 (M^+, 100), 218 (5), 192 (8), 160 (30), 91 (10). IR (KBr, cm^{-1}) ν_{max}: 1651 (C=O), 1233 (C-O). Anal. Calcd for $C_{22}H_{19}ClO_2$: C, 75.32; H, 5.46. Found: C, 75.55; H, 5.31.

(2*E*)-3-(6-Chloro-2*H*-chromen-3-yl)-1-(4-cyanophenyl)prop-2-en-1-one (B6)

Yeild 57%; mp 266-268°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.04 (d, 2H, J = 8.5 Hz, H_2 and H_6 phenyl), 7.81 (d, 2H, J = 8.5 Hz, H_3 and H_5 phenyl), 7.54 (d, 1H, J = 16 Hz, H_3 propenone), 7.16 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.10 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.83 (brs, 1H, H_4 chromene), 6.81 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.79 (d, 1H, J = 16 Hz, H_2 propenone), 5.08 (s, 2H, OCH_2). MS (m/z, %): 326 (M+4, 10), 324 (M+2, 24), 322 (M^+, 76), 286 (7), 240 (6), 227 (5), 191 (100), 165 (25), 128 (72), 102 (91), 75 (40). IR (KBr, cm^{-1}) ν_{max}: 1664 (C=O). Anal. Calcd for $C_{19}H_{12}ClNO_2$: C, 70.92; H, 3.76; N, 4.35. Found: C, 70.74; H, 4.62; N, 4.11.

(2*E*)-3-(6-Chloro-2*H*-chromen-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (B7)

Yeild 88%; mp 212-213°C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.92 (dd, 2H, J = 9 and 2 Hz, H_2 and H_6 phenyl), 7.81 (dd, 2H, J = 9 and 2 Hz, H_3 and H_5 phenyl), 7.52 (d, 1H, J = 16 Hz, H_3 propenone),7.14 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.09 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.81 (d, 1H, J = 16 Hz, H_2 propenone), 6.80 (d, 1H, J = 8.5 Hz, H_8 chromene), 6.73 (s, 1H, H_4 chromene), 5.08 (s, 2H, OCH_2). MS (m/z, %): 330 (M^+, 55), 191 (100), 165 (27), 139 (92), 127 (28), 111 (55), 75 (32). IR (KBr, cm^{-1}) ν_{max}: 1649 (C=O). Anal. Calcd for $C_{18}H_{12}Cl_2O_2$: C, 65.28; H, 3.65. Found: C, 65.48; H, 3.53.

(2*E*)-3-(6-Chloro-2*H*-chromen-3-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (B8)

Yeild 54%; mp 224-225°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.99 (d, 2H, J = 8.9 Hz, H_2 and H_6 phenyl), 7.50 (d, 1H, J = 15.65 Hz, H_3 propenone), 7.14 (dd, 1H, J = 8.5 and 2.5 Hz, H_7 chromene), 7.09 (d, 1H, J = 2.5 Hz, H_5 chromene), 6.99 (d, 2H, J = 8.9 Hz, H_3 and H_5 phenyl), 6.88 (d, 1H, J = 15.65 Hz, H_2 propenone), 6.80 (d, 1H, J = 8.9 Hz, H_8 chromene), 6.76 (brs, 1H, H_4 chromene), 5.01 (s, 2H, OCH_2), 3.91 (s, 3H, OMe). MS (m/z, %): 326 (M^+, 81), 191 (32), 165 (15), 135 (100), 107 (16), 92 (37), 77 (52), 64 (18). IR (KBr, cm^{-1}) ν_{max}: 1662 (C=O), 1219 (C-O). Anal. Calcd for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63. Found: C, 69.76; H, 4.88.

