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Research Letter

Design, Synthesis, and Cytotoxicity of Novel 3-Arylidenones Derived from Alicyclic Ketones

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Forty-four novel chalcone-inspired analogs having a 3-aryl-2-propenoyl moiety derived from alicyclic ketones were designed, synthesized, and investigated for cytotoxicity against murine B16 and L1210 cancer cell lines. The analogs belong to four structurally divergent series, three of which (series g, h, and i) contain differently substituted cyclopentanone units and the fourth (series j) contains a 3,3-dimethyl-4-piperidinone moiety. Of these, the analogs in series j showed potential cytotoxic activity against murine B16 (melanoma) and L1210 (lymphoma) cells. The most active compounds 5j, 11j, 15j, and 12h produced IC_{50} values from 4.4 to 15 μM against both cell lines. A single-crystal X-ray structure analysis and molecular modeling studies confirmed that these chalcones have an *E*-geometry about the alkene bond and possess a slightly 'twisted' conformation similar to that of combretastatin A-4. At a concentration of 30 μM, compounds 5j, 11j, and 15j did not cause microtubule depolymerization in cells, suggesting that they have a different mechanism of action.

Key words: 3-aryl-2-propenoyl, chalcones, combretastatin A-4, cyclopentanone, cytotoxicity, tubulin

Despite recent advances in the understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to help bring this disease under control. Among the recently identified antitumor agents, combretastatins and chalcones represent important classes of molecules. Combretastatin A-4 (CA-4 or **1** as shown in Figure 1) is a natural product, which consists of a core *cis*-stilbene moiety. It originates from the African willow tree, *Combretum caffrum*, and is known to inhibit tubulin polymerization via interaction with the colchicine binding site of tubulin (1–4). Like combretastatins, chalcones (**2**, Figure 1) also derive their antitumor properties through the inhibition of tubulin polymerization (5–8). Chalcones differ from combretastatin by having a *trans*-enone moiety between the aromatic rings (Figure 1). This 1,3-diarylprop-1-enone system is essential for chalcones to elicit their cytotoxic properties. The ease of synthesis of chalcones, from substituted benzaldehydes and acetophenones, makes them an attractive drug scaffold. Many of the chalcone analogs exhibit powerful anticancer activities, causing renewed interest in this class of molecules (9–15).

Structurally, chalcones are open-chained molecules bearing two aromatic rings linked by a three-carbon enone pharmacophore. We envisaged that the attachment of this 3-aryl-2-propenoyl pharmacophore to alicyclic scaffolds would lead to additional series of compounds, which may exhibit cytotoxic activities against malignant cells. This rationale was inspired by several reports that described superior cytotoxic effects of the cyclic chalcone analogs (**3**, Figure 2) (16–21). Some of these analogs were found to display significant CYP1A inhibitor activity (22,23).

This study was aimed at preparing analogs that contained a 3-aryl-2-propenoyl core structure **4** (Figure 2) and at investigating their ability to inhibit the proliferation of cancer cells in culture. A diverse group of compounds were included in this study because earlier structure–activity studies on cyclic chalcone analogs have revealed that the cytotoxicity of these compounds was influenced by the shape of the molecules (22,23). Accordingly, four structurally divergent series of compounds, each differing significantly in the substitution pattern of cyclic ketone moiety, were synthesized and evaluated for their cytotoxic activity against murine B16 and L1210 cancer cell lines. Selected molecules were also assayed in comparison with a known tubulin inhibitor, CA-4, for their ability to cause microtubule depolymerization in A-10 cells.

Material and Methods

Solvents and organic reagents were purchased from Aldrich and used without further purification. Melting points (mp) were determined using a Büchi B-545 melting point apparatus, and the results were uncorrected. Infrared (IR) spectra were recorded on a Midac M1700 FT-IR instrument as films on KBr disks, unless stated otherwise. Proton NMR spectra were recorded on a Varian INOVA

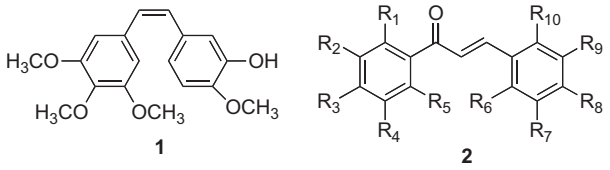


Figure 1: Structures of combretastatin A-4 and chalcone.

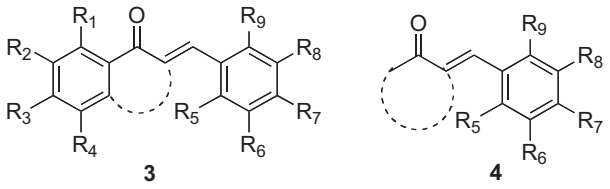


Figure 2: Structures of cyclic chalcone analogs.

