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Mechanism of cell death mediated by 3 – aryl α, β – unsaturated ketone involving Michael adduct formation and induction of endoplasmic reticulum stress in human lung cancer A549 cells

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Abstract: Six natural products were applied to investigate the mechanism that the 3 – aryl α, β – unsaturated ketone induced human lung cancer A549 cell apoptosis. The effects of these compounds with or without the structure of 3 – aryl α, β – unsaturated ketone on human lung cancer A549 cells by the MTT assay were examined. It is found that these natural products with a fragment of 3 – aryl α, β – unsaturated ketone, including curcumin (CUR), chalcone (CC) and dehydrozingerone (DHZ), were cytotoxic, with CUR > CC > DHZ, whereas the compounds without the 3 – aryl α, β – unsaturated ketone including tetrahydrocurcumin (THC), dihydrochalcone (DHCC) and zingerone (ZG) were not. *In vitro*, 3 – aryl α, β – unsaturated ketone formed Michael adducts with the thiol nucleophile N – acethlcysteine. In cultured cells, preincubation of the NAC with CUR, CC and DHZ decreased the cytotoxicity significantly except the THC, DHCC and ZG. Moreover, CUR and DHZ induced A549 cell apoptosis by activating the proteins JNK, P – JNK, CHOP, Bcl – 2, Bax and cyto – *c*. These results suggested that 3 – aryl α, β – unsaturated ketone mediated cell apoptosis involve in the mechanism of Michael adduct formation and induction of endoplasmic reticulum stress in human lung cancer A549 cells.

Key words: 3 – aryl α, β – unsaturated ketone; NAC; Michael adduct; apoptosis
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0 Introduction

A lot of natural products containing 3 – aryl α, β – unsaturated ketone possess a variety of pharmacological activities and therapeutic properties. Curcumin, derived from the *Curcuma longa L.*, a major yellow pigment and active component of turmeric, exhibits numerous biological activities including anti – tumor, anti – inflammatory, and anti – angiogenesis activity [1]. Curcumin is a homodimer of feruloylmethane containing 3 – aryl α, β – unsaturated ketone, a methoxy group, and a hydroxyl group. Dehydrozingerone, another natural product derived from the *Curcuma longa L.*, is a curcumin analog containing 3 – aryl α, β – unsaturated ketone. The structure of α, β – unsaturated ketone, as a Michael acceptor, can form adducts with the sulfhydryl group and generate reactive oxygen spe-

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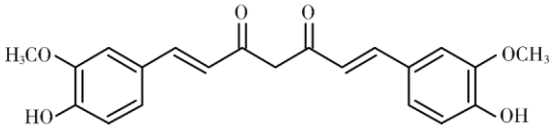
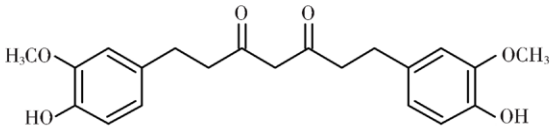
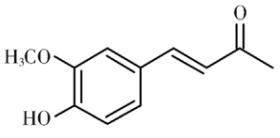
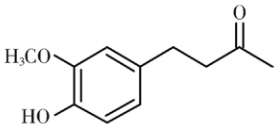
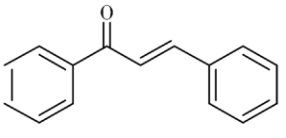
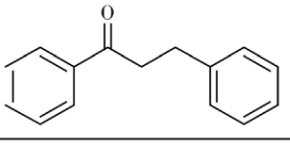
cies [2-5]. However, the underlying molecular and biochemical mechanisms responsible for 3 – aryl α, β – unsaturated ketone inducing apoptosis are not well understood and most probably involve several pathways. In particular, the role of Michael adduct formation in dehydrozingerone and chalcone cytotoxicity is not clear.

In the present study, curcumin, dehydrozingerone, chalcone, tetrahydrocurcumin, zingerone, and dihydrochalcone, six natural products with 3 – aryl α, β – unsaturated ketone group or without 3 – aryl α, β – unsaturated ketone group, were applied to investigate the mechanism that the 3 – aryl α, β – unsaturated ketone induced human lung cancer A549 cell apoptosis (Table 1). Comparisons among three 3 – aryl α, β – unsaturated ketone congener compounds, methyl substituted dehydrozingerone (DHZ), aryl substituted chalcone (CC), and two Michael acceptors curcumin (CUR), allow us to estimate contributions from 3 – aryl α, β – unsaturated ketone structure to the extent and rate of Michael adduct formation, to 3 – aryl α, β – unsaturated ketone cytotoxicity, and to cellular mechanisms associated with it.