MTT-based cytotoxiciy assay

Cell viability following exposure to synthetic compounds was estimated by using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay (Mosmann, 1983). Breast cancer cell lines (MDA-MB-231 and MCF-7) were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco BRL) and 100 μg/mL streptomycin and 100 U/mL penicillin at 37°C in a humidified atmosphere with 5% CO_2 in air. Cultures in the exponential growth phase were trypsinized and diluted in complete growth medium to give a total cell count of 5 × 10^4 cells/mL. 195 μL of suspension was added to wells of sterile 96-well plates (NUNC) and allowed to attach for overnight. After plating, 5 μL of a serial dilution of every compound was added. Each compound dilution was assessed in triplicate. Etoposide was used as a positive control for cytotoxiciy while three wells containing tumor cells cultured in 200 μL of complete medium and 0.5% DMSO was used as controls for cell viability. Compounds were all first dissolved in DMSO and then diluted in the growth medium. The maximum concentration of DMSO in the wells was 0.5%. Cells were further incubated for 48 h and at the end of the incubation time; the medium was replaced with fresh medium containing 0.5 mg/mL of MTT (Sigma-Aldrich). Plates were incubated for another 4 h at 37°C. Then the Formazan crystals formed in the cells dissolved in DMSO (100 μL). Then the absorbance of each well was measured by using a microplate reader (Power wave XS2) at 492 nm wavelengths. The percentage inhibition of viability for each concentration of compound was calculated compared to the control wells and IC_{50} values (concentration of the compound that induces 50% inhibition of cell viability) were calculated by linear regression analysis, expressed in mean ± S.D.

QSAR analysis

Descriptor generation

The chemical structure of molecules was constructed by using HyperChem (Version 7, Hypercube Inc., http://www.hyper.com). The Z-matrices of the structures were provided by the software and were then transferred to the Gaussian 98 program (Frisch et al., 1998). Complete geometry optimization was performed by taking the most extended conformations as starting geometries. Semi-empirical molecular orbital calculations (AM1) of the structures were performed by using the Gaussian 98 program.

A large number of molecular descriptors were calculated by using HyperChem, Gaussian 98 and Dragon (Todeschini http://www.disat.unimib.it/vhm/) Packages. Gaussian 98 was employed to calculate different quantum chemical descriptors including dipole moment

(DM), local charges, HOMO and LUMO energies, hardness (η); softness (S); electronegativity (χ); and electrophilicity (ω). Dragon software was used to calculate different descriptors including functional groups, topological, geometrical, constitutional and charge descriptors as well as aromaticity indices for each molecule. Some chemical parameters including molecular volume (MV), molecular surface area (SA), hydrophobicity (logP) and hydration energy (HE) were calculated by using HyperChem software.

Data processing and modeling

The calculated descriptors were collected in a data matrix, **D**. First the descriptors were checked for constant or near constant values and those detected were removed from the original data matrix. Then, the correlation of descriptors with each other and with the activity data was determined. Among the collinear descriptors detected (r >0.9), the one with the highest correlation with activity was retained and the rest were omitted.

MLR analysis with stepwise selection and elimination of variables was applied for developing QSAR models using SPSS software (SPSS Inc., version 17). The resulted models were validated by a leave-one out cross-validation procedure (using MATLAB software) to check their predictivity and robustness.

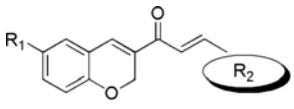
RESULTS AND DISCUSSION

Chemistry

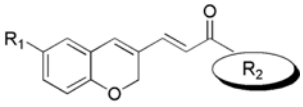
The general procedures for synthesis of type **A** (**A1**-**A9**) and type **B** (**B1**-**B14**) chromene-chalcone hybrids are presented in Scheme 1. These two series of chromene-based chalcones were prepared by slightly different established methods. Preparation of prototype **A** consists of two steps: treatment of 5-chloro or 5-methoxy-2-hydroxybenzaldehyde **1** with methyl vinyl-ketone in refluxing dioxane in the presence of potassium carbonate to afford 1-(6-chloro or 6-methoxy-2*H*-chromen-3-yl)ethanone **2**. Claisen-Schmidt condensation of compound **2** with different aldehydes in ethanolic solution of NaOH yielded corresponding 1-(6-chloro or 6-methoxy-2*H*-chromen-3-yl)propen-1-ones **A1**-**A9**. Type **B** of chromene-chalcone derivatives is prepared by reaction of 5-chloro or 5-methoxy-2-hydroxybenzaldehyde **1** by acrolein in refluxing dioxane in the presence of potassium carbonate to afford corresponding chromene-3-carbaldehyde **3** as the intermediate product. Claisen-Schmidt condensation of compound **3** with various acetophenones in ethanolic solution of NaOH afforded 3-(6-chloro- or 6-methoxy-2*H*-chromen-3-yl)propen-1-ones (**B1**-**B14**). The structures of all intermediates and desired products were confirmed with IR, ^{1}H-NMR and mass spectroscopy analysis methods.

