7228 J. Med. Chem. 2009, 52, 7228–7235
DOI: 10.1021/jm901278z

Journal of Medicinal Chemistry Article

Structure−Activity Relationship Studies of Chalcone Leading to 3-Hydroxy-4,3′,4′,5′-tetramethoxychalcone and Its Analogues as Potent Nuclear Factor κB Inhibitors and Their Anticancer Activities

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Received August 26, 2009

Chalcone is a privileged structure, demonstrating promising anti-inflammatory and anticancer activities. One potential mechanism is to suppress nuclear factor kappa B (NF-κB) activation. The structures of chalcone-based NF-κB inhibitors vary significantly that there is minimum information about their structure−activity relationships (SAR). This study aims to establish SAR of chalcone-based compounds to NF-κB inhibition, to explore the feasibility of developing simple chalcone-based potent NF-κB inhibitors, and to evaluate their anticancer activities. Three series of chalcones were synthesized in one to three steps with the key step being aldol condensation. These candidates demonstrated a wide range of NF-κB inhibitory activities, some of low micromolar potency, establishing that structural complexity is not required for NF-κB inhibition. Lead compounds also demonstrate potent cytotoxicity against lung cancer cells. Their cytotoxicities correlate moderately well with their NF-κB inhibitory activities, suggesting that suppressing NF-κB activation is likely responsible for at least some of the cytotoxicities. One lead compound effectively inhibits lung tumor growth with no signs of adverse side effects.

Introduction

Chalcone-based compounds (1,3-diaryl-2-propen-1-ones, Figure 1), natural or synthetic, have been widely reported to exhibit various biological activities, especially with regard to anti-inflammatory and anticancer activities.[1−14] For instance, chalcone (**1**) and isoliquiritigenin (**3**) demonstrated significant chemopreventive activities against lung, breast, prostate, and colorectal cancers.[1,15−18] Flavokawain A (**6**) suppressed bladder tumor growth at a dose of 50 mg/kg of body weight in a mouse xenograft model.[9] Cardamonin (**5**) inhibited inflammation.[19] Chalcones thus comprise a class of compounds with important therapeutic potential. The ease of preparation, the potential of oral administration,[1,15,16,20] and safety[20] also support the feasibility of chalcone-based compounds as therapeutic agents. Tremendous effort has been devoted to elucidate the mechanisms of chalcone-based compounds for their promising biological activities, including their interference in microtubule formation[2,11,12,21−28] and cellular signaling pathways,[7,29,30] such as nuclear factor kappa B (NF-κB)[a] inhibition. Although interfering microtubule formation is one mechanism potentially responsible for the anticancer activities of chalcone-based compounds, such a mechanism cannot account for their anti-inflammatory activities. On the other hand, the anti-inflammatory properties of chalcone-based compounds is probably, at least partially, responsible for their anticancer activities because of the close linkage between inflammation and tumorigenesis.[31−34] Therefore, the anti-inflammatory and anticancer activities of chalcone-based compounds may be a result of its inhibitory activities against the NF-κB signaling pathways.[32−37] In fact, quite a few chalcone-based compounds have been reported to inhibit the NF-κB signaling pathway, some of them being shown in Figure 1.[14,38−49] Very interestingly, these chalcone-based NF-κB inhibitors possess diverse structural properties with varied substitutions, most of which being electron donating functionalities, such as hydroxy and methoxy functional groups, at different positions of both aromatic systems. Many of these chalcones are easily obtainable through synthesis (**1**−**7**, one-step aldol condensation), while others are comparatively more challenging to prepare. For instance, **8** has never been synthesized before while **12**−**15** require four to seven steps of syntheses.[46,50−52] It is therefore important to elucidate the structural requirements for chalcone-based compounds with respect to NF-κB inhibition to determine whether potent and easily accessible chalcone-based NF-κB inhibitors can be developed for future therapeutic evaluation.

In this research, we explored the structure−activity relationship (SAR) of three series of chalcone-based compounds regarding their NF-κB inhibitory activities by using a luciferase-based in vitro NF-κB reporter assay with the goal to determine the essential functionalities on the chalcone core for

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[a] Abbreviations: NF-κB, nuclear factor kappa B; IKKβ, I kappa B kinase 2; IRAK4, interleukin-1 receptor-associated kinase 4; SAR, structure−activity relationship; *N*-Boc, *N*-*tert*-butyloxycarbonyl; TNF-α, tumor necrosis factor α; IκBα, I kappa B alpha; IKKα, I kappa B kinase 1; IRAK1, interleukin-1 receptor-associated kinase 1; TAK1, Tat-associated kinase 1; SCID, severe combined immunodeficiency; PBS, phosphate-buffered saline; PEG, polyethylene glycol.

 Published on Web 11/02/2009

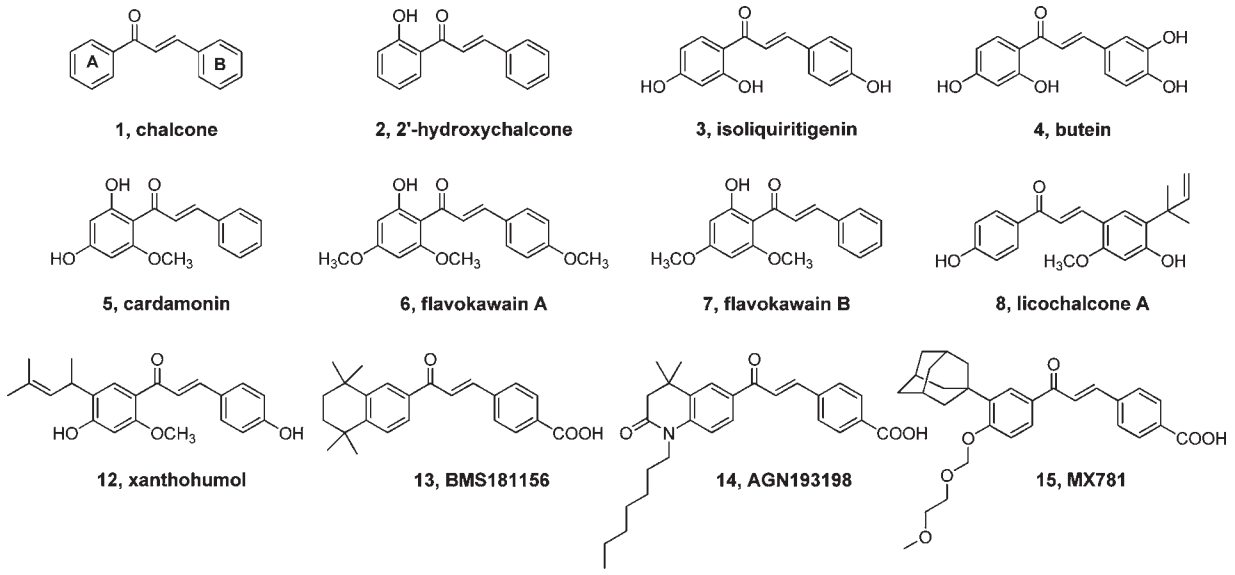
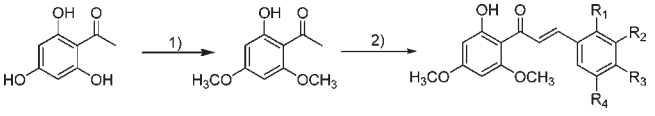


Figure 1. Structures of representative chalcone-based NF-κB inhibitors.

