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cis-Terpenones as an Effective Chemopreventive Agent against Aflatoxin B1 Induced Cytotoxicity and TCDD-induced P450 1A/B activity in HepG2 Cells

Qibing Zhou[†,*], **Hang Xie**[‡], **Lin Zhang**[†], **Jennifer K. Stewart**[§], **Xing-Xing Gu**[‡], and **John J. Ryan**[§]

†*Department of Chemistry, Virginia Commonwealth University, 1001 West Main Street, Richmond, Virginia 23284-2006*

‡*National Institute on Deafness and Other Communication Disorders, National Institute of Health, 5 Research Court, Rockville, Maryland 20850*

§*Department of Biology, Virginia Commonwealth University, 1000 West Cary Street, Richmond, Virginia 23284-2012*

Abstract

Aflatoxin B1 (AFB1) is a potent carcinogen, which can significantly increase the risk of hepatocellular carcinoma development through food contamination. In past decades, chemopreventive agents such as oltipraz and chlorophyllins have demonstrated that chemo-intervention is an effective approach to reduce the hepatotoxicity by AFB1. However, due to the potential adverse effects of these agents, alternative novel mechanism-based chemopreventive agents are needed. We report here that novel *cis*-terpenones **1-3,** which were synthesized as the precursors of natural product analogues in our laboratory, showed promising protective effects against AFB1 induced cytotoxicity in HepG2 cells. Chemo-protection was observed with increasing concentrations of *cis*-terpenones in the co-treatment of AFB1, and no cytotoxicity was observed with *cis*-terpenones alone. In addition, *cis*-terpenones **1-3** at 10 μM effectively inhibited induced cytochrome P450 1A/1B activity by 50% in HepG2 cells, as indicated by an EROD assay. P450 1A/B is involved in the activation of many pre-carcinogens and is highly inducible in liver cells. These results suggested that novel terpenones **1-3** are candidates for the development of novel mechanism-based chemopreventive agents against AFB1 and other carcinogenic stimuli.

Introduction

Aflatoxin B1 (AFB1)[1] is a major mycotoxin produced by the fungus *A. flavus* and is commonly found as a crop contaminant (1). The potent cytotoxicity of AFB1 is attributed to the covalent DNA modification by the exo-epoxide metabolite (2,3), resulting in liver damage and possibly hepatocellular carcinoma (4-8**)**. In past decades, several effective chemoprevention agents, including oltipraz and chlorophyllins, have been developed and showed promising results in clinical trials (9-12). Unfortunately, adverse effects were the major concern for oltipraz (9, 10), while more mechanistic studies are required for chlorophyllins (12). Recently, screening of natural products, such as extracts from algae (13), onion bulbs (14), and kola seeds (15), has identified several alternatives as potential candidates against AFB1. However, it is difficult to

*To whom correspondence should be addressed. Phone: (804) 828-3520. Fax (804) 828-8599. Email: qzhou@vcu.edu.

predict at this time whether these agents will be effective drugs. Thus, novel mechanism-based chemoprevention agents against AFB1 are needed.

cis-Terpenones **1-3** were designed as precursors of analogues of the natural product tingenone (16,17), which could be metabolically converted by Phase I enzymes through hydroxylation and oxidation, based on the metabolism of similar compounds, such as estrogens (18,19). On the other hand, *cis*-terpenones **1-3** differ from estrogens due to the unique bent conformation (17). The hypothesized hydroxylated *cis*-terpenone metabolite resembles partially the structure of the chemopreventive agent resveratrol, which is a potent inhibitor of P450 1A/B activity (20,21). Because P450 1A/B is highly inducible in liver cells and is involved in the activation of many pre-carcinogens, including benzopyrenes, estrogens and even AFB1 (20-24); it is important to evaluate the inhibitory potential of these *cis*-terpenones on P450 1A/B.

We report in this paper that novel *cis*-terpenones **1-3** (Scheme 1) showed promising protective effects against AFB1 induced cytotoxicity in HepG2 cells, while no cytotoxicity was observed by *cis*-terpenones alone. The chemo-protective effect of *cis*-terpenones **1-3** was observed with both cell viability and apoptosis assays when co-treated with AFB1. In addition, *cis*-terpenones **1-3** at 10 μM effectively inhibited the induced activity of Phase I P450 1A/B in HepG2 cells as indicated by an EROD assay. These results suggest that *cis*-terpenones **1-3** are potential candidates for novel mechanism-based preventive agents against both AFB1 and other carcinogenic stimuli.

Experimental Procedures

All chemicals were purchased from Fisher Scientific (Pittsburg, PA) or Sigma-Aldrich (Milwaukee, WI) and used without further purification. NMR spectra of the synthesized compounds were obtained by Variant NMR spectrometers. Electrospray ionization mass spectroscopy (ESI-MS) analysis was carried out with Q-TOF2 from Micromass (Manchester, UK).