400 MHz Fourier transform spectrometer. Chemical shifts are quoted in parts per million downfield from tetramethylsilane (TMS). Low- and high-resolution mass spectra were provided by the Mass Spectrometry Laboratory, University of South Carolina, Columbia, SC. Reactions were monitored by thin-layer chromatography (TLC) using commercially available precoated plates (Merck Kieselgel 60 F254 silica), and developed plates were examined under UV light (254 nm) or using iodine vapor staining. Column chromatography was performed using 200-mesh silica gel. Crystallographic data for compound **10j** were collected using a Bruker AXS SMART APEX CCD diffractometer with monochromatic Mo Kα radiation (Wavelength λ = 0.71073 Å) with the Ω scan technique. The crystal was mounted on a Mitegen micromesh mount using a trace of mineral oil and cooled *in situ* to 100(2) K for data collection. Cell refinement, data reduction, and absorption corrections were carried out using APEX2. The structure was solved by direct methods using SHELXTL 6.14 and was refined by full-matrix least-squares calculations on F^2 with SHELXTL. Non-hydrogen atoms were refined with anisotropic displacement parameters. Carbon-bound hydrogen atoms were placed in geometrically idealized positions with $U_{iso}(H)$ = 1.2–1.5 $U_{eq}(C)$. The position of the amine H atom was refined; $U_{iso}(H)$ was set to 1.2 $U_{eq}(N)$.

General procedure for the synthesis of chalcone analogs in the series g and h

Sodium hydroxide solution (3% in ethanol, 2.0 mL) was added dropwise to the solution of bicyclo[2.2.1]heptan-2-one (for series **g**) or 2-methylcyclopentanone (for series **h**) (1.0 mmol) and the appropriate aryl aldehyde (1.0 mmol) in ethanol (4.0 mL) at room temperature. The reaction mixture was stirred at room temperature for 12 h. The reaction was monitored by TLC using 20% ethyl acetate in hexane as the eluent system. Upon completion of the reaction, water (10.0 mL) was added to the reaction mixture and stirred for 10 min. The reaction mixture was then extracted with ethyl acetate (2 × 15.0 mL). The ethyl acetate layer was washed with water (2 × 10.0 mL), dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator to obtain the crude product, which was purified by column chromatography using silica gel and 10% ethyl acetate in hexane as the eluent system. The spectral characterization of compounds that were selected for detailed cytotoxicity studies is given below.

3-(3-Methoxybenzylidene)bicyclo[2.2.1]heptan-2-one (6g)

Yield: 77%; white solid; mp: 93–95 °C; FTIR (KBr, /cm) 1727, 1643, 1596, 1577, 1434, 1266, 1207, 1092, 942, 916, 810, 739; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (t, 1H, J = 8.0 Hz), 7.12 (s, 1H), 7.08 (dt, 1H, J = 1.0, 8.0 Hz), 7.01 (dd, 1H, J = 1.0, 2.6 Hz), 6.91 (ddd, 1H, J = 1.0, 2.6, 8.0 Hz), 3.83 (s, 3H), 3.64 (m, 1H), 2.77 (m, 1H), 2.09–1.99 (m, 2H), 1.78–1.63 (m, 4H); MS (ESI, *m/z*) 229 ($M+H^+$).

3-(4-Chlorobenzylidene)bicyclo[2.2.1]heptan-2-one (9g)

Yield: 65%; white solid; mp: 88–90 °C; FTIR (KBr, /cm) 1724, 1640, 1490, 1087, 914, 830, 788, 719; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (d, 2H, J = 8.0 Hz), 7.37 (d, 2H, J = 8.0 Hz), 7.09 (s, 1H), 3.58 (m, 1H), 2.80 (m, 1H), 2.08–1.97 (m, 2H), 1.79–1.62 (m, 4H); MS (EI, *m/z*) 232 (M^+); HRMS *m/z* calc. for $C_{14}H_{13}ClO$ 232.0655, found 232.0657.

3-(3,4,5-Trimethoxybenzylidene)bicyclo[2.2.1]heptan-2-one (11g)

Yield: 55%; light yellow solid; mp: 57–60 °C; FTIR (KBr, /cm) 1725, 1639, 1593, 1472, 1255, 1111, 943, 776, 732; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28 (s, 1H), 6.73 (s, 2H), 3.89 (s, 9H), 3.64 (m, 1H), 2.79 (m, 1H), 2.06–1.90 (m, 2H), 1.80–1.68 (m, 4H); MS (ESI, *m/z*) 289 ($M+H^+$).

3-(3-Bromobenzylidene)bicyclo[2.2.1]heptan-2-one (14g)

Yield: 64%; white solid, mp: 102–105 °C; FTIR (KBr, /cm) 1732, 1650, 1558, 1447, 1241, 1098, 954, 767, 719; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (t, 1H, J = 1.6 Hz), 7.47 (dd, 1H, J = 1.6, 8.0 Hz), 7.39 (dd, 1H, J = 1.6, 8.0 Hz); 7.27 (t, 1H, J = 8.0 Hz), 7.06 (s, 1H), 3.59 (m, 1H), 2.78 (m, 1H), 2.09–1.95 (m, 2H), 1.79–1.64 (m, 4H); MS (ESI, *m/z*) 277 ($M+H^+$).