The chemical structures of test compounds are presented in Table I.

Table I. Chemical structure and cytotoxic activity of synthetic compounds assessed by the MTT reduction assay



Type A (A1-A9)



Type B (B1-B14)

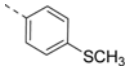
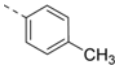
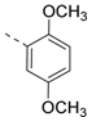
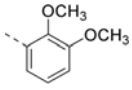
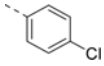
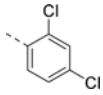
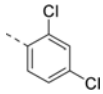
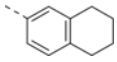
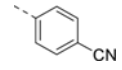
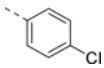
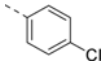
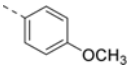
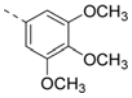
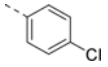
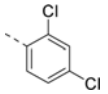
Compound	R_1	R_2	IC_{50} (μM)	
			MDA-MB-231	MCF-7
A1	Cl		7.02 ± 0.34	14.50 ± 1.92
A2	Cl		26.01 ± 1.99	27.97 ± 2.91
A3	Cl		7.97 ± 0.82	14.19 ± 0.74
A4	Cl		55.53 ± 6.83	48.90 ± 3.45
A5	Cl		9.83 ± 0.48	66.94 ± 1.78
A6	CH_3O		39.38 ± 0.25	14.97 ± 2.40
A7	CH_3O		8.04 ± 0.29	17.84 ± 1.39
A8	CH_3O		14.08 ± 1.23	22.96 ± 1.53
A9	CH_3O		69.81 ± 2.68	>100
B1	Cl		26.05 ± 1.32	>100
B2	Cl		>100	>100
B3	Cl		20.64 ± 0.29	>100
B4	Cl		22.15 ± 2.32	>100
B5	Cl		>100	>100
B6	Cl		>100	>100
B7	Cl		>100	>100

Table I. Continued

Compound	R_1	R_2	IC_{50} (μM)	
			MDA-MB-231	MCF-7
B7	Cl		>100	>100
B8	Cl		>100	>100
B9	Cl		9.35 ± 1.71	38.65 ± 3.82
B10	CH_3O		>100	>100
B11	CH_3O		30.47 ± 2.94	>100
B12	CH_3O		>100	>100
B13	CH_3O		>100	>100
B14	CH_3O		>100	>100
Etoposide	-	-	34.86 ± 2.47	31.31 ± 1.55

Cytotoxic activity

The cytotoxic activities of both series of synthesized compounds (**A1**-**A9** and **B1**-**B14**) were evaluated by MTT reduction assay against MCF-7 (breast adenocarcinoma) and MDA-MB-231 (human breast cancer) cell lines. Data are demonstrated in Table I. Most of compounds in prototype **A** showed moderate to strong cytotoxic activity (7 μM < IC_{50} valuses < 70 μM), especially against MDA-MB-231 cell line. The comparison of IC_{50} values of type **A** with those of etoposide revealed that the growth inhibitory potential of test compounds is more than that of the reference drug etoposide in most cases. It is also clear that type **A** of chromene-chalcone derivatives in which the phenyl ring is closed to the double bond of propenone linkage (**A1**-**A9**) are more potent than type **B** series (**B1**-**B14**) in the most cases. The results indicate that the cytotoxic activity of compounds in both series is partly influenced by the type of substitutes positioned on the phenyl ring attached to 1- or 3-position of the propenone linker. The most active compounds of type **A** chromene-chalcones against MDA-MB-231 cell line appeared to be 3-bromophenyl derivative **A1** (IC_{50} = 7.02 μM) and 4-methylphenyl analog **A3** (IC_{50} = 7.97 μM). Compound **A1** is also among the most potent derivatives against MCF-7 cell line with IC_{50} value of 14.50 μM. The growth inhibitory activity of this compound is 2 to 5-fold higher than that of etoposide.