Table 1　Chemical structures of the six natural products

Compound	Structure
Curcumin(CUR)	
Tetrahydrocurcumin(THC)	
Dehydrozingerone(DHZ)	
Zingerone(ZG)	
Chalcone(CC)	
Dihydrochalcone(DHCC)	

1　Materials and methods

1.1　Chemical synthesis

^{1}H and ^{13}C NMR spectra were recorded in ppm on a Bruker Biospin A G 400 MHz spectrometer (Bruker Optics, Germany) with TMS as an internal standard. Coupling constants were reported in Herz (Hz). NMR abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, br = broad, ap = approximate. ESI – MS was performed on an Applied Biosystems 3 200 Q Trap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) via a TurboIonSpray eletrospray ionization (ESI) interface for mass analysis and detection. Infrared (IR) spectra were recorded on a Nicolet Avatar 380 FT – IR spectrometer (Nicolet Instrument Co., Madison, WI, USA). The purification of compounds was ac–

complished by chromatography (Silica Gel GF 254) with petroleum ether and ethyl acetate as eluents. All chemicals were purchased as AR reagents and were used without further purification. Reactions were carried out under an atmosphere of nitrogen with a septum cap in ovendried glassware with magnetic stirring.

1.1.1 Preparation of Dehydrozingerone (DHZ)

To a solution of acetophene (99 mg, 0.83 mmol) , vanillic aldehyde (126 mg, 0.83 mmol) and water (10 mL) at room temperature was added NaOH (40 mg, 1.00 mmol) . The reaction mixture was stirred 24 h. Yellow solids separated were filtered, washed with ice water and purified by chromatography using petroleum ether and ethyl acetate(6∶1) as eluent to give a yellow solid (143 mg, 90% yield) [6] .

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 2.38 (s, 3H) , 3.92 (s, 3H) , 6.20 (d, J = 16.2 Hz, 1H) , 6.58 (d, J = 16.2 Hz, 1H) , 6.95 (d, J = 8.18 Hz, 1H) , 7.03 (d, J = 1.89 Hz, 1H) , 7.09 (dd, J = 8.18, 1.89 Hz, 1H) . ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 27.3, 55.9, 109.4, 114.9, 123.6, 124.9, 126.8, 143.9, 146.9, 148.4, 198.6. IR (KBr, cm^{-1}) : 3 416, 1 624, 1 590, 1 512, 1 422, 1 255, 1 160, 1 031, 981. MS (ESI, m/z) : 193.1 [M + H^+] , 215.0 [M + Na^+] , 231.1 [M + K^+] .

1.1.2 Preparation of Tetrahydrocurcumin (THC)

Curcumin (1.0 g, 2.7 mmol) in methanol (20 mL) and 10% palladium on activated charcoal (0.30 g) was stirred under hydrogen for 60 h at room temperature. The reaction solution was concentrated in vacuo and the residue was purified by chromatography using petroleum ether and ethyl acetate (8∶1) as eluent to give a yellow solid (0.68 g, 67% yield) [7] .

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 2.53 ~ 2.59 (m, 4H) , 2.73 ~ 2.89 (m, 4H) , 3.52 (s, 2H) , 3.86 (s, 6H) , 5.48 (s, 2H) , 6.65 ~ 6.77 (m, 4H) , 6.83 ~ 6.89 (dd, J = 8.4 Hz, 2H) , 7.31 (s, 1H) , 16.91 (brs, 1H, enol) . ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 31.3, 40.3, 55.8, 99.8, 110.9, 114.3, 120.8, 132.3, 143.9, 146.4, 185.2, 193.2. IR (KBr, cm^{-1}) : 3 435, 2 955, 2 919, 2 850, 1 618, 1 518, 1 462, 1 270, 1 032, 852, 808, 791. MS (ESI, m/z) : 373.2 [M + H^+] , 395.2 [M + Na^+] , 411.2 [M + K^+] .

1.1.3 Preparation of Zingerone (ZG)

Dehydrozingerone (96 mg, 0.5 mmol) in methanol (10 mL) and 10% palladium on activated charcoal (0.10 g) was stirred under hydrogen for 12 h at room temperature. The reaction solution was concentrated in vacuo and the residue was purified by chromatography using petroleum ether and ethyl acetate (5∶1) as eluent to give a yellow oil (92 mg, 95% yield) [8] .