Scheme 1. Representative Synthetic Schemes of Chalcone-Based Analogues, Using Series **10** as an Example[a]



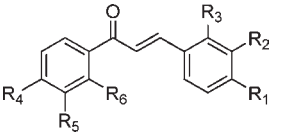
[a] Reagents and conditions: (1) Me_2SO_4, K_2CO_3, acetone, reflux; (2) substituted aromatic aldehyde, KOH, MeOH.

NF-κB inhibition. The lead compound with low micromolar inhibitory activity was evaluated for its inhibitory activities against kinases upstream of NF-κB and in vitro and in vivo anticancer activities. One lead compound, **11a**, directly inhibits the kinase activities of I kappa B kinase 2 (IKKβ) and interleukin-1 receptor-associated kinase 4 (IRAK4), potentially responsible for its NF-κB inhibitory activities. **11a** also demonstrates potent cytotoxicity against lung cancer cells in vitro and safely suppresses lung tumor growth in vivo. The results of these SAR studies also identified positions on the chalcone core structure that can accommodate modifications without significant loss of the NF-κB inhibitory activities, which will guide the future development of probe-based chalcones to identify their cellular targets responsible for NF-κB inhibition, to confirm whether IKKβ and IRAK4 are in fact the cellular targets.

Results

Synthesis of Chalcone Analogues. Three series of chalcone-based compounds were synthesized according to procedures previous described with the condensation step yielding ~50−90% (Scheme 1).[2,6] Compounds of the first series have substituents of varied electron density on both rings with a goal to explore whether the electron density on chalcone may influence the enone's electrophilicity (Table 1, **9a**−**e**), potentially responsible for its NF-κB inhibition through modification of its target(s), such as IKKβ, via Michael addition (Figure 2). Compounds of the second series have the same substituents on the A ring as flavokawains A and B and varied substituents on the B ring (Table 2, **10a**−**j**) with a goal to determine whether modification of the B ring could improve NF-κB inhibitory activity, since flavokawains

Table 1. NF-κB Inhibitory Activities[a] of Chalcones with Substituents of Varied Electron Densities on A and B Rings



	R_1	R_2	R_3	R_4	R_5	R_6	$IC_{50} \pm SD$ (μM)
1	H	H	H	H	H	H	> 20
2	H	H	OH	H	H	H	> 20
3	OH	H	H	OH	H	OH	> 20
4	OH	OH	H	OH	H	OH	> 20
9a	H	H	H	CH_3	H	H	> 20
9b	H	H	H	F	H	H	> 20
9c	H	H	H	CF_3	H	H	> 20
9d	H	H	H	F	F	H	> 20
9e	OH	H	H	H	H	H	> 20

[a] Results are given as the mean of three independent experiments with triplicates in each experiment.

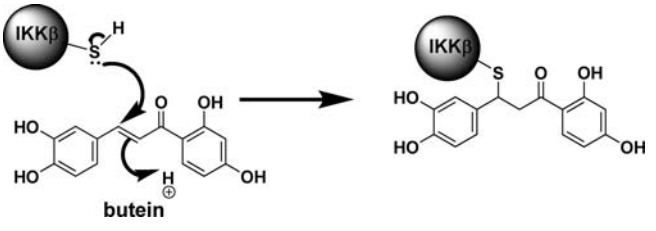


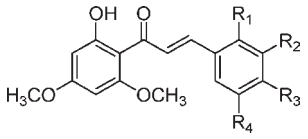
Figure 2. Potential mechanism of chalcone-based compounds to inhibit NF-κB via covalent modification of IKKβ, using butein as the example of chalcone-based compounds.

A and B were reported to be the most potent NF-κB inhibitors in kava by Folmer et al.[43] On the basis of the observation that most substituents on natural chalcone-based NF-κB inhibitors are hydroxyl and methoxy functional groups, a third chalcone series was synthesized to have varied methoxy and hydroxyl substituents on both rings (Table 3, **11a**−**m**). **11k** was prepared from **11a** through Zn dust reduction (Scheme 2). Such a candidate can help

determine whether the α,β unsaturated carbonyl functional group is essential for NF-κB inhibition, potentially revealing the chemical mechanism of NF-κB inhibition. **11l** was synthesized by reacting **11a** with 2-bromoethanol. **11m** was synthesized by reacting **11a** with *N*-Boc-2-bromoethanamine followed by Boc deprotection.

In Vitro Inhibition of TNF-α-Induced NF-κB Activation in A549 Lung Adenocarcinoma Cell Line. Human lung adenocarcinoma A549 cell line stably transfected with NF-κB-luc allows easy monitoring of the effects of chalcone-based compounds on tumor necrosis factor-alpha (TNF-α)-induced NF-κB activation in vitro. Chalcone-based compounds were first evaluated at a single concentration of 20 μM. Candidates with >50% inhibitory activities at 20 μM were further evaluated in a dose-dependent manner to determine the concentration needed to inhibit 50% of TNF-α-induced NF-κB activation (IC_{50}). Disappointingly, none of the chalcones in series 9, including **1**, **2**, **3**, and **4**, the reported NF-κB inhibitors, showed significant NF-κB inhibitory activities (IC_{50} > 20 μM). Among the chalcones in series **10**, only two candidates (**10f** and **10g**) demonstrate moderate NF-κB inhibitory activities (10 μM < IC_{50} < 20 μM). The rest of the candidates, including **6** and **7**, the most potent NF-κB inhibitors identified from kava, have minimum inhibitory activities with IC_{50} values higher than 20 μM. However, **11a** was found to potently inhibit NF-κB activation with an IC_{50} of 5.1 μM (Table 3). SAR studies revealed that removal of methoxy functional groups (**11b**−**d**) or demethylation (**11e**) on the A ring leads to a 2- to 3-fold decrease in activities. Removal of 3′-hydroxy on the B ring also leads to a slight to moderate decrease in activities (**11g**, **11i**, and **11j**), while demethylation or removal of 4′-methoxy leads to 2- to 3-fold increase in activities (**11f** and **11h**). Reducing the α,β-unsaturated carbonyl double bond in **11a** leads to significant loss of activity (**11k** more than 4-fold less active). Lastly, modification of 3′-hydroxy of **11a** with 2-hydroxyethyl (**11l**) or 2-aminoethyl functional (**11m**) groups leads to analogues of similar NF-κB inhibitory activities. Both of these compounds can be further modified by affinity tags, such as biotin, that can be used to identify the cellular targets of series **11** that are responsible for their NF-κB inhibition.

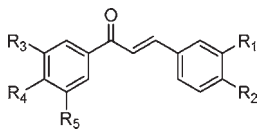
Table 2. NF-κB Inhibitory Activities[a] of Chalcones with the Same A-Ring as Flavokawains

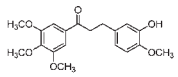


	R_1	R_2	R_3	R_4	IC_{50} ± SD (μM)
6	H	H	OCH_3	H	>20
7	H	H	H	H	>20
10a	H	H	$OCH_2CH_2CH_3$	H	>20
10b	H	H	F	H	>20
10c	H	H	Cl	H	>20
10d	Br	H	H	H	>20
10e	CH_3	H	H	H	>20
10f	H	Br	H	H	12.3 ± 2.8
10g	H	CH_3	H	H	17.0 ± 2.9
10h	H	OH	OCH_3	H	>20
10i	H	CH_3	OCH_3	H	>20
10j	H	OCH_3	OCH_3	OCH_3	>20

[a] Results are given as the mean of three independent experiments with triplicates in each experiment.