3-(5-Bromo-2-methylbenzylidene)bicyclo[2.2.1]heptan-2-one (15g)

Yield: 64%; white solid; mp: 86–89 °C; FTIR (KBr, /cm) 1719, 1599, 1432, 1067, 979, 749, 722; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (d, 1H, J = 2.0 Hz), 7.36 (dd, 1H, J = 2.0, 8.0 Hz), 7.21 (s, 1H), 7.08 (d, 1H, J = 8.0 Hz), 3.45 (m, 1H), 2.81 (m, 1H), 2.31 (s, 3H), 2.07–1.94 (m, 2H), 1.78–1.64 (m, 4H); MS (ESI, *m/z*) 291 ($M+H^+$).

2-(4-Fluorobenzylidene)-5-methylcyclopentanone (12h)

Yield: 58%; yellow solid; mp: 60–64 °C; FTIR (KBr, /cm) 1706, 1631, 1600, 1507, 1415, 1229, 1101, 835, 912; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (dd, 2H, J_{H-H} = 8.7 Hz, J_{H-F} = 5.4 Hz), 7.39 (s, 1H); 7.11 (dd, 2H J_{H-H} = 8.7 Hz, J_{H-F} = 8.6 Hz), 2.96–2.88 (m, 1H), 2.84–2.64 (m,

1H), 2.42–2.23 (m, 2H), 1.58–1.52 (m, 1H), 1.18 (d, 3H, *J* = 6.4 Hz); MS (EI, *m/z*) 204 (M^+, 100); HRMS *m/z* calc. for $C_{13}H_{13}FO$ 204.0948, found 204.0950.

General procedure for the synthesis of chalcone analogs in the series i

To a solution of the appropriate 2-arylidene-5-methylcyclopentanone **5h**–**15h** (1 mmol) in dry tetrahydrofuran (5.0 mL) was added potassium *tert*-butoxide (2 mmol) and stirred for 30 min at room temperature. To the reaction mixture was then added methyl iodide (1.2 mmol) and stirred at room temperature for 12 h. The reaction was monitored and worked up in the same procedure as described for series **g** and **h**, and the characterization data for 'active' compounds according to the cytotoxicity screen are given below.

2-(2-Chlorobenzylidene)-2,2-dimethylcyclopentanone (8i)

Yield: 68%; white solid; mp: 82–85 °C; FTIR (KBr, /cm) 1717, 1626, 1476, 1436, 1183, 1095, 989, 766, 745; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.75 (t, 1H, *J* = 8.0 Hz), 7.56–7.53 (m, 1H), 7.45–7.43 (m, 1H), 7.33–7.28 (m, 2H), 2.82 (dt, 2H, *J* = 4.0, 8.0 Hz), 1.84 (t, 2H, *J* = 8.0 Hz), 1.15 (s, 6H); MS (ESI, *m/z*) 235 ($M+H^+$).

5-(4-Chlorobenzylidene)-2,2-dimethylcyclopentanone (9i)

Yield: 71%; off-white solid; mp: 110–114 °C; FTIR (KBr, /cm) 1706, 1623, 1492, 1265, 1183, 1089, 1012, 988, 735, 705; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.48 (dt, 2H, *J* = 2.6, 8.0 Hz), 7.38 (d, 2H, *J* = 8.0 Hz), 7.36 (t, 1H, *J* = 2.6 Hz), 2.86 (dt, 2H, *J* = 4.0, 8.0 Hz), 1.86 (t, 2H, *J* = 8.0 Hz), 1.14 (s, 6H); MS (EI, *m/z*) 234 (M^+); HRMS *m/z* calc. for $C_{14}H_{15}ClO$ 234.0811, found 234.0807.

5-(2,6-Dimethoxybenzylidene)-2,2-dimethylcyclopentanone (10i)

Yield: 60%; white solid; mp: 78–81 °C; FTIR (KBr, /cm) 1711, 1628, 1593, 1472, 1254, 1119, 1106, 990, 887, 776, 751; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51 (t, 1H, *J* = 2.7 Hz), 7.28 (t, 1H, *J* = 8.0 Hz), 6.57 (d, 2H, *J* = 8.0 Hz), 3.84 (s, 6H), 2.48 (dt, 2H, *J* = 4.0, 8.0 Hz), 1.72 (t, 2H, *J* = 8.0 Hz), 1.13 (s, 6H); MS (ESI, *m/z*) 261 ($M+H^+$).