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[1]Abbreviations:

AFB1 aflatoxin B1

ANOVA analysis of variance

EROD ethoxyresorufin-O-deethylase

ESI-MS electrospray ionization mass spectroscopy

FITC fluorescein isothiocyanate

MTT methylthiazolyldiphenyl-tetrazolium bromide

ROESY rotating-frame Overhauser spectroscopy

PI propidium iodine

TCDD 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

2-(3-Methoxybenzyl)-2-(2,6-dimethylhepta-1,5-dienyl)-1,3-dithiane (5)

To a solution of thiol-protected citral as a 1:1 cis/trans mixture (1.10 g, 4.54 mmol) in dry THF (20 mL) at -40 °C (acetonitrile/dry ice bath) was slowly added a solution of 1.05 equiv *n*-BuLi (1.6 M in hexanes, 2.84 mL) under N_2. The resulting reaction solution was stirred at -40 °C for 1 h, and then a solution of 3-methoxybenzyl chloride (650 mg, 4.16 mmol) in dry THF (10 mL) was added. The reaction mixture was maintained at -40 °C for 8 h and then slowly warmed to room temperature over 16 h under N_2. The reaction solution was quenched with brine (100 mL) and extracted with ether (100 mL × 2). The organic layers were collected, dried with $MgSO_4$ and concentrated. A flash column separation (0-2% EtOAc in hexanes) afforded the desired product as a cis/trans diastereoisomeric mixture (0.60 g) in 40% yield. Further separation of the isomers was not carried out because the subsequent cyclization step afforded diastereoisomerically pure *cis*-compounds. ^{1}H NMR ($CDCl_3$, 300 MHz) for the cis/trans diastereomeric mixture: δ 7.23-7.14 (m, 1H), 6.95-6.72 (m, 3H), 5.40 (s, 1H), 5.19-5.05 (m, 1H), 3.83-3.76 (m, 3H), 3.37-3.25 (m, 2H), 3.00-2.74 (m, 4H), 2.54-2.44 (m, 1H), 2.17-1.90 (m, 5H), 1.84-1.52 (m, 9H). ^{13}C NMR ($CDCl_3$, 75 MHz) observed: δ 159.1, 143.2, 142.5, 137.8, 137.5, 132.0, 128.8, 128.7, 127.9, 127.4, 124.5, 124.3, 123.7, 117.0, 116.9, 112.5, 112.4, 55.3, 54.4, 47.0, 46.8, 41.8, 32.8, 28.2, 28.0, 26.9, 26.4, 26.0, 25.6, 24.7, 18.0, 17.9, 17.3. ESI-MS calcd for $C_{21}H_{31}OS_2$ ($M - H^+$), 361.17; found, 361.07.

1-(3-Methoxyphenyl)-4,8-dimethylnona-3,7-dienyl-2-one (6)

To a solution of **5** (300 mg, 0.83 mmol) in MeOH/H_2O (9:1, 25 mL) was added 1.1 equiv HgO and $HgCl_2$. The resulting reaction solution was stirred at room temperature for 4 h. The reaction solution was diluted with CH_2Cl_2 and the precipitation was filtered through a 5 μm Acrodisc filter. The solution was washed with brine, dried with $MgSO_4$ and concentrated. The desired product was purified by a flash column separation (0-10 % EtOAc in hexanes) as a cis/trans diastereomeric mixture (160 mg) in 71% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) for the cis/trans diastereomeric mixture: δ 7.24-7.14 (m, 1H), 6.84-6.74 (m, 2H), 6.09 (s, 1H), 5.05-4.98 (m, 1H), 3.79 (s, 3H), 3.74-3.67 (m, 2H), 2.18-2.09 (m, 7H), 1.17-1.56 (m, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) observed: δ 198.1, 160.3, 160.0, 136.7, 132.7, 129.7, 129.5, 123.2, 123.1, 122.6, 122.1, 121.9, 119.7, 115.3, 115.2, 114.1, 113.0, 112.5, 64.1, 55.5, 55.3, 51.7, 46.7, 41.5, 34.2, 26.9, 26.3, 26.0, 25.9, 19.7, 17.9. ESI-MS calcd for $C_{18}H_{25}O_2$ ($M + H^+$), 273.19; found, 273.07.