Table 3. NF-κB Inhibitory Activities[a] of Chalcones with Varied Methoxy and Hydroxyl Substituents on A and B Rings



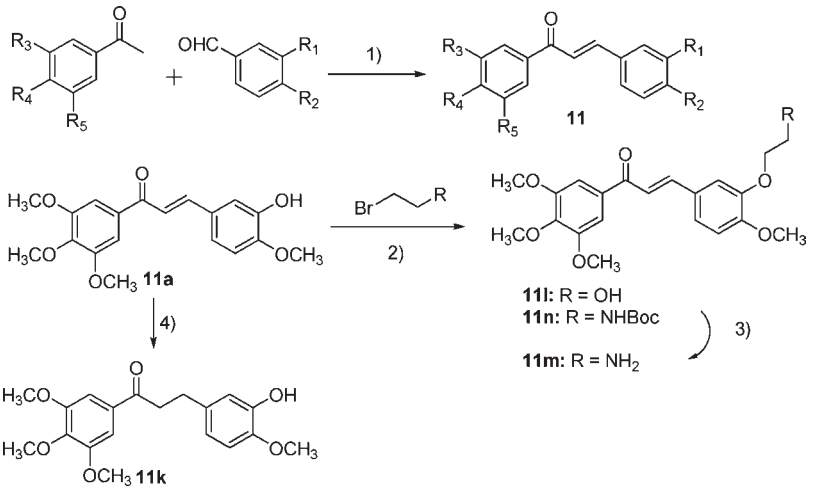
	R_1	R_2	R_3	R_4	R_5	IC_{50} ± SD (μM)
11a	OH	OCH_3	OCH_3	OCH_3	OCH_3	5.1 ± 1.9
11b	OH	OCH_3	OCH_3	OCH_3	H	8.0 ± 2.1
11c	OH	OCH_3	OCH_3	H	OCH_3	8.1 ± 0.9
11d	OH	OCH_3	H	OCH_3	H	15.4 ± 0.8
11e	OH	OCH_3	OCH_3	OH	OCH_3	14.7 ± 3.4
11f	OH	H	OCH_3	OCH_3	OCH_3	2.3 ± 0.3
11g	H	OH	OCH_3	OCH_3	OCH_3	11.6 ± 2.7
11h	OH	OH	OCH_3	OCH_3	OCH_3	1.9 ± 0.1
11i	H	OCH_3	OCH_3	OCH_3	OCH_3	19.3 ± 3.8
11j	H	H	OCH_3	OCH_3	OCH_3	8.9 ± 1.4
11k						>20
11l	$O(CH_2)_2OH$	OCH_3	OCH_3	OCH_3	OCH_3	9.1 ± 1.2
11m	$O(CH_2)_2NH_2$	OCH_3	OCH_3	OCH_3	OCH_3	5.9 ± 0.8

[a] Results are given as the mean of at least three independent experiments with triplicates in each experiment.

Inhibitory Activities of 11a against Kinases Modulating NF-κB Activation. The NF-κB pathway can be activated by multiple signaling pathways, most probably through upstream kinase-based phosphorylation of IκBα (an inhibitory protein of the NF-κB pathway), leading to IκBα ubiquitination and proteasome degradation, p65 enrichment in nucleus, and gene transcription. Chalcone **11a** and its analogues may induce NF-κB inhibition through inhibiting kinases upstream of IκBα, such as IKKβ, like **4** does. We therefore evaluated the effect of **11a**, the initial lead compound, against six upstream kinases that have been reported to activate the NF-κB pathway, including I kappa B alpha (IKKα), IKKβ, interleukin-1 receptor-associated kinase 1 (IRAK1), IRAK4, Tat-associated kinase 1 (TAK1), and cSrc (Figure 3).[53,54] It was found that **11a** at 10 μM significantly inhibits IKKβ and IRAK4 by 46% and 41%, respectively, while it has no inhibitory activities against the other four kinases.

In Vitro Cytotoxicities of Chalcone Series 11 against H2009 and A549 Lung Cancer Cells. As chronic lung inflammation induced by tobacco usage through NF-κB activation is one potential mechanism of lung cancer development, NF-κB inhibitors will have therapeutic potential for lung cancer treatment.[55] Since chalcones in series **11** have potent NF-κB inhibitory activities, these chalcones were chosen from the three chalcone series and evaluated for their in vitro cytotoxicities against two lung cancer cell lines, H2009 (Figure 4) and A549. The concentrations to inhibit the cancer cell growth by 50% (GI_{50}) are reported in Table 4. Chalcones in series **11** demonstrated significant cytotoxicities, with GI_{50} mainly in the single-digit micromolar range, similar to their in vitro NF-κB inhibitory potencies. Their in vitro cytotoxicities also correlate positively with their NF-κB inhibitory activities (Figure 5). Representative chalcones from series **9** and **10** were evaluated for

Scheme 2. Synthesis of Chalcone Analogues (Series **11**)[a]



[a] Reagents and conditions: (1) KOH, MeOH, room temp; (2) DMF, K_2CO_3, room temp; (3) trifluoroacetic acid, CH_2Cl_2, room temp; (4) Zn, NH_4OAc, EtOH, H_2O, room temp.

their cytotoxicity in these two cell lines as well with $IC_{50} > 20\ \mu M$ (data not shown).

Anticancer Activities of 11a against H2009 Lung Cancer Cell Induced Tumor Growth in Nude Mouse Xenograft Model. To determine whether chalcone-based NF-κB inhibitors can be potentially used to treat lung cancer, **11a**, the first lead compound, was evaluated for its effect on lung tumor growth in vivo in a xenograft model. H2009 lung cancer cells were inoculated in the right flank of severe combined immunodeficiency (SCID) nude female mice. **11a** was administered to mice at a dose of 1 mg/mouse/day 6 days a week through intraperitoneal injection. Bodyweights of mice were measured twice a week. At the end of week 5, the mice were weighed and euthanized by CO_2 and the tumor dissected and weighed. All animals tolerated the treatments well with no observable signs of toxicity, and increase in body weight occurred at a similar rate among control mice and **11a** treated mice. No gross pathologic abnormalities were noted at the time of necropsy as well (data not shown). **11a** significantly suppressed H2009-induced lung tumor growth by 57%.

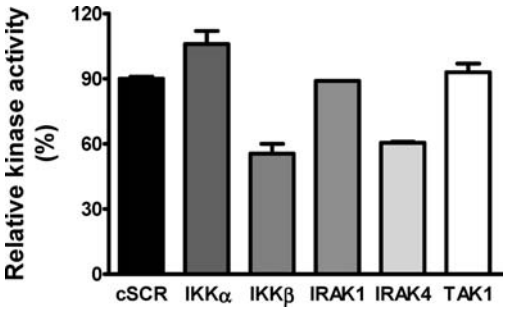


Figure 3. Inhibitory activities of chalcone **11a** at 10 μM against six kinases upstream of the NF-κB pathway.

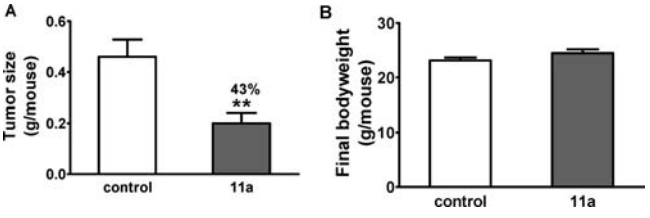


Figure 4. Effect of ip **11a** at 1 (mg/mouse)/day on the growth of H2009 tumors in nude mice. Each mouse was inoculated sc in the right flank with 2×10^6 H2009 cells suspended in 0.1 mL of PBS/metrigel (v/v 1:1). Mice were given **11a** at 1 (mg/mouse)/day in a carrier made of PEG 400/EtOH (v/v 2:1, 50 μL) with intraperitoneal administration for 5 weeks, 6 days per week. Control mice received carrier alone. (A) Tumor volumes: columns, mean; bars, SE ($n = 5$; (**) $p < 0.01$ compared with the control group). (B) Average body weight: columns, mean; bars, SE ($n = 5$; $p > 0.05$ compared with the control group).