General procedure for the synthesis of chalcone analogs in the series j

To the solution of *tert*-butyl 3,3-dimethyl-4-piperidone-1-carboxylate **j** (1 mmol) and the appropriate aryl aldehyde (1 mmol) in ethanol (5.0 mL) at room temperature was added sodium hydroxide solution (10%, 2.0 mL), and the reaction mixture was stirred at room temperature for 12 h. The reaction was monitored by TLC using 20% ethyl acetate in hexane as the eluent system. On completion of the reaction, the precipitated solid was filtered off and washed with cold ethanol (3.0 mL). The solid was dried and then stirred with 20% HCl in dioxane (5.0 mL) for 3 h at room temperature. The precipitate was filtered and washed with cold methanol (3.0 mL). It was then stirred in saturated sodium carbonate solution (10.0 mL) for 30 min and again filtered, washed with water, and dried. The crude compound was purified by column chromatography using silica gel and 40% ethyl acetate in hexane as the eluent system. The spectral characterization of compounds selected for cytotoxicity studies is given below.

5-Benzylidene-3,3-dimethylpiperidin-4-one (5j)

Yield: 57%; pale yellow solid; mp: 89–93 °C; FTIR (KBr, /cm) 1693, 1594, 1421, 1265, 895, 739, 704; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (s, 1H), 7.46–7.42 (m, 3H), 7.33–7.30 (m, 2H), 4.41 (s, 2H), 3.31 (s, 2H), 1.37 (s, 6H); MS (EI, *m/z*) 215 (M^+); HRMS *m/z* calc. for $C_{14}H_{17}NO$ 215.1310, found 215.1314.

5-(3-Methoxybenzylidene)-3,3-dimethylpiperidin-4-one (6j)

Yield: 67%; pale yellow solid; mp: 98–101 °C; FTIR (KBr, /cm) 1705, 1682, 1597, 1489, 1317, 1160, 1048, 993, 781; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.39–7.33 (m, 1H), 7.24 (s, 1H), 7.03–6.95 (m, 3H), 3.94 (s, 2H), 3.77 (s, 3H), 2.81 (s, 2H), 1.53 (s br, 1H), 1.07 (s, 6H); MS (ESI, *m/z*) 246 ($M+H^+$).

5-(2-Chlorobenzylidene)-3,3-dimethylpiperidin-4-one (8j)

Yield: 71%; off-white solid; mp: 89–92 °C; FTIR (KBr, /cm) 1687, 1605, 1469, 1436, 1317, 1150, 1054, 970, 762, 737; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.46–7.45 (m, 1H), 7.44–7.41 (m, 1H), 7.28–7.25 (m, 2H), 7.16–7.13 (m, 1H), 3.88 (s, 2H), 2.96 (s, 2H), 1.73 (s br, 1H), 1.20 (s, 6H); MS (ESI, *m/z*) 250 ($M+H^+$).

5-(4-Chlorobenzylidene)-3,3-dimethylpiperidin-4-one (9j)

Yield: 55%; off-white solid; mp: 142–144 °C; FTIR (KBr, /cm) 1711, 1684, 156, 1283, 1095 1013, 811, 745; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.39 (s, 1H), 7.36 (d, 2H, *J* = 8.0 Hz), 7.23 (d, 2H, *J* = 8.0 Hz), 4.02 (s, 2H), 2.97 (s, 2H), 1.21 (s, 6H); MS (ESI, *m/z*) 250 ($M+H^+$).

5-(2,6-Dimethoxybenzylidene)-3,3-dimethylpiperidin-4-one (10j)

Yield: 57%; off-white solid; mp: 108–111 °C; FTIR (KBr, /cm) 1681, 1602, 1582, 1471, 1254, 1180, 778, 747; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30 (s, 1H), 7.27 (t, 1H, *J* = 8.0 Hz), 6.55 (d, 2H, *J* = 8.0 Hz), 3.80 (s, 6H), 3.60 (s, 2H), 2.97 (s, 2H), 1.19 (s, 6H); MS (EI, *m/z*) 275 (M^+); HRMS *m/z* calc. for $C_{16}H_{21}NO_3$ 275.1521, found 275.1520.

5-(3,4,5-Trimethoxybenzylidene)-3,3-dimethylpiperidin-4-one (11j)

Yield: 67%; light yellow solid; mp: 99–103 °C; FTIR (KBr, /cm) 1700, 1654, 1265, 1103, 1083,738, 704; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (m, 1H), 6.54 (s, 2H), 4.14 (s, 2H), 3.88 (s, 3H), 3.86 (s, 6H), 2.99 (s, 2H), 1.16 (s, 6H); MS (EI, *m/z*) 305 (M^+); HRMS *m/z* calc. for $C_{17}H_{23}NO_4$: 305.1627, found 305.1624.

5-(4-Fluorobenzylidene)-3,3-dimethylpiperidin-4-one (12j)

Yield: 74%; light brown solid; mp: 92–95 °C; FTIR (KBr, ⁄cm) 1724, 1599, 1508, 1225, 1158, 1085, 836, 668; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (s, 1H), 7.28 (dd, 2H, $J_{H–H}$ = 8.7 Hz, $J_{H–F}$ = 5.4 Hz), 6.90 (dd, 2H $J_{H–H}$ = 8.7 Hz, $J_{H–F}$ = 8.6 Hz), 4.05 (s, 2H), 3.06 (s, 2H), 1.32 (s, 6H); MS (ESI, *m/z*) 234 ($M+H^+$).