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 2.53 ~ 2.85 (m, 6H) , 3.94 (s, 3H) , 5.91 (s, 1H) , 6.61 ~ 6.83 (m, 3H) . ^{13}C NMR ($CDCl_3$, 100 MHz) : δ 29.3, 30.4, 45.4, 55.8, 111.1, 114.4, 120.7, 132.8, 143.8, 146.5, 208.3. IR (KBr, cm^{-1}) : 3 426, 2 945, 2 918, 2 851, 1 617, 1 523, 1 235, 1 160, 1 021, 881, 780. MS (ESI, m/z) : 195.0 [M + H^+] , 217.1 [M + Na^+] , 233.0 [M + K^+] .

1.1.4 Preparation of Chalcone (CC)

To a solution of acetophene (99 mg, 0.83 mmol) , benzaldehyde (88 mg, 0.83 mmol) and methanol (1 mL) at room temperature was added NaOH (40 mg, 1.00 mmol) . The reaction mixture was stirred 40 min. Yellow solids separated were filtered, washed with ice water and purified by chromatography using petroleum ether and ethyl acetate(6∶1) as eluent to give a yellow solid (140 mg, 83% yield) [9] .

^{1}H – NMR (400 MHz, $CDCl_3$) : δ 8.16 (d, J = 7.3 Hz, 2H) , 7.96 (d, J = 15.8 Hz, 1H) , 7.91 (dd, J = 7.5, 2H, 3.5 Hz) , 7.76 (d, J = 15.8 Hz, 1H) , 7.69 (tt, J = 7.5, 1H, 1.3 Hz) , 7.59 (dt, 2H, J = 7.5, 1.3 Hz) , 7.49 ~ 7.46 (m, 3H) ^{13}C NMR (100 MHz, $CDCl_3$) : δ 189.2, 144.0, 137.5, 134.6, 133.1, 130.6, 128.9, 128.9, 128.8, 128.5, 122.0. IR (KBr, cm^{-1}) : 3 462, 3 059, 3 026, 1 664, 1 606, 1 576, 1 495, 1 448, 1 335, 1 306, 1 215, 1 176, 978, 746, 689, 565. MS (ESI, m/z) : 209.3 [M + H^+] , 230.9 [M + Na^+] , 247.1 [M + K^+] .

1.1.5 Preparation of Dihydrochalcone (DHCC)

A chalcone (0.1 g, 0.48 mmol) in methanol (10 mL) of 10% palladium on activated charcoal (50 mg) was stirred under hydrogen for 6 h at room temperature. The reaction solution was concentrated in vacuum and the resi–

due was purified by chromatography using petroleum ether and ethyl acetate (5∶1) as eluent to give a light yellow solid (42.6 mg, 42.3% yield).

^{1}H – NMR (400 MHz, $CDCl_3$): δ 7.98 (d, 2H, J = 8.0 Hz), 7.64 ~ 7.60 (m, 1H), 7.51 (dd, 2 H, J = 8.0, 4.0 Hz), 7.28 ~ 7.24 (m, 4H), 7.19 ~ 7.14 (m, 1H), 3.37 (t, 2H, J = 8.0 Hz), 2.93 (t, 2H, J = 8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.1, 141.2, 136.6, 133.1, 128.7, 128.3, 128.2, 127.9, 125.8, 40.1, 29.4. IR (KBr, cm^{-1}): 3 423, 3 342, 3 083, 3 024, 2 951, 2 924, 2 854, 1 963, 1 894, 1 682, 1 595, 1 495, 1 448, 1 261, 1 032, 602, 744, 689, 561, 519. MS (ESI, m/z): 211.2 [M + H$^+$], 233.1 [M + Na$^+$].

1.2 Biological study

1.2.1 Materials

Dehydrozingerone, chalcone, tetrahydrocurcumin, zingerone, and dihydrochalcone were synthesized, purified and characterized. The purity of all chemicals was proved above 98% (identified by NMR and HPLC). Curcumin and NAC (B.R) were purchased from Sinopharm Chemical Reagent Co.. RPMI – 1640 was purchased from GIBCO (Invitrogen, USA). Calf serum, penicillin, streptomycin were purchased from Hyclone (USA). Anti – cytochrome c, anti – Bcl – 2, anti – Bax, anti – JNK, anti – p – JNK, antibodies, Goat anti – Mouse IgG (H + L), and Goat anti – Rabbit IgG (H + L) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). CHOP antibody was purchased from Afinity Bioreagents (ABR). Other reagents used were of analytical grade and were procured locally.

1.2.2 Cell lines and cell culture

Human lung cancer cells A549 were acquired from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cancer cells were maintained in RPMI1640 medium containing 10% fetal bovine serum, 100 U · mL^{-1} penicillin and 0.1 mg · mL^{-1} streptomycin. The cells were cultured in a humidified incubator in 5% CO_2 at 37 ℃.