Discussion and Conclusion

We have synthesized and evaluated three series of chalcone-based compounds with different substitutions on both aromatic rings and reduction of the alkene linkage with the goal to determine the structural requirements for chalcone to inhibit the NF-κB pathway, potentially leading to candidates with anti-inflammatory and anticancer activities. Disappointingly, many of the reported simple chalcone-based NF-κB inhibitors, such as **1**−**7**, were found to have rather weak inhibitory activities ($IC_{50} > 20\ \mu M$). Varying electron density on chalcone also has no effect with respect to NF-κB inhibition. However, several simple chalcone candidates with only hydroxyl and methoxy substitutions in series **11** demonstrate single-digit micromolar NF-κB inhibitory activities. The reduction of the alkene functionality in chalcone completely abolishes this activity, supporting the notion that the mechanism of inhibition is likely through a covalent Michael addition of nucleophiles (such as SH from cysteine) from protein candidate(s) to chalcones. Despite its simple structure and ease of synthesis, **11a** in fact inhibits the kinase activity of IKKβ of similar potency compared to **15**.[46] **11a** also inhibits the kinase activity of IRAK4. Chalcones in series **11** also demonstrate potent cytotoxicity against lung cancer cells in vitro with most GI_{50} in single-digit micromolar range, consistent with their NF-κB inhibitory potency, which also correlates positively with each other. These results suggest that inhibiting NF-κB is likely responsible for the in vitro cytotoxicity of chalcones **11**. The nonperfect correlation may be due to potential experimental errors, or more likely there are other mechanisms involved for the cytotoxicities of chal-

cones in series **11**. **11a** also effectively suppresses lung cancer growth in vivo at a dose of 1 mg/mouse/day via ip administration. Such treatment reveals no signs of adverse side effect.

These data collectively demonstrate that chalcones as simple as those in series **11** can potently inhibit NF-κB activation via inhibiting the kinase activities of IKKβ and/or IRAK4. These simple chalcones are promising anticancer candidates against lung cancer, warranting further investigations.

Materials and Methods

Chemistry. All commercial reagents and anhydrous solvents were purchased from vendors and were used without further purification or distillation, unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed on EM Science silica gel 60 F_{254} (0.25 mm). Compounds were visualized by UV light and/or stained with either *p*-anisaldehyde, potassium permanganate, or cerium molybdate solutions followed by heating. Flash column chromatography was performed on Fisher Scientific silica gel (230−400 mesh). HPLC purity analyses were performed on a Beckman Coulter system Gold 126 solvent module with a 168 detector. A Clipeus C-18 column (5 μm, 250 mm × 4.6 mm) was used for the analyses. The flow rate used was 0.5 mL/min. The mobile phase A was water, while B was acetonitrile. The time program used for the analyses was 55% B (0−5 min) and 55−85% B (5−25 min) and 99% B (25−40 min). Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K and were uncorrected. IR spectra were recorded on a Nicolet Portege 460 FT-IR instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 spectrometer. Proton chemical shifts are reported in ppm from an internal standard of residual $CHCl_3$ (7.26 ppm), and carbon chemical shifts are reported using an internal standard of residual $CHCl_3$ (77.16 ppm). Proton chemical data are reported as follows: chemical shift, multiplicity, coupling constant, and integration. High resolution mass spectra were obtained at the University of Minnesota, Department of Chemistry Mass Spectrometry Facility, on a Bruker BioTOF II ESI-TOF/MS utilizing polyethylene glycol (PEG) as an internal high resolution calibration standard. All compounds synthesized were at least 95% pure as analyzed by HPLC.

Table 4. GI_{50}[a] of Chalcones against Two Lung Cancer Cells

	H2009 (μM)		A549 (μM)	
	GI_{50}	SE	GI_{50}	SE
11a	5.2	0.7	2.1	0.5
11b	4.6	0.3	5.2	1.7
11c	2.3	0.6	3.3	0.3
11d	8.1	0.9	10.0	1.2
11e	7.5	1.7	4.0	0.7
11f	4.2	0.8	1.7	0.2
11g	4.5	0.9	8.5	0.5
11h	1.1	0.3	2.6	0.4
11i	8.4	0.9	12.4	0.6
11j	6.6	0.8	8.8	0.4
11k	47	7.6	17.6	2.0
11l	3.3	0.8	4.7	0.5
11m	3.6	0.04	5.6	0.5

[a] Results are given as the mean of at least three independent experiments with triplicate in each experiment.

General Procedure for the Synthesis of Chalcones. A mixture of the corresponding acetophenone (1 equiv) and the corresponding aldehyde (1 equiv) in anhydrous EtOH (3 mL for 1 mmol of acetophenone) was stirred at room temperature for 5 min. Then NaOH (3 equiv) was added. The reaction mixture was stirred at room temperature until aldehyde was consumed (usually 6−12 h). After that, HCl (10%) was added until pH 5 was obtained. In the case of the chalcones that precipitated, they were filtered and crystallized from MeOH. In the other cases, the product was purified by using silica gel chromatography. The physical data for the prepared chalcone compounds **1**−**4**, **6**, **7**, **9a**−**e**, **10a**−**d**, **10f**, **10h**, **10j**, **11a**, **11c**, **11d**, **11f**−**j** nicely match literature reported physical data (see Supporting Information).

(*E*)**-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-*m*-tolylprop-2-en-1-one (10g). 10g** was recrystallized from MeOH. Yield: 32%. HPLC t_R = 19.6 min; R_f = 0.23 (10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.39 (s, 3H), 3.83 (s, 3H), 3.92 (s, 3H), 5.96 (d, J = 2.7 Hz, 1H), 6.11 (d, J = 2.7 Hz, 1H), 7.19−7.43 (ovlp m, 4H), 7.75 (d, J = 15.6 Hz, 1H), 7.88 (d, J = 15.3 Hz, 1H), 14.33, (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.6, 55.8, 56.1, 91.5, 94.0, 106.6, 125.6, 127.6, 129.0, 129.5, 131.2, 135.7, 138.7, 142.8, 162.7, 166.4, 168.6, 192.9. HRMS calcd ($C_{18}H_{18}O_4 + Na^+$): 321.1097, found 321.1119.

(*E*)**-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-*o*-tolylprop-2-en-1-one (10e). 10e** was recrystallized from MeOH. Yield: 16%. HPLC t_R = 19.8 min; R_f = 0.23 (10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.49 (s, 3H), 3.84 (s, 3H), 3.91 (s, 3H), 5.96 (d, J = 2.1 Hz, 1H), 6.11 (d, J = 2.7 Hz, 1H), 7.21−7.31 (m, 3H),7.64 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 15.9 Hz, 1H), 8.07 (d, J = 15.6 Hz, 1H), 14.31 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.2, 55.8, 56.1, 91.5, 94.0, 106.6, 126.5, 126.8, 128.8, 130.0, 131.1, 134.8, 138.4, 140.2, 162.8, 166.5, 168.6, 193.0. HRMS calcd ($C_{18}H_{18}O_4 + Na^+$): 321.1097, found 321.1126.