5-(5-Bromo-2-methylbenzylidene)-3,3-dimethylpiperidin-4-one (15j)

Yield: 65%; off-white solid; mp: 111–114 °C; FTIR (KBr, ⁄cm) 1695, 1604, 1475, 1319, 1138, 1098, 813; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.61 (s, 1H), 7.33 (d, 1H, J = 8.0 Hz), 7.17 (s, 1H), 7.06 (d, 1H, J = 8.0 Hz), 4.14 (s, 2H), 3.24 (s, 2H), 2.21 (s, 3H), 1.23 (s, 6H); MS (EI, *m/z*) 307 (M^+); HRMS *m/z* calc. for $C_{15}H_{18}BrNO$ 307.0572, found 307.0558.

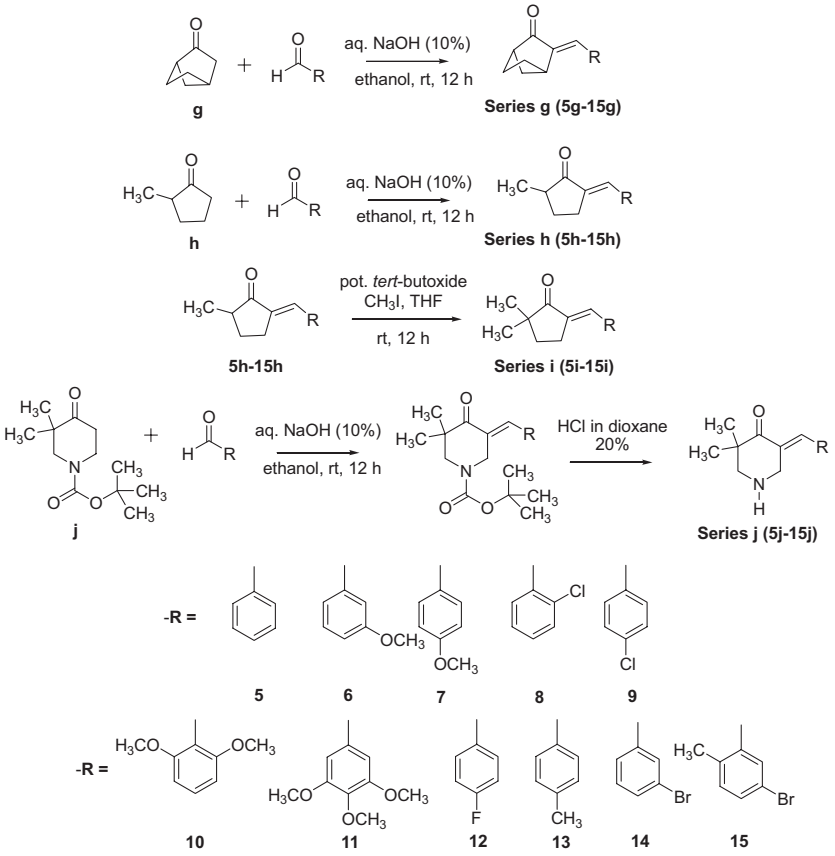
Results and Discussion

Synthesis of novel chalcone-inspired analogs

Four series of cyclic chalcone-inspired analogs (**g**, **h**, **i**, and **j**) were synthesized as outlined in Scheme 1. Three (**g**, **h**, and **i**) of the four series were derived from cyclopentanones substituted differently at the α-position, while the fourth series (**j**) was derived from a nitrogen-containing, α-substituted, six-membered cyclic ketone (3,3-dimethyl-4-piperidinone). The compounds in series **g** and **h** were synthesized in good-to-excellent yields (50–85%) by aldol cross-condensation of the appropriate ketone with appropriately substituted benzaldehydes. The series **h** compounds were stirred with potassium *tert*-butoxide in THF for 30 min at room temperature, and the resulting solution was treated with methyl iodide and stirred for 12 h to obtain series **i** compounds in good yields (60–70%). To synthesize the analogs of series **j**, commercially available Boc-protected 3,3-dimethyl-4-piperidone was treated with an appropriate aldehyde in ethanol and aqueous sodium hydroxide at ambient conditions for 12 h. The Boc protection was removed by stirring the resulting compounds in 20% HCl in dioxane to afford the desired analogs (**5j**–**15j**) in moderate-to-good yields (35–50%). All compounds were purified by column chromatography using silica gel.