1.2.3 Measurement of cell viability

The inhibitory effect of compounds on the growth of A549 cells was measured by MTT assay as described [10]. The cells were dispensed in 96 – well flat bottom microtiter plates (NUNC, Roskilde, Denmark) at a density of 1×10^4 cells per well. After 12 h incubation, they were treated with various concentrations of compounds for the indicated time periods. Cell growth was measured with an ELISA reader (TECAN SPECTRA, Wetzlar, Germany) by MTT assay at the indicated time points.

$$\text{Viability}\ (\%) = 100 - [A_{490,\,\text{control}} - A_{490,\,\text{compounds}}) / A_{490,\,\text{control}} \times 100\%]$$

1.2.4 NAC detoxification

For detoxification without preincubation, the six compounds (160 μmol · mL^{-1}) and 1 mmol · mL^{-1} NAC were added to cell cultures at the same time, and then incubated with cells for an additional 48 h. For detoxification with preincubation, the NAC/compounds mixture was incubated at room temperature for indicated time periods, and then added to cell cultures for a 48 h incubation period. Cell viability was measured by the MTT assay.

1.2.5 ESI – MS analysis of adducts

1 mmol · mL^{-1} curcumin, dehydrozingerone and chalcone in 20 mL methanol with 5 mmol · L^{-1} NAC in 5mL water were used for the experiments, respectively. The solutions were stirred on a magnetic stirrer for about 9 h at room temperature, and then the mixed solution of 10 μL was aspirated and diluted with 500 μL of methanol. Mass spectrometry was applied to detect the adducts.

1.2.6 Western blot analysis

A549 cells were plated in 100 mm dish at a density of 4×10^6 cells/plate for 24 h, then incubated with 90 μmol · mL^{-1} compounds for an additional 0, 3, 6, 9, 12, and 24 h. The cells were washed with PBS for 3 times and collected. After centrifugation, cell lysis was carried out on the ice for 15 min in RIPA buffer, every 5 mins, the cells were shaked in vortex for about 30 s. Then cells were centrifugated at 14 000 g for 1 min, the supernatant was separated and stored at – 78 ℃ until use. After addition of sample loading buffer, protein samples were electro–

phoresed on a 10% SDS – polyacrylamide gel. Proteins were transferred to polyvinylidene difluoride blots at 300 mA for 1.5 h. The blots were blocked in fresh blocking buffer (5% W/V 5 g non – fat milk dissolved in 100 mL Tris – buffered saline) at room temperature for 1 h. The blots were incubated with primary antibodies, anti – β – actin (1∶1 000, V/V), anti – cyto – *c* (1∶1 000, V/V), anti – Bax (1∶1000, V/V), anti – Bcl – 2 (1∶750, V/V), anti – JNK (1∶1 000, V/V), anti – p – JNK (1∶1 000, V/V), anti – CHOP (1∶1 000, V/V) at 4 ℃ for 12 h. Following three times washed with TBST (TBS and 0.05% Tween 20), the blots were incubated with goat anti – mouse or goat anti – rabbit secondary antibodies (1∶3 000, V/V) at room temperature for 1 h. The blots were washed again three times in TBST buffer, and transferred proteins were incubated with ECL substrate solution for 1 min, followed by visualization with X – ray film.

1.2.7 Statistical Analysis

All data represent at least three independent experiments and expressed as the mean ± SD unless otherwise indicated. Statistical comparisons were made by Students' s *t* – test. Significance was considered as $p < 0.05$.

2 Results and Discussion

2.1 Higher cytotoxicity associated with 3 – aryl α, β – unsaturated ketone

To evaluate the inhibitory effects of six natural products on the growth of A549 cells, the cells were treated with 10 ~ 160 μmol · mL^{-1} of compounds for 48 h, and the cell viability was determined by MTT assay. Curcumin, dehydrozingerone and chalcone inhibited the growth of A549 cells in a dose – dependent manner. As shown in Fig. 1, CUR, CC, and DHZ were cytotoxic, with CUR > CC > DHZ, severe cytotoxicity was observed in cells treated with CUR and CC, and 160 μmol · mL^{-1} DHZ caused 60% of the cells to lose viability, whereas little change in viability was observed in cells treated with THC, DHCC, and ZG. The results suggested that 3 – aryl α, β – unsaturated ketone plays a key role in natural products – induced cell death in A549 cells.