The IC_{50} values of Type **B** compounds against MDA-MB-231 cells revealed that 6-chlorochromene derivatives **B1**, **B3**, **B4**, **B9** and 6-methoxychromene analog **B11** showed good activity at concentrations of less than 30 μM. Among this type of derivatives, the **B9** containing three methoxy groups on the phenyl ring demonstrated better cytotoxic profile in MDA-MB-231 cell line (IC_{50} = 9.35 μM). This compound with the IC_{50} value of 38.65 μM was the only active derivative of type B against MCF-7 cell line.

QSAR study

QSAR analysis of synthetic compounds (**A1-A9** and **B1-B14**) was carried out by MLR analysis and the resulted QSAR models (E1 and E2) are demonstrated in Table II.

The correlation coefficient (R^2), standard error of regression (SE), correlation coefficient for cross-validation significance (Q^2) and root mean square error (RMS) were employed to judge the validity of the regression equation. As colinearity degrades the performances of the MLR-based QSAR equation, correlation analysis was performed to detect the colinear descriptors (Mager, 1983). Therefore; the correlation of descriptors with each other and with activity data was evaluated and among the colinear descriptors one of them that represented the highest correlation with activity was retained and the rest were omitted.

Equation 1 (E1) was obtained for MDA-MB-231 cell line. Stepwise selection and elimination of variables produced a one-parametric QSAR equation, with moderate statistical quality (R^2 = 0.76, SE = 15.12, Q^2 = 0.67 and RMS_{CV} = 0.17). Selected variables demonstrated that "surface area" of chromen-based chalcones derivatives affect the cytotoxic activity of studied compounds in MDA-MB-231 cell line. The positive

Table II. Statistical equations obtained by QSAR analysis

Cell line	Equation	R^2	S.E[a]	F[b]	Q^2	RMScv	n[c]
MDA-MB-231	**E1:** Y = 0.12 (±0.02) SA + 5.05 (±0.06)	0.76	0.15	28.42	0.67	0.17	11
MCF-7	**E2:** Y = – 0.40 (±0.10) LUMO + 0.41 (±0.01) SA – 0.52 (±0.07) PHI + 6.72 (±0.25)	0.98	0.03	84.72	0.97	0.15	8

[a]Standard error of regression; [b]Fisher ration; [c]Number of compounds used for QSAR analysis.

Table III. Data of the selected descriptors used in this study and the experimental and predicted values of pIC_{50} in each cell line

Compound	Descriptors			Experimental pIC_{50}		Predicted pIC_{50}	
	SA	PHI	LUMO	MDA-MB-231	MCF-7	MDA-MB-231	MCF-7
A1	1.90	4.58	-0.16	5.15	4.84	5.27	4.73
A2	0.49	5.02	3.14	4.58	4.55	outlier	4.4
A3	-1.13	4.23	0.41	5.10	4.85	4.91	4.70
A4	-6.08	5.46	1.31	4.25	4.31	4.32	4.18
A5	0.31	5.46	-1.26	5.01	4.77	5.08	4.64
A6	-0.63	4.29	-0.12	4.40	4.82	outlier	outlier
A7	-1.42	4.72	0.04	5.09	4.75	4.88	4.68
A8	-2.57	4.72	-0.34	4.85	4.64	4.74	4.55
A9	-2.66	5.17	-1.38	4.17	-	outlier	
B1	-1.81	4.00	0.71	4.58	-	4.83	
B2	-0.84	4.88	0.25	-	-	-	
B3	-2.28	4.19	0.09	4.68	-	4.78	
B4	-3.10	4.44	1.63	4.65	-	4.68	
B5	-2.41	4.56	0.25	-	-	-	
B6	2.12	4.39	-1.35	-	-	-	
B7	0.41	4.44	-1.09	-	-	-	
B8	-1.66	4.72	1.12	-	-	-	
B9	-1.22	6.22	1.78	5.03	4.41	4.90	4.29
B10	-3.44	4.29	1.37	-	-	-	
B11	-4.25	4.72	-0.63	4.51	-	4.54	
B12	-2.27	4.72	1.31	-	-	-	
B13	-1.29	4.72	2.68	-	-	-	
B14	-0.25	5.17	-0.14	-	-	-	