Cytotoxicity of the chalcone-inspired analogs

With the target molecules **5**–**15** (series **g**, **h**, **i**, and **j**) at hand, their ability to inhibit the growth of murine L1210 lymphoma cells in culture was screened at a concentration of 10 μM. For comparison, the previously reported cytotoxic agent *seco*-hydroxy-CBI-TMI **16** (24–26) (Figure 3) was used as a positive control. Screening of the compounds was conducted after the cells were incubated continuously for 72 h, and growth inhibition was measured using an MTT assay (27). For each compound, the average growth inhibition from four wells was determined by calculating the inverse of cell viability. Viable, living cells readily convert MTT to formazan, which absorbs strongly at 570 nm. The inverse of cell viability is therefore an inverse of the absorbance at 570 nm after adjustment of the



Scheme 1: Synthetic pathway of the compounds in series **g**, **h**, **i**, and **j**.

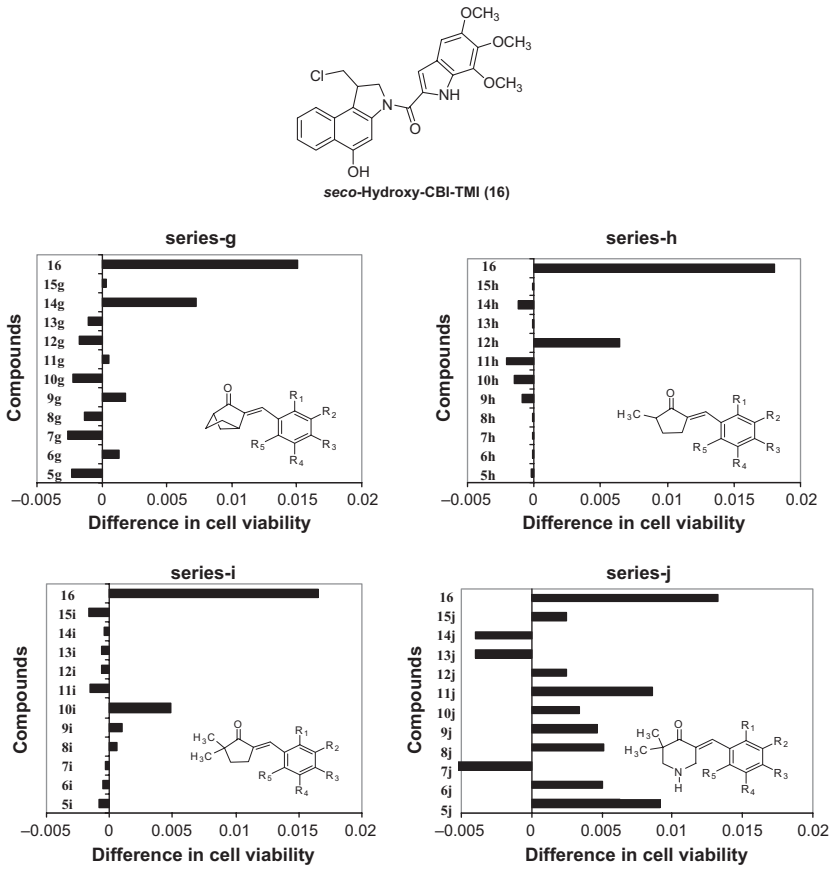


Figure 3: Structure of the cytotoxic agent *seco*-hydroxy-CBI-TMI **16** and charts showing the results from an MTT-based *in vitro* screening of the compounds at 10 μM concentration for activity against murine L1210 (lymphoma) cells. Difference in cell viability (*x*-axis) is defined as $A - B$, in which $A = 1/$(absorbance at 570 nm for an individual compound – absorbance at 570 nm for the negative untreated control). B = average of A for all compounds within each series. Bars to the right of the average indicate compounds that are more active than the average for each series of molecules. The activity of compound **16** was not included in the calculations.