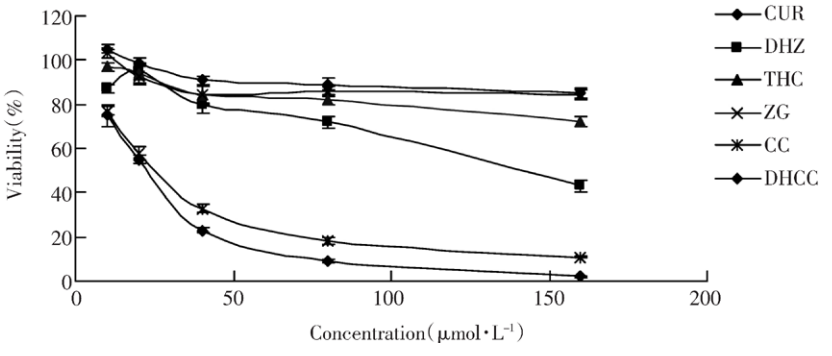
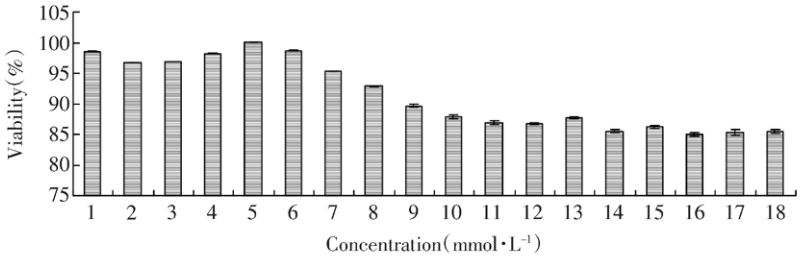


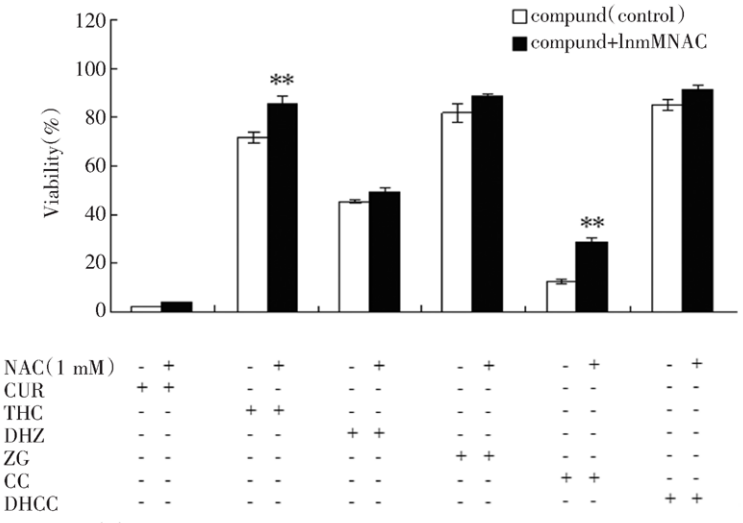
Fig. 1 Inhibitory effects of the six natural products on the growth of A549 cells
(The data are presented as the mean ± S. D. of the results for three independent experiments)

2.2 NAC prevents the cytotoxicity of curcumin, dehydrozingerone and chalcone by prior Michael adduct formation

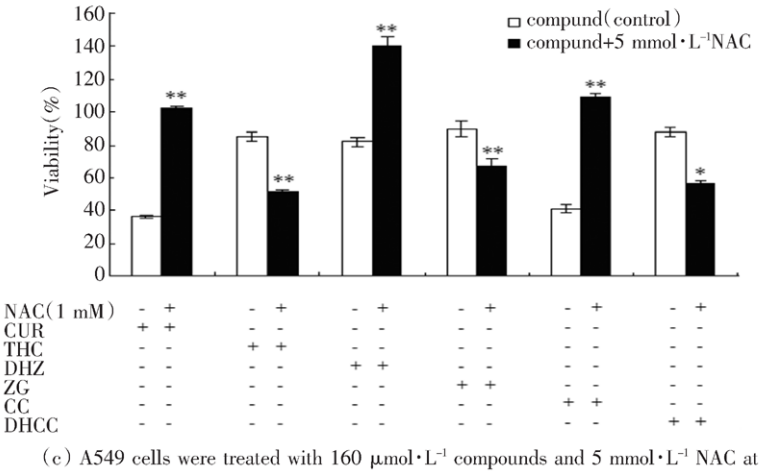
The α, β – unsaturated ketone, as a Michael acceptor, could form adducts with the sulfhydryl groups and generate reactive oxygen species. To illustrate whether Michael adduct formation was responsible for the 3 – aryl α, β – unsaturated ketone cytotoxicity observed, we investigated the role of NAC, a simple and cell – permeable thiol nucleophile, in the alleviation of cytotoxicity [11 – 13]. It was not clear whether the NAC protective effect was because of its formation of a Michael adduct with CUR, CC, and DHZ. In the present study, we preincubated the compound and NAC mixtures at room temperature for 9 h to allow Michael adduct formation before its addition to cells. A significant increase in cell viability was observed with an equimolar mixture after preincubation of NAC with CUR, CC, and DHZ (Fig. 2). In contrast, NAC enhanced THC, DHCC, and ZG – induced cell death in A549 cells. The results illustrated that the Michael adducts formation were responsible for the cytotoxicity of CUR, CC, and DHZ, and 3 – aryl α, β – unsaturated ketone played an important role in the cell death process.