(*E*)**-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-(4-methoxy-3-methylphenyl)prop-2-en-1-one (10i). 10i** was recrystallized from MeOH. Yield: 20%. HPLC t_R = 19.3 min; R_f = 0.12 (10% EtOAc/hexanes). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.25 (s, 3H), 3.83 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 5.96 (d, J = 2.4 Hz, 1H), 6.11 (d, J = 2.4 Hz, 1H), 6.84 (d, J = 8.1 Hz, 1H), 7.42−7.44 (m, 2H), 7.78, (s, 2H), 14.46, (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.6, 55.7, 55.8,

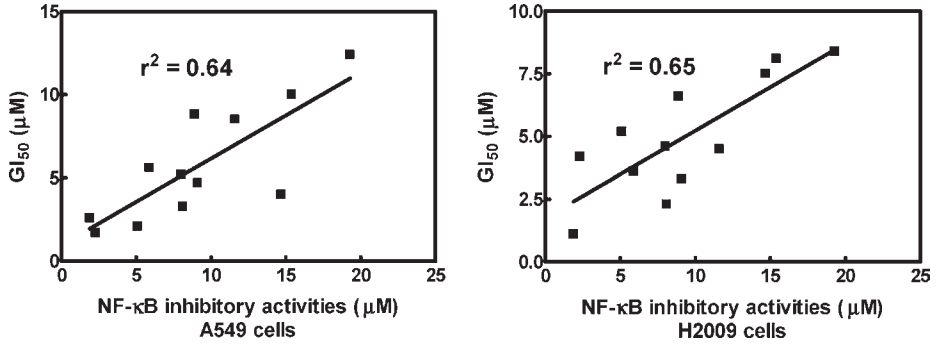


Figure 5. Correlation of the in vitro NF-κB inhibitory activities and cytotoxicities of chalcones in series **11** in A549 and H2009 cells. The mean values of the GI_{50} (the concentration to inhibit cancer cell growth by 50%) of each chalcone compounds were analyzed for their linear relationship with their mean IC_{50} of NF-κB inhibition (the concentration to inhibit the luciferase-based NF-κB activities by 50%) by using a linear regression model in GraghPad.

56.1, 91.4, 94.0, 106.6, 110.2, 125.0, 127.4, 128.0, 128.5, 130.7, 143.2, 159.9, 162.7, 166.2, 168.6, 192.9. HRMS calcd ($C_{19}H_{20}O_5 + Na^+$): 351.1203, found 351.1197.

(*E*)-1-(3,4-Dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (11b). Yellow solid. Yield: 52%. TLC (acetone/hexane = 1:1), R_f = 0.35. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, J = 15.5 Hz, 1H), 7.69 (dd, J = 8.4, 2.0 Hz, 1H), 7.64 (d, J = 1.96 Hz, 1H), 7.44 (d, J = 15.5 Hz, 1H), 7.32 (d, J = 2.1 Hz, 1H), 7.15 (dd, J = 2.0, 8.4 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 6.89 (d, J = 8.3 Hz, 1H), 5.71 (s, 1H), 3.99 (s, 3H), 3.98 (s, 3H), 3.96 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.6, 153.1, 149.2, 148.7, 145.9, 143.9, 131.5, 128.7, 122.9, 122.7, 119.8, 112.9, 110.7, 110.6, 109.9, 76.7, 56.1, 56.0. MS (ESI, positive) m/z 337.05. Calcd for $C_{18}H_{18}O_5Na$: 337.12.

(*E*)-1-(4-Hydroxy-3,5-dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one (11e). Yellow solid. Yield: 48%. TLC (acetone/hexane = 1:1), R_f = 0.31. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J = 15.5 Hz, 1H), 7.37 (d, J = 15.5 Hz, 1H), 7.33 (s, 2H), 7.31 (d, J = 2.1 Hz, 1H), 7.13 (dd, J = 2.1, 8.4 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 5.95 (s, 1H), 5.68 (s, 1H), 3.99 (s, 6H), 3.95 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 188.5, 148.7, 146.8, 145.8, 144.2, 119.5, 129.9, 128.7, 122.9, 119.8, 112.6, 110.5, 105.8, 76.7, 56.56, 56.1. MS (ESI, positive) m/z 353.05. Calcd for $C_{18}H_{18}O_6Na$: 353.11.

3-(3-Hydroxy-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)-propan-1-one (11k). A 0.01 M solution of **11a** in 95% EtOH (10 mL) was added to 50 mM ammonium acetate aqueous solution (2 mL) and stirred vigorously with 60 mg of zinc powder added in three equal intervals of 15 min. Stirring was continued for further 15 min (monitored by TLC). The suspended material was removed by filtration and washed with ethanol, and the filtrate was evaporated to dryness under reduced pressure. The crude product was subjected to column chromatography on silica gel using 2:3 acetone/hexanes to afford pure **11k** as faint yellow solid in 75% yield. TLC (acetone/hexane = 1:1), R_f = 0.40. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 1H), 6.87 (d, J = 2 Hz, 1H), 6.82 (d, J = 8 Hz, 1H), 6.76 (dd, J = 8 Hz, 2 Hz, 1H), 5.62 (s, 1H), 3.93 (s, 3H), 3.93 (s, 6H), 3.90 (s, 3H), 3.25 (t, J = 8 Hz, 2H), 3.00 (t, J = 8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.1, 153.1, 145.6, 145.0, 142.5, 134.6, 132.2, 119.8, 114.5, 110.7, 76.7, 60.9, 56.0, 40.4, 29.8. MS (ESI, positive) m/z 369.06. Calcd for $C_{19}H_{22}O_6Na$: 369.14.

(*E*)-3-(3-(2-Hydroxyethoxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (11l). Yellow solid. Yield: 98%. TLC (acetone/hexane = 1:1), R_f = 0.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, J = 15.6 Hz, 1H), 7.25 (d, J = 15.6 Hz, 2H), 7.16−7.21 (m, 3H), 6.86 (d, J = 8.4 Hz, 1H), 4.12 (t, J = 4.3 Hz, 2H), 3.90 (t, J = 4.3 Hz, 2H), 3.82 (s, 6H), 3.87 (s, 3H), 3.85 (s, 3H), 2.42 (t, J = 5.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.3, 153.1, 152.2, 148.4, 144.6, 142.4, 133.7, 128.0, 123.9, 119.9, 114.1, 111.7, 106.1, 76.7, 71.6, 61.3, 60.9, 56.5, 55.9. MS (ESI, positive) m/z 411.08. Calcd for $C_{21}H_{24}O_7Na$: 411.15.

(*E*)-3-(3-(2-Aminoethoxy)-4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (11m). To a mixture of **11a** (50 mg, 0.145 mmol) and K_2CO_3 (30 mg, 0.22 mmol) in dry DMF, *N*-Boc-2-bromoethanamine (33 mg, 0.145 mmol) was added and stirred under N_2 atmosphere at room temperature overnight. Solvent was evaporated under reduced pressure. The reaction mass was dispersed in ethyl acetate and washed by saturated aqueous solution of NaCl. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford the crude product. The crude was subjected to column chromatography using hexanes/ethyl acetate (1:1) to afford the pure **11n** in 96% yield. TLC (MeOH/hexane = 0.1:1), R_f = 0.21. ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.74 (d, J = 15.6 Hz, 1H), 7.32 (d, J = 15.6 Hz, 1H), 7.25 (m, 4H), 6.92 (d, J = 8.3 Hz, 1H), 4.13 (t, J = 4.8 Hz, 2H), 3.95 (s, 6H), 3.93 (s, 3H), 3.91 (s, 3H), 3.52 (t, J = 4.8 Hz, 2H), 1.44 (s, 9H). A mixture of compound **11n** (50 mg) and 10% trifluoroacetic acid in dry methylene chloride (2 mL) was stirred at room temperature for 4 h. The reaction mass was basified to pH 8.5 using saturated aqueous $NaHCO_3$ solution and extracted using methylene chloride. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product was subjected to column chromatography using 10% methanol in methylene chloride to afford pure **11m** as a yellow solid in 84% yield. TLC (MeOH/hexane = 0.1:1), R_f = 0.21. ^{1}H NMR (400 MHz, MeOH-d_4): δ 7.74 (d, J = 15.5 Hz, 1H), 7.63 (d, J = 15.5 Hz, 1H), 7.46 (m, 2H), 7.39 (s, 2H), 7.09 (d, J = 8.4 Hz, 1H), 4.30 (t, J = 4.9 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 6H), 3.87 (s, 3H), 3.36 (t, J = 4.9 Hz, 2H). ^{13}C NMR (100 MHz, MeOH-d_4): δ 191.1, 154.6, 153.6, 149.6, 146.3, 143.9, 135.0, 129.6, 125.6, 120.8, 115.1, 113.0, 107.5, 107.0, 70.2, 61.2, 56.9, 56.5, 41.3. MS (ESI, positive) m/z 388.11. Calcd for $C_{21}H_{25}NO_6H$: 388.17