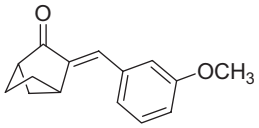
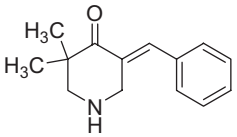
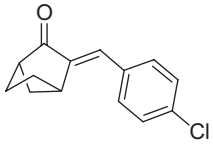
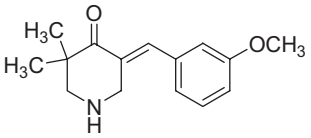
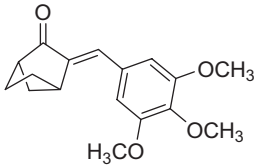
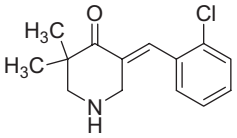
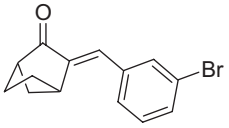
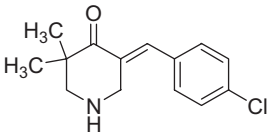
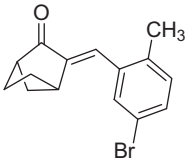
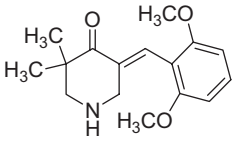
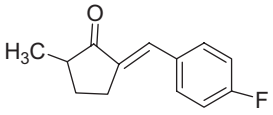
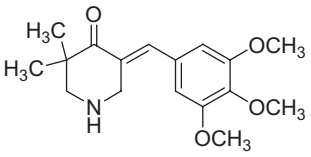
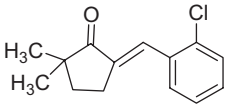
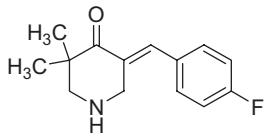
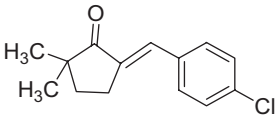
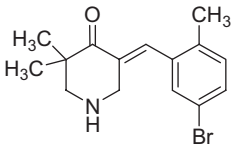
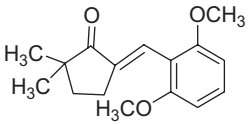
controls (see legend of Figure 3). The screening results are depicted in Figure 3. Compounds that are more cytotoxic than the average for each series are indicated by bars pointing to the right from the average. Accordingly, the following compounds were selected for detailed cytotoxicity studies: five compounds in series **g** (**6g**, **9g**, **11g**, **14g**, and **15g**), one compound in series **h** (**12h**), three compounds in series **i** (**8i**, **9i**, and **10i**), and eight compounds in series **j** (**5j**, **6j**, **8j**, **9j**, **10j**, **11j**, **12j**, and **15j**). These compounds were tested against the growth of murine B16 (melanoma) and L1210 cell lines using a 72-h continuous exposure MTT assay technique (27). The concentration at which 50% cell growth was inhibited (IC_{50}, μM) was determined for each compound in triplicate experiments; the values were averaged and are presented in Table 1.

The results given in Table 1 showed that none of the compounds in series **g**, **h**, and **i** was active against murine L1210 and B16 cancer cells with the sole exception of the compound **12h**, derived from 2-methylcyclopentanone. Compound **12h**, which contains a fluorine atom in the *para* position of the phenyl moiety, showed remarkable cytotoxicity against both B16 and L1210 cell lines. The recorded IC_{50} values are 12 and 6.8 μM, respectively. The series **j** compounds furnished more promising results. Five compounds, **5j**, **8j**, **11j**, **12j**, and **15j**, were cytotoxic against both B16 and L1210 cell lines (IC_{50} values between 4.4 and 41 μM). On the other hand, three compounds **6j**, **9j**, and **10j** showed moderate cytotoxicity against B16 melanoma cell lines with IC_{50} values of 60, 59, and 50 μM, respectively. The simple non-substituted compound **5j** and 3,4,5-trimethoxy-substituted compound **11j** emerged as the most cytotoxic compounds in the series with IC_{50} values of 9.2 and 8.4 μM, respectively, for B16. These compounds gave even lower IC_{50} values of 5.1 and 4.4 μM, respectively, for L1210 cell lines. The monohalogenated compounds **8j**, **9j**, and **12j** were only moderately active against the B16 cell line (IC_{50} = 30, 59, and 41 μM, respectively). Compounds **8j** and **12j** showed remarkable cytotoxicity against the L1210 cell line with lower IC_{50} values of 4.4 and 4.9 μM, respectively. However, the *para*-chloro-substituted compound **9j** did not show cytotoxicity against L1210 cells, indicating that the position of substitution on the phenyl ring has an impact on activity. It was interesting to note that compound **15j**, which has a 2-methyl-5-bromo substitution, was equally active against B16 and L1210 cancer cell lines (IC_{50} = 14 and 15 μM, respectively). It is noteworthy that any substitution on the aromatic ring decreased cytotoxicity except for the trimethoxy analog. Interestingly, the 3,4,5-trimethoxy substitution pattern in compound **11j** is consistent with the A-ring of CA-4.

Conformation

The configuration about the double bond between the alicyclic moiety and the aromatic ring was determined by aromatic solvent–induced shift (ASIS) data by recording the ^{1}H NMR spectrum in $CDCl_3$ and in an aromatic solvent (C_7D_8, deuterated toluene-d_8). For example, in $CDCl_3$, the peak for the β-alkene proton on compound **10j** appeared as a singlet at 7.30 ppm, which shifted

Table 1: IC_{50} values of the selected compounds in the series **g**, **h**, **i**, and **j**

Compounds	Structure	IC_{50} (μM) B16	IC_{50} (μM) L1210	Compounds	Structure	IC_{50} (μM) B16	IC_{50} (μM) L1210
6g		>100	>100	**5j**		9.2	5.1
9g		>100	>100	**6j**		60	>100
11g		>100	>86	**8j**		30	4.4
14g		>100	>100	**9j**		59	>100
15g		>100	>100	**10j**		50	>100
12h		12	6.8	**11j**		8.4	4.4
8i		>100	>100	**12j**		41	4.9
9i		>100	>100	**15j**		14	15
10i		>100	>100				

downfield (7.74 ppm) when the solvent was changed from $CDCl_3$ to deuterated toluene, while all other peaks shifted upfield. This downfield shift of 0.44 ppm of the β-alkene proton in an aromatic solvent suggests that the proton is *syn* to a keto group (Figure 4) (28,29). Unambiguous evidence for the *E*-configuration of compound **10j** was gained from a single-crystal X-ray crystallographic analysis