(a) Inhibitory effects of NAC on the growth of A549 cells. Cells were treated with various concentrations (1 mmol·L^{-1} to 18 mmol·L^{-1}) of NAC for 48 h



(b) A549 cells were treated with 160 μmol·L^{-1} compounds, and 1 mmol·L^{-1} NAC added immediately without preincubation for 48 h



(c) A549 cells were treated with 160 μmol·L^{-1} compounds and 5 mmol·L^{-1} NAC at room temperature for 9 h, and then the cells were incubated with this mixture for another 48 h. Cell viability was measured by MTT assay as described. (*P<0.005, **P<0.001, significant difference between NAC groups and the control)

Fig. 2　Effects of NAC on cell death induced by the six natural products

2.3　Formation of Michael adducts

To further confirm whether the cause of A549 cell death induced by CUR, CC, and DHZ was by Michael adduct formation of α, β – unsaturated ketone, ESI – MS was performed. The solutions of CUR, CC, and DHZ with NAC were stirred at room temperature for 9 h, and then the mixtures were detected by ESI – MS analyses. The results showed that Michael addition of NAC to CUR, CC, and DHZ gave rise to adducted [M + H$^+$] at 532.0, [M + K$^+$] at 569.9 (Fig. 3a), [M + H$^+$] at 356.0 and [M + K$^+$] at 393.9 (Fig. 3b) and [M + H$^+$] at 372.3 and [M + Na$^+$] at 394.2 (Fig. 3c), respectively. The results demonstrated that CUR, CC, and DHZ induced cell death involving the mechanism of Michael adduct formation of 3 – aryl α, β – unsaturated ketone. (a) 1 mmol ·

L^{-1} CUR, (b) 1 mmol · L^{-1} DHZ or (c) 1 mmol · L^{-1} CC in 20 mL methanol and 5 mmol · L^{-1} NAC in 5 mL water were mixed and analyzed by ESI – MS in the positive ion, respectively.