relation of activity and "surface area" indicates that the substitution of groups with larger surface area on the phenyl ring of chromen-based chalcones resulted in increasing cytotoxic activity of compounds in MDA-MB-231 cell line.

When we used the pIC_{50} of the chromene-based chalcones against MCF-7 cell line as the dependant variable, equation E2 was obtained from the pool of calculated descriptors. This model with good statistical quality (R^2 = 0.98, SE = 0.03, Q^2 =0.97) indicated that the cytotoxic activity of compounds is affected by topological parameters (surface area and flexibility indices) and LUMO eigene value as representative of quantum chemical descriptors in this cell line (Equation E2). The aromaticity indices of compounds demonstrated the negative relation with the cytotoxic activity of the compound. Moreover, the effect of surface area of chromene-chalcone scaffold on the cytotoxic potential of compounds is similar to MDA-MB-231 cell line.

The data of selected descriptors used in this study together with the experimental and corresponding predicted value of activity of compounds as pIC_{50} (-Log IC_{50}) are listed in Table III. As an example, the respective predicted values of activity, refined from the calibration and cross-validation, by using equation 2 is plotted against the experimental values. As it could be seen, there is a close agreement between the experimental and predicted activities obtained by QSAR model in MCF-7cell line (Fig. 3).

In conclusion, two types of regioisomeric chromene-chalcone hybrids have been prepared and evaluated for their cytotoxic activity against breast cancer cell lines including MCF-7 and MDA-MB-231. Type **A** re-

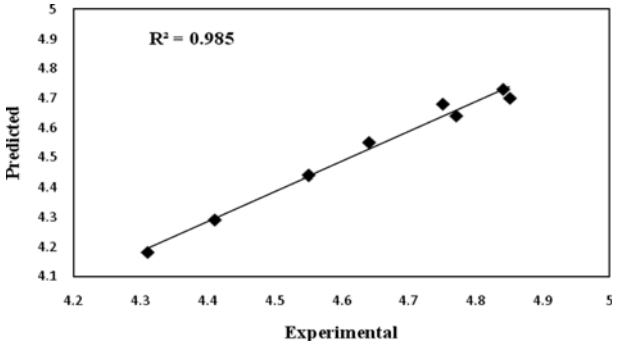


Fig. 3. Plot of the predicted activity against the experimental activity for the QSAR models obtained by Equation 2 in MCF-7 cell line.

gioisomer of chromene-chalcone hybrids, in which the chromene moiety is attached close to the carbonyl group of propenone linkage of chalconoid scaffold, demonstrated better cytotoxic profile than the type B regioisomer. The results indicated that the cytotoxic activity of both regioisomeric compounds is highly dependent on the type of substitutes positioned on the phenyl ring of chalconoid scaffold. Quantitative analysis of structure and cytotoxic activity relationships of these novel compounds demonstrated that topological parameters are among the important descriptors that should be considered in design of potent cytotoxic derivatives of chromene-chalcone hybrids. The notable cytotoxic activity profile of 1-(6-chloro or 6-methoxy-2*H*-chromen-3-yl)-3-phenyl-prop-2-en-1-ones along with valuable QSAR equation suggest this novel type of regioisomeric hybrids as promising chemotherapeutic agents for further optimization. The obtained QSAR equation can be used for prediction of the cytotoxic activity of further novel derivatives of this type. It is important that the obtained topological parameters are considered as important structural parameters in the design of future chromene-chalcone derivatives.

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