In Vitro Inhibition of TNF-α-Induced NF-κB Activation in A549 Lung Adenocarcinoma Cell Line. Human lung adenocarcinoma A549 cell line stably transfected with NF-κB-luc was purchased from Panomics (catalog no. RC0002, Fremont, CA). This cell line was obtained by cotransfecting A549 cells with pNFκB-luc (Panomics P/N LR0051) and pHyg followed by hygromycin selection, which can monitor NF-κB activity in vitro. This cell line was cultured using DMEM medium supplemented with 10% FBS, penicillin (100 units/mL), streptomycin (100 μg/mL), and hygromycin (100 μg/mL) at 37 °C with 5% CO_2. Cells were plated in a 96-well plate at a density of 5000 cells/well. Twenty-four hours after plating, the cell medium was replaced with fresh medium and incubated at 37 °C with 5% CO_2 for 1 h to minimize the potential stimulation to the cells induced by medium change. Cells were then treated with TNF-α (15 ng/mL) and chalcone-based compounds simultaneously for 7 h. Cells treated with TNF-α alone served as positive controls, while cells without TNF-α treatment served as negative controls. Luciferase activities from these cells were then measured by using the Bright-Glo luciferase assay kit from Promega (catalog no. E2620, Madison, WI), following the manufacturer's protocol (catalog no. TM052). The relative NF-κB activities of the cells treated by chalcone candidates were obtained as the ratio of its luciferase activity to that from the positive controls, both of which have been corrected with background (signals from negative controls) and cell viability.[56] Under these experimental conditions, none of the chalcone compounds induced significant toxicity to A549 cells (< 5% reduction of cell viability). The IC_{50} of each fraction was determined by fitting the relative NF-κB activity to the drug concentration by using a sigmoidal dose−response model of varied slope in GraphPad. The IC_{50} reported herein is the average of at least three replicates with standard error, in most cases, of no more than 20% of the average IC_{50}.

In Vitro Cytotoxicity Assay. Both cell lines were cultured in RPMI medium with 10% FBS, penicillin (100 units/mL), and streptomycin (100 μg/mL) at 37 °C with 5% CO_2. The in vitro cytotoxicity of the small molecules was assayed by determining the GI_{50} (the concentration of the small molecules to reduce the cell growth by 50%). In brief, the cells were plated in a 96-well plate (2.5×10^3 cells/well). The cells were treated with the small molecules with a series of 3-fold dilution with 0.5% DMSO in the final cell media (cells treated with media containing 0.5% DMSO served as a control). After 48 h of treatment, the relative cell viability in each well was determined by using CellTiter-Blue cell viability assay kit (a fluorescence assay that measures the reduction of a dye (resazurin) into a fluorescent end product (resorufin) by metabolically active cells − viable cells, Promega, CA). The GI_{50} of each agent was determined by fitting the relative viability of the cells to the drug concentrations by using a sigmoidal dose−response model of varied slope in GraphPad. The GI_{50} reported herein is the average of at least three replicates with standard error, in most cases, of no more than 20% of the average GI_{50}.

Upstream Kinase Inhibitory Screening. Compound **11a** was evaluated by Millipore Kinase Profiler service (Dundee, U.K.)

for its inhibitory activities against six upstream kinases of the NF-κB pathway, including IKKα, IKKβ, IRAK1, IRAK4, TAK1, and cSrc that have been reported to regulate the activation of the NF-κB signaling pathway. **11a** was evaluated at a single concentration of 10 μM in duplicate with ATP at a concentration of 10 μM.

Xenograft Tumor Growth and 11a Treatment. Female athymic nude mice obtained from Harland were maintained in a laminar airflow cabinet under pathogen-free conditions and used at 6−12 weeks of age. All facilities were approved by the American Association for Accreditation of Laboratory Animal Care in accordance with the current regulations and standards of the U.S. Department of Agriculture, U.S. Department of Health and Human Services, and NIH. Compound **11a** was reconstituted in a carrier made of PEG400/EtOH (v/v 2:1) to a concentration of 20 mg/mL. H2009 cells (60−70% confluent) were used for injection. Viable tumor cells (2×10^6 in 0.1 mL of PBS/metrigel (v/v 1:1)) were implanted subcutaneously into the right flank. Formation of a bulla indicated a satisfactory injection. Beginning on the same day of implantation, one group of mice (5 mice per group) were treated with the carrier (50 μL) through ip. injection and the other group of mice (5 mice per group) were treated with **11a** at 1 (mg/mouse)/day in the carrier (50 μL) through ip injection for 5 weeks, 6 days per week. The body weights of mice were monitored twice a week. Mice were euthanized by CO_2 at the end of week 5. Tumor mass and mouse final body weight were determined.

Statistical Analysis. All biological experiments, including the NF-κB inhibitory activities and the in vitro cytotoxicity, were performed at least three times with triplicates in each experiment. Average results are depicted in this report. Data were analyzed and presented using the GraphPad software. Student's *t*-test with a 95% confidence was applied for comparison between groups using the GraphPad software. Significance was set at $P < 0.05$. The correlation study was performed using a linear fitting model in GraphPad.

Acknowledgment. NIH Grant CA114294 and NIH Chemistry-Biology interface training grant are gratefully acknowledged. T.E.J. also gratefully acknowledges a 3M Science & Technology Fellowship.

Supporting Information Available: NMR spectra of new compounds and literature information of known chalcone compounds used for the study. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wattenberg, L. W.; Coccia, J. B.; Galbraith, A. R. Inhibition of carcinogen-induced pulmonary and mammary carcinogenesis by chalcone administered subsequent to carcinogen exposure. *Cancer Lett*. **1994**, *83*, 165–169.

(2) Ducki, S.; Forrest, R.; Hadfield, J. A.; Kendall, A.; Lawrence, N. J.; McGown, A. T.; Rennison, D. Potent antimitotic and cell growth inhibitory properties of substituted chalcones. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1051–1056.

(3) Baba, M.; Asano, R.; Takigami, I.; Takahashi, T.; Ohmura, M.; Okada, Y.; Sugimoto, H.; Arika, T.; Nishino, H.; Okuyama, T. Studies on cancer chemoprevention by traditional folk medicines XXV. Inhibitory effect of isoliquiritigenin on azoxymethane-induced murine colon aberrant crypt focus formation and carcinogenesis. *Biol. Pharm. Bull*. **2002**, *25*, 247–250.

(4) Kapadia, G. J.; Azuine, M. A.; Tokuda, H.; Hang, E.; Mukainaka, T.; Nishino, H.; Sridhar, R. Inhibitory effect of herbal remedies on 12-*o*-tetradecanoylphorbol-13-acetate-promoted Epstein−Barr virus early antigen activation*1. *Pharm. Res*. **2002**, *45*, 213–220.