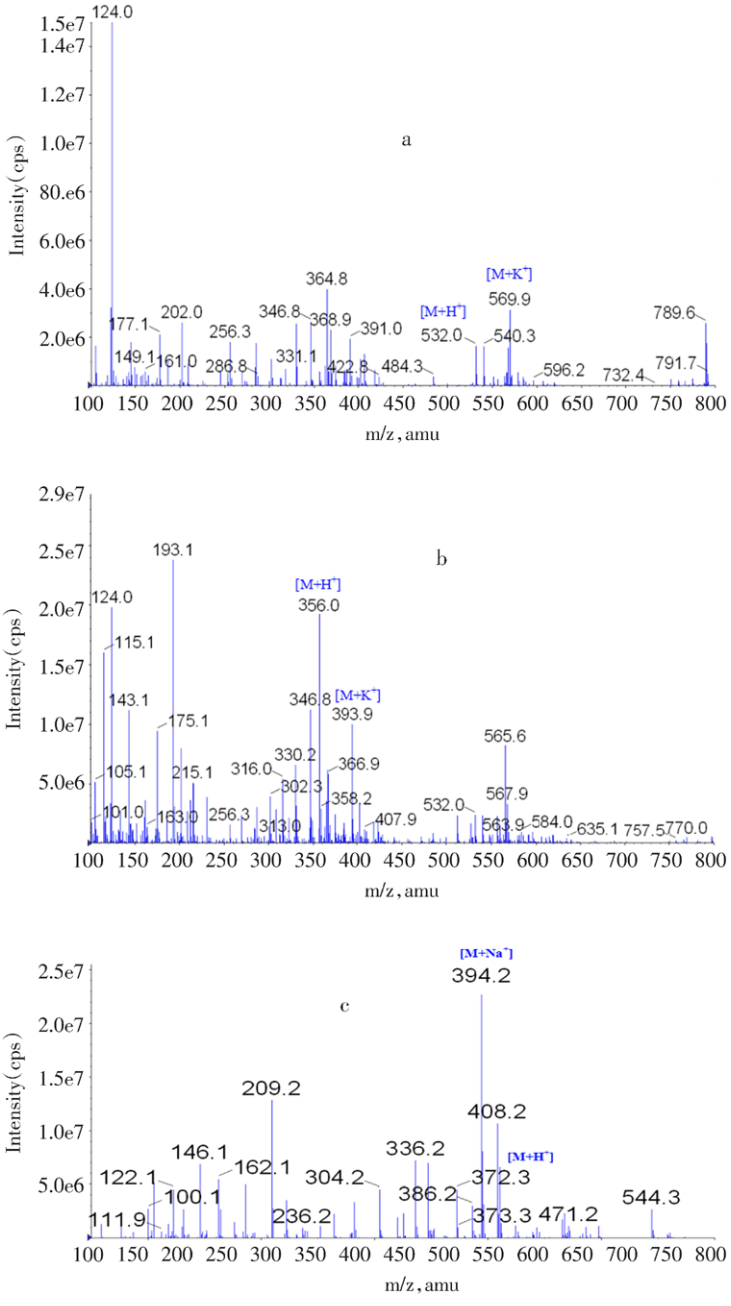


Fig. 3 Michael adduct formation of CUR, DHZ, and CC with NAC

2.4 Induction of ER stress is associated with 3 – aryl α, β – unsaturated ketone cytotoxicity

It was reported that disruption of disulfide bonding by Michael adduct formation should lead to protein misfolding and ER stress [5]. To investigate whether ER stress was associated with these natural products toxicity, and CHOP and JNK were required for the 3 – aryl α, β – unsaturated ketone – induced cell death, Western blot analysis was performed to examine CHOP, JNK and p – JNK expression during cell death. A time – course analysis of CHOP, JNK and phosphorylated JNK in A549 cells treated with 90 μmol · L^{-1} CUR or DHZ was applied to investigate the role of these proteins. After A549 cells were treated with CUR, the expression level of CHOP significantly increased within 9 h, whereas treatment with lower cytotoxicity DHZ, CHOP increased in 12 h (Fig. 4a). Exposure of the cells to CUR or DHZ for 24 h increased the expression of p – JNK, suggesting that the activation of JNK signaling pathway by CUR or DHZ involved in the mechanism of ER stress induced by 3 – aryl α, β – unsaturated ketone in A549 cells.

2.5　α, β – unsaturated ketone – induced mitochondrial pathway dependent cell death

To investigate whether the cytochrome c/Apaf – 1/caspase – 9 'apoptosome' was required for two compounds – induced cell death, Western blot analysis was performed. The expression level of Bax slightly increased within 12 h, and Bcl – 2 protein decreased with culturing time periods. Cytochrome c dissipated much later, 12 to 24 h after CUR or DHZ treatment (Fig. 4). It was suggested that these apoptotic pathways were involved in mitochondrial function, and the mechanism of DHZ – induced cell death was coincident with that of CUR. These results demonstrated that 3 – aryl α, β – unsaturated ketone – induced cell death involved in the same mechanism and 3 – aryl α, β – unsaturated ketone played a crucial role in natural product – induced cell death. The cells were treated with 90 μmol · L^{-1} CUR or DHZ for 0, 3, 6, 9, 12 and 24 h. Cell lysates were separated by 12% SDS – PAGE electrophoresis. JNK, p – JNK, CHOP, Bc1 – 2, Bax and cyto – *c* protein bands were detected by Western blot analysis.