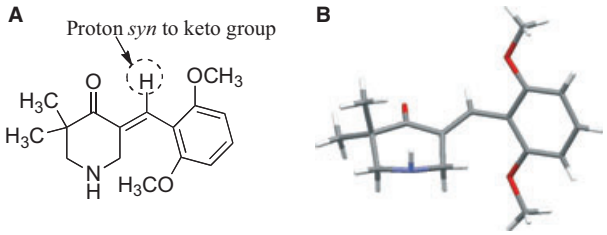


Figure 4: Chemical structure (A) and single-crystal X-ray crystallographic structure (B) of the compound **10j**. The crystals of **10j** were grown from a solution in methanol that was kept in the refrigerator. Complete X-ray structural data for **10j** were deposited with the Cambridge Structural Database.[a]

(Figure 4). The X-ray structure of **10j** confirmed the *E*-geometry about the alkene and showed that the two rings are almost perpendicular to each other.[a]

The conformation of the compounds **5j** and **11j** was also examined by molecular modeling studies using the suite of programs in MacSpartan, version '04. Upon optimization of the structure using molecular mechanics (MMFF) and molecular dynamics (equilibrium conformer search option and molecular mechanics), the structure was energy-optimized using Hartree–Fock (3-21G), followed by density function theory (B3LYP and 6-31G*) calculations. For comparison, the conformation of CA-4 **1**, determined using the same protocol and as previously reported, was used in the analysis (30). The resulting conformations of compounds **5j** and **11j** are depicted in Figure 5, and the conformations are remarkably similar to the X-ray diffraction-derived conformation of **10j**, indicating the molecular modeling can quite reasonably predict the conformation of the compounds described herein. It is evident from these results that in general the steric hindrance between the methylene-hydrogen atoms of the piperidino unit and the phenyl moiety on the alkene causes the molecule to adopt a slightly 'twisted' conformation between 5 and 10°. Interestingly, this conformation is similar to that observed in an X-ray structure of CA-4 (31) and prompted us to investigate whether some of the active compounds may enhance microtubule depolymerization in cells in a similar way as CA-4.

Microtubule depolymerization activity

The effects of compounds **5j**, **11j**, and **15j** on interphase cellular microtubules were evaluated with A-10 smooth muscle cells (32). Exposure of the cells to the three compounds did not show any significant microtubule depolymerization up to 30 μM concentration. In contrast, CA-4 strongly causes microtubule depolymerization giving an EC_{50} value of 0.007 μM (33). This indicates that the novel compounds described herein do not act via a similar mechanism of inhibiting cell growth as CA-4. This is interesting because related bisarylidene analogs of the target compounds, which gave equal cytotoxicity, also did not display microtubule disruption properties (34).

Conclusions

Forty-four alicyclic chalcone-inspired analogs belonging to four unique series were designed and synthesized for the investigation of their cytotoxicity. Generally, the chalcone analogs derived from substituted cyclopentanones (series **g**, **h**, and **i**) were inactive at inhibiting the growth of cancer cells in the culture. However, several chalcones containing a 3,3-dimethyl-4-piperidinone unit (series **j**) showed significant cytotoxicity against both murine L1210 and B16 cancer cell lines. These chalcone analogs possess an *E*-configuration and a twisted conformation similar to that of combretastatin A-4 as revealed by single-crystal X-ray structure analysis and molecular modeling studies. However, their mechanism of action was found to be different from that of combretastatin A-4 as they failed to cause microtubule depolymerization. Further studies on the **j** series of compounds are planned which include experiments to determine their mechanism of action and *in vivo* studies.

Acknowledgments

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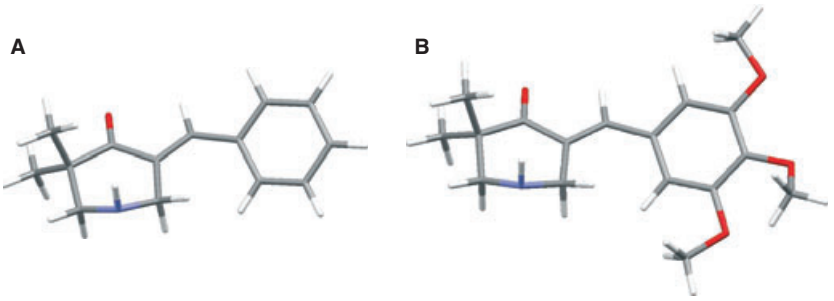

Figure 5: Molecular models of the compounds **5j** (A) and **11j** (B) determined using Hartree–Fock and density functional calculations.

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Note

[a]X-ray structural information for 10j in cif format, CCDC 807546. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.