(5) Kinghorn, A. D.; Su, B. N.; Jang, D. S.; Chang, L. C.; Lee, D.; Gu, J. Q.; Carcache-Blanco, E. J.; Pawlus, A. D.; Lee, S. K.; Park, E. J.; Cuendet, M.; Gills, J. J.; Bhat, K.; Park, H. S.; Mata-Greenwood, E.; Song, L. L.; Jang, M.; Pezzuto, J. M. Natural inhibitors of carcinogenesis. *Planta Med*. **2004**, *70*, 691–705.

(6) Rao, Y. K.; Fang, S. H.; Tzeng, Y. M. Differential effects of synthesized 2′-oxygenated chalcone derivatives: modulation of human cell cycle phase distribution. *Bioorg. Med. Chem*. **2004**, *12*, 2679–2686.

(7) Takahashi, T.; Takasuka, N.; Iigo, M.; Baba, M.; Nishino, H.; Tsuda, H.; Okuyama, T. Isoliquiritigenin, a flavonoid from licorice, reduces prostaglandin E2 and nitric oxide, causes apoptosis, and suppresses aberrant crypt foci development. *Cancer Sci*. **2004**, *95*, 448–453.

(8) Ye, C. L.; Liu, J. W.; Wei, D. Z.; Lu, Y. H.; Qian, F. In vivo antitumor activity by 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone in a solid human carcinoma xenograft model. *Cancer Chemother. Pharmacol*. **2005**, *55*, 447–452.

(9) Zi, X.; Simoneau, A. R. Flavokawain A, a novel chalcone from kava extract, induces apoptosis in bladder cancer cells by involvement of Bax protein-dependent and mitochondria-dependent apoptotic pathway and suppresses tumor growth in mice. *Cancer Res*. **2005**, *65*, 3479–3486.

(10) Achanta, G.; Modzelewska, A.; Feng, L.; Khan, S. R.; Huang, P. A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. *Mol. Pharmacol*. **2006**, *70*, 426–433.

(11) Hsu, Y. L.; Kuo, P. L.; Tzeng, W. S.; Lin, C. C. Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis. *Food Chem. Toxicol*. **2006**, *44*, 704–713.

(12) Kim, D. Y.; Kim, K. H.; Kim, N. D.; Lee, K. Y.; Han, C. K.; Yoon, J. H.; Moon, S. K.; Lee, S. S.; Seong, B. L. Design and biological evaluation of novel tubulin inhibitors as antimitotic agents using a pharmacophore binding model with tubulin. *J. Med. Chem*. **2006**, *49*, 5664–5670.

(13) Modzelewska, A.; Pettit, C.; Achanta, G.; Davidson, N. E.; Huang, P.; Khan, S. R. Anticancer activities of novel chalcone and bis-chalcone derivatives. *Bioorg. Med. Chem*. **2006**, *14*, 3491–3495.

(14) Shen, K. H.; Chang, J. K.; Hsu, Y. L.; Kuo, P. L. Chalcone arrests cell cycle progression and induces apoptosis through induction of mitochondrial pathway and inhibition of nuclear factor kappa B signalling in human bladder cancer cells. *Basic Clin. Pharmacol. Toxicol*. **2007**, *101*, 254–261.

(15) Wattenberg, L. W. Chalcones, myo-inositol and other novel inhibitors of pulmonary carcinogenesis. *J. Cell Biochem., Suppl*. **1995**, *22*, 162–168.

(16) Baba, M.; Asano, R.; Takigami, I.; Takahashi, T.; Ohmura, M.; Okada, Y.; Sugimoto, H.; Arika, T.; Nishino, Y.; Okuyama, T. Studies on cancer chemoprevention by traditional folk medicines. XXV. Inhibitory effect of isoliquiritigenin on azoxymethane-induced murine colon aberrant crypt focus formation and carcinogenesis. *Biol. Pharm. Bull*. **2002**, *25*, 247–250.

(17) Kanazawa, M.; Satomi, Y.; Mizutani, Y.; Ukimura, O.; Kawauchi, A.; Sakai, T.; Baba, M.; Okuyama, T.; Nishino, H.; Miki, T. Isoliquiritigenin inhibits the growth of prostate cancer. *Eur. Urol*. **2003**, *43*, 580–586.

(18) Yamazaki, S.; Morita, T.; Endo, H.; Hamamoto, T.; Baba, M.; Joichi, Y.; Kaneko, S.; Okada, Y.; Okuyama, T.; Nishino, H.; Tokue, A. Isoliquiritigenin suppresses pulmonary metastasis of mouse renal cell carcinoma. *Cancer Lett*. **2002**, *183*, 23–30.

(19) Israf, D. A.; Khaizurin, T. A.; Syahida, A.; Lajis, N. H.; Khozirah, S. Cardamonin inhibits COX and iNOS expression via inhibition of p65NF-kappaB nuclear translocation and Ikappa-B phosphorylation in RAW 264.7 macrophage cells. *Mol. Immunol*. **2007**, *44*, 673–679.

(20) Phillpotts, R. J.; Higgins, P. G.; Willman, J. S.; Tyrrell, D. A.; Lenox-Smith, I. Evaluation of the antirhinovirus chalcone Ro 09-0415 given orally to volunteers. *J. Antimicrob. Chemother*. **1984**, *14*, 403–409.

(21) Peyrot, V.; Leynadier, D.; Sarrazin, M.; Briand, C.; Rodriquez, A.; Nieto, J. M.; Andreu, J. M. Interaction of tubulin and cellular microtubules with the new antitumor drug MDL 27048. A powerful and reversible microtubule inhibitor. *J. Biol. Chem*. **1989**, *264*, 21296–21301.

(22) Edwards, M. L.; Stemerick, D. M.; Sunkara, P. S. Chalcones: a new class of antimitotic agents. *J. Med. Chem*. **1990**, *33*, 1948–1954.

(23) Lawrence, N. J.; McGown, A. T.; Ducki, S.; Hadfield, J. A. The interaction of chalcones with tubulin. *Anti-Cancer Drug Des*. **2000**, *15*, 135–141.

(24) Bhat, B. A.; Dhar, K. L.; Puri, S. C.; Saxena, A. K.; Shanmugavel, M.; Qazi, G. N. Synthesis and biological evaluation of chalcones and their derived pyrazoles as potential cytotoxic agents. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3177–3180.

(25) Lawrence, N. J.; Patterson, R. P.; Ooi, L. L.; Cook, D.; Ducki, S. Effects of alpha-substitutions on structure and biological activity