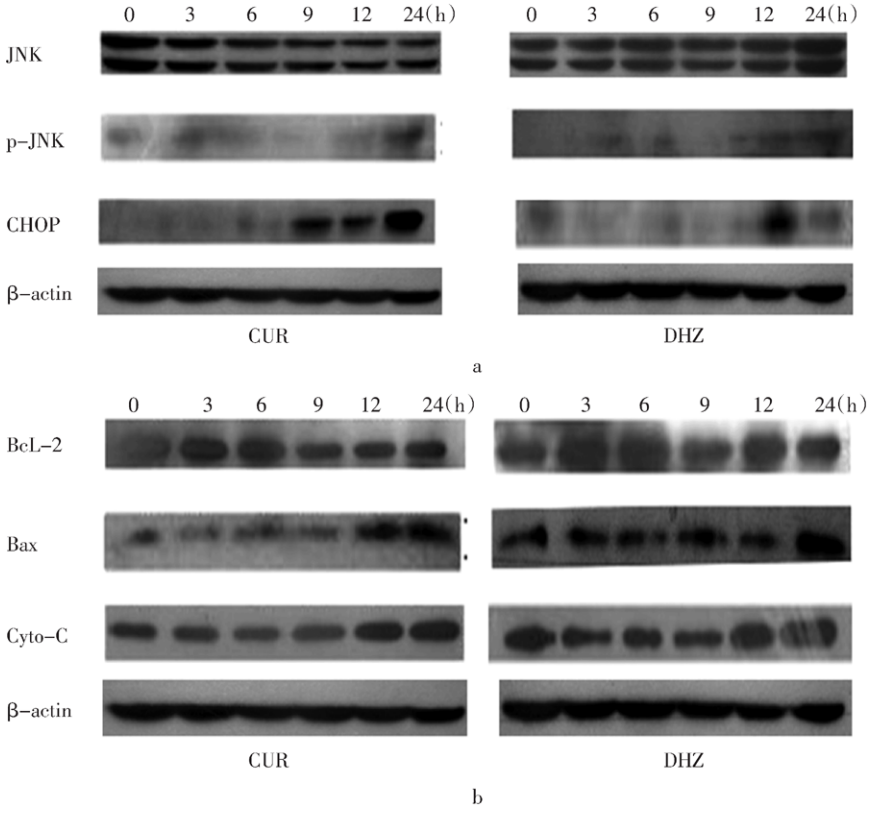


Fig. 4　(a) Effects of CUR and DHZ on the expression of JNK, p – JNK, and CHOP; (b) Effects of CUR and DHZ on the expression of Bcl – 2, Bax and cyto – *c*

2.6　The lipophilicities (Clog *P*) of the six compounds

N – Octanol/water partition coefficient (log *P*) of compounds has long been recognized as the key molecular property and is widely used in such diverse areas as pharmaceutics, biochemistry, environmental chemistry, toxicology, chemistry and chemical engineering. The lipophilicities of the six compounds were calculated to correlate their values with the cell toxicity. In this study, lipophilicity is given as Clog *P* and the values (Table 2) were calculated as described [14]. The IC_{50} value was performed using software SPSS 17.0. The Clog *P* value was calculated using software ACD/ChemSketch 8.0. The results indicated that the in vitro cytotoxicity of these 3 – aryl α, β – unsaturated ketones did not correlate with the calculated Clog *P* values.

Table 2　The lipophilicity and in vitro cytotoxicity activity in A549 cells

Compounds	Clog *P*	IC_{50} (μmol · L^{-1})
CUR	4.35	31.69
THC	4.20	>200
DHZ	2.16	117.1
ZG	2.08	> 200
CC	4.01	42.17
DHCC	3.83	> 200

3　Conclusions

In the present study, it was shown that 3 – aryl α, β – unsaturated ketone mediated cell death involve in the

mechanism of Michael adduct formation and induction of endoplasmic reticulum stress in human lung cancer A549 cells. Higher toxicity associated with CUR, DHZ, and CC containing 3 – aryl α, β – unsaturated ketone in contrast to its congener reduced products THC, DHCC, and ZG, and detoxification by prior formation of a Michael adduct with the thiol nucleophile NAC, unambiguously reveal the important role of Michael adduct formation in 3 – aryl α, β – unsaturated ketone cytotoxicity. 3 – aryl α, β – unsaturated ketone induced A549 cell apoptosis mediated by Michael adduct formation, through up – regulation of Bax and down – regulation of Bcl – 2 to release of cytochrome c, which contributed to A549 cell death. In addition, the induction of ER stress is tightly coupled to 3 – aryl α, β – unsaturated ketone cytotoxicity in curcumin and dehydrozingerone induced A549 cell apoptosis, indicating ER stress as a cellular mechanism for 3 – aryl α, β – unsaturated ketone cytotoxicity.

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3 – 芳基 – α, β – 不饱和酮介导的细胞凋亡机理研究

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摘 要: 选择了与 3 – 芳基 – α, β – 不饱和酮结构关联的六个天然产物, 研究了 3 – 芳基 – α, β – 不饱和酮在诱导人肺癌细胞 *A*549 凋亡的作用与分子机制。结果表明, 六个天然产物的细胞毒活性来源于 3 – 芳基 – α, β – 不饱和酮结构, 缺失该结构, 活性明显降低; NAC 明显抑制 3 – 芳基 – α, β – 不饱和酮诱导的细胞毒活性, 3 – 芳基 – α, β – 不饱和酮诱导的 A549 细胞凋亡涉及迈克尔加成、内质网应急和线粒体依赖的细胞凋亡分子机理。

关键词: 3 – 芳基 – α, β – 不饱和酮; NAC; 迈克尔加成; 细胞凋亡