of anticancer chalcones. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5844–5848.
(26) Miglarese, M. R.; Carlson, R. O. Development of new cancer therapeutic agents targeting mitosis. *Expert Opin. Invest. Drugs* **2006**, *15*, 1411–425.
(27) Rowinsky, E. K.; Calvo, E. Novel agents that target tublin and related elements. *Semin. Oncol.* **2006**, *33*, 421–435.
(28) Liu, X.; Go, M. L. Antiproliferative properties of piperidinylchalcones. *Bioorg. Med. Chem.* **2006**, *14*, 153–163.
(29) Lee, Y. S.; Lim, S. S.; Shin, K. H.; Kim, Y. S.; Ohuchi, K.; Jung, S. H. Anti-angiogenic and anti-tumor activities of 2′-hydroxy-4′-methoxychalcone. *Biol. Pharm. Bull.* **2006**, *29*, 1028–1031.
(30) Luo, Y.; Eggler, A. L.; Liu, D.; Liu, G.; Mesecar, A. D.; van Breemen, R. B. Sites of alkylation of human Keap1 by natural chemoprevention agents. *J. Am. Soc. Mass. Spectrom.* **2007**, *18*, 2226–2232.
(31) Coussens, L. M.; Werb, Z. Inflammation and cancer. *Nature* **2002**, *420*, 860–867.
(32) Maeda, S.; Omata, M. Inflammation and cancer: role of nuclear factor-kappaB activation. *Cancer Sci.* **2008**, *99*, 836–842.
(33) Lu, H.; Ouyang, W.; Huang, C. Inflammation, a key event in cancer development. *Mol. Cancer Res.* **2006**, *4*, 221–233.
(34) Federico, A.; Morgillo, F.; Tuccillo, C.; Ciardiello, F.; Loguercio, C. Chronic inflammation and oxidative stress in human carcinogenesis. *Int. J. Cancer.* **2007**, *121*, 2381–2386.
(35) Zhang, Z.; Rigas, B. NF-kappaB, inflammation and pancreatic carcinogenesis: NF-kappaB as a chemoprevention target. *Int. J. Oncol.* **2006**, *29*, 185–192.
(36) Pikarsky, E.; Porat, R. M.; Stein, I.; Abramovitch, R.; Amit, S.; Kasem, S.; Gutkovich-Pyest, E.; Urieli-Shoval, S.; Galun, E.; Ben-Neriah, Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature* **2004**, *431*, 461–466.
(37) Karin, M. NF-kappaB and cancer: mechanisms and targets. *Mol. Carcinog.* **2006**, *45*, 355–361.
(38) Funakoshi-Tago, M.; Tanabe, S.; Tago, K.; Itoh, H.; Mashino, T.; Sonoda, Y.; Kasahara, T. Licochalcone A potently inhibits TNF-{alpha}-induced NF-{kappa}B activation through the direct inhibition of IKK activation. *Mol. Pharmacol.* **2009**, DOI: 10.1124/mol.109.057448.
(39) Harikumar, K. B.; Kunnumakkara, A. B.; Ahn, K. S.; Anand, P.; Krishnan, S.; Guha, S.; Aggarwal, B. B. Modification of the cysteine residues in IkappaBalpha kinase and NF-kappaB (p65) by xanthohumol leads to suppression of NF-kappaB-regulated gene products and potentiation of apoptosis in leukemia cells. *Blood* **2009**, *113*, 2003–2013.
(40) Kim, J. Y.; Park, S. J.; Yun, K. J.; Cho, Y. W.; Park, H. J.; Lee, K. T. Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* inhibits LPS-induced iNOS and COX-2 expression via the attenuation of NF-kappaB in RAW 264.7 macrophages. *Eur. J. Pharmacol.* **2008**, *584*, 175–184.
(41) Shen, K. H.; Chang, J. K.; Hsu, Y. L.; Kuo, P. L. Chalcone arrests cell cycle progression and induces apoptosis through induction of mitochondrial pathway and inhibition of nuclear factor kappa B signalling in human bladder cancer cells. *Basic Clin. Pharmacol. Toxicol.* **2007**, *101*, 254–261.
(42) Pandey, M. K.; Sandur, S. K.; Sung, B.; Sethi, G.; Kunnumakkara, A. B.; Aggarwal, B. B. Butein, a tetrahydroxychalcone, inhibits nuclear factor (NF)-kappaB and NF-kappaB-regulated gene expression through direct inhibition of IkappaBalpha kinase beta on cysteine 179 residue. *J. Biol. Chem.* **2007**, *282*, 17340–17350.
(43) Folmer, F.; Blasius, R.; Morceau, F.; Tabudravu, J.; Dicato, M.; Jaspars, M.; Diederich, M. Inhibition of TNFalpha-induced activation of nuclear factor kappaB by kava (*Piper methysticum*) derivatives. *Biochem. Pharmacol.* **2006**, *71*, 1206–1218.
(44) Israf, D. A.; Khaizurin, T. A.; Syahida, A.; Lajis, N. H.; Khozirah, S. Cardamonin inhibits COX and iNOS expression via inhibition of p65NF-kappaB nuclear translocation and Ikappa-B phosphorylation in RAW 264.7 macrophage cells. *Mol. Immunol.* **2007**, *44*, 673–679.
(45) Madan, B.; Batra, S.; Ghosh, B. 2′-Hydroxychalcone inhibits nuclear factor-kappaB and blocks tumor necrosis factor-alpha- and lipopolysaccharide-induced adhesion of neutrophils to human umbilical vein endothelial cells. *Mol. Pharmacol.* **2000**, *58*, 526–534.
(46) Lorenzo, P.; Alvarez, R.; Ortiz, M. A.; Alvarez, S.; Piedrafita, F. J.; de Lera, A. R. Inhibition of IkappaB kinase-beta and anticancer activities of novel chalcone adamantyl arotinoids. *J. Med. Chem.* **2008**, *51*, 5431–5440.
(47) Shishodia, S.; Amin, H. M.; Lai, R.; Aggarwal, B. B. Curcumin (diferuloylmethane) inhibits constitutive NF-kappaB activation, induces G1/S arrest, suppresses proliferation, and induces apoptosis in mantle cell lymphoma. *Biochem. Pharmacol.* **2005**, *70*, 700–713.
(48) Ban, H. S.; Suzuki, K.; Lim, S. S.; Jung, S. H.; Lee, S.; Ji, J.; Lee, H. S.; Lee, Y. S.; Shin, K. H.; Ohuchi, K. Inhibition of lipopolysaccharide-induced expression of inducible nitric oxide synthase and tumor necrosis factor-alpha by 2′-hydroxychalcone derivatives in RAW 264.7 cells. *Biochem. Pharmacol.* **2004**, *67*, 1549–1557.
(49) Kumar, S.; Sharma, A.; Madan, B.; Singhal, V.; Ghosh, B. Isoliquiritigenin inhibits IkappaB kinase activity and ROS generation to block TNF-alpha induced expression of cell adhesion molecules on human endothelial cells. *Biochem. Pharmacol.* **2007**, *73*, 1602–1612.
(50) Vogel, S.; Ohmayer, S.; Brunner, G.; Heilmann, J. Natural and non-natural prenylated chalcones: synthesis, cytotoxicity and anti-oxidative activity. *Bioorg. Med. Chem.* **2008**, *16*, 4286–4293.
(51) Khupse, R. S.; Erhardt, P. W. Total synthesis of xanthohumol. *J. Nat. Prod.* **2007**, *70*, 1507–1509.
(52) Tsang, K. Y.; Sinha, S.; Liu, X.; Bhat, S.; Chandraratna, R. A. Preparation of Disubstituted Chalcone Oximes as Selective Agonists of RAR-r Retinoid Receptors. U.S. Pat. Appl. Publ. US 2005148590, 2005.
(53) Schmid, J. A.; Birbach, A. IkappaB kinase beta (IKKbeta/IKK2/IKBKB), a key molecule in signaling to the transcription factor NF-kappaB. *Cytokine Growth Factor Rev.* **2008**, *19*, 157–165.
(54) Häcker, H.; Karin, M. Regulation and function of IKK and IKK-related kinases. *Sci. STKE* **2006**, *357*, 1–19.
(55) Ballaz, S.; Mulshine, J. L. The potential contributions of chronic inflammation to lung carcinogenesis. *Clin. Lung Cancer* **2003**, *5*, 46–62.
(56) Doshi, J. M.; Tian, D.; Xing, C. Ethyl-2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4*H*-chromene-3-carboxylate (HA 14-1), a prototype small-molecule antagonist against anti-apoptotic Bcl-2 proteins, decomposes to generate reactive oxygen species (ROS) that induce apoptosis. *Mol. Pharmaceutics* **2007**, *4*, 919–928.