4b,5,6,7,8,8a-*cis*-Hexahydro-2-methoxy-4b,8,8-trimethylphenanthren-9(10*H*)-one (1)

To a solution of **6** (130 mg, 0.48 mmol) in dry CH_3NO_2 (10 mL) at room temperature was added 10 equiv $BF_3{\cdot}Et_2O$, and the resulting reaction solution was stirred under N_2 for 2 h. The reaction solution was diluted with saturated $NaHCO_3$ (150 mL) and extracted with CH_2Cl_2 (150 mL × 3). The organic layers were collected, dried with $MgSO_4$ and concentrated. The desired compound was purified by a flash column separation (0-5% EtOAc in hexanes) as a diastereoisomeric pure product (50 mg) as an oil in 38% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.23 (d, J = 8.7 Hz, 1H), 6.80 (dd, J_1 = 8.7 Hz, J_2 = 2.7 Hz, 1H), 6.61 (d, J = 2.7 Hz, 1H), 3.80 (s, 3H), 3.69 (d, J = 22.8 Hz, 1H), 3.48 (d, J = 22.8 Hz, 1H), 2.48 (d, J = 14.1 Hz, 1H), 2.10 (s, 1H), 1.54-1.46 (m, 2H), 1.34-1.23 (m, 3H), 1.05 (s, 3H), 0.95 (s, 3H), 0.34 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 212.5, 158.2, 135.6, 134.0, 125.2, 113.9, 112.7, 66.7, 55.4, 44.4, 42.4, 38.3, 36.4, 34.4, 33.8, 32.4, 22.7, 19.0. ESI-MS calcd for $C_{18}H_{25}O_2$ ($M + H^+$), 273.19; found, 273.12.

4b,5,6,7,8,8a-*cis*-Hexahydro-2-hydroxy-4b,8,8-trimethylphenanthren-9(10*H*)-one (2)

To a solution of **1** (15 mg, 0.05 mmol) in CH_2Cl_2 (2.0 mL) under N_2 was added a solution of BBr_3 (1.0 M in heptane, 1.0 mL). The resulting solution was stirred at room temperature for 2 h. The reaction solution was quenched with brine (100 mL) and extracted with EtOAc (100

mL × 2). The organic layers were collected, dried with $MgSO_4$ and concentrated. A flash column separation (5-35 % EtOAc in hexanes) afforded the desired product (8 mg) as an oil in 57% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.18 (d, J = 8.7 Hz, 1H), 6.73 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H), 6.57 (d, J = 2.4 Hz, 1H), 4.78 (bs, 1H), 3.67 (d, J = 22.8 Hz, 1H), 3.45 (d, J = 22.8 Hz, 1H), 2.48 (d, J = 12.3 Hz, 1H), 2.10 (s, 1H), 1.56-1.47 (m, 2H), 1.35-1.23 (m, 3H), 1.05 (s, 3H), 0.95 (s, 3H), 0.35 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 212.6, 154.1, 135.9, 134.0, 125.4, 115.3, 114.2, 66.7, 44.2, 42.4 38.3, 36.4, 34.4, 33.8, 32.4, 22.7, 19.0. ESI-MS calcd for $C_{17}H_{21}O_2$ (M - H^+), 257.15; found, 257.33.

4b,5,6,7,8,8a-*cis*-Hexahydro-2-acetyloxy-4b,8,8-trimethylphenanthren-9(10*H*)-one (3)

To a solution of **2** (5 mg, 0.02 mmol) in CH_3CN (2.0 mL) was added solutions of Ac_2O and Et_3N (1.0 M, 1.0 mL each). The resulting solution was stirred at room temperature for 4 h. The reaction solution was extracted with Et_2O (50 mL × 2). The organic layers were collected, dried with $MgSO_4$ and concentrated. A flash column separation (5-20 % EtOAc in hexanes) afforded the desired product (5 mg) as an oil in 84% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33 (d, J = 8.7 Hz, 1H), 6.99 (dd, J_1 = 8.7 Hz, J_2 = 2.4 Hz, 1H), 6.83 (d, J = 2.4 Hz, 1H), 3.71 (d, J = 22.8 Hz, 1H), 3.50 (d, J = 22.8 Hz, 1H), 2.50 (d, J = 14.1 Hz, 1H), 2.29 (s, 3H), 2.12 (s, 1H), 1.56-1.48 (m, 2H), 1.37-1.23 (m, 3H), 1.07 (s, 3H), 0.95 (s, 3H), 0.33 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 211.7, 169.7, 149.2, 139.3, 135.8, 125.2, 121.5, 120.3, 66.5, 44.2, 42.3, 38.7, 36.4, 34.5, 33.5, 32.3, 22.7, 21.4, 18.9. ESI-MS calcd for $C_{19}H_{23}O_3$ (M - H^+), 299.16; found, 299.33.

Cell Culture Study

Human HepG2 cells were maintained in the growth medium at 37 °C with 5% CO_2. The growth medium contained 90% Minimum Essential Eagle Medium (Invitrogen, CA) and 10% heat inactivated fetal bovine serum, and was enriched with 2 mM *L*-glutamine, 0.75 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 50 U/mL penicillin, and 50 μg/mL streptomycin. Stock solutions of TCDD, AFB1, and *cis*-terpenones **1-3** were prepared in DMSO and stored at -20 °C. The MTT solution was prepared at 5 mg/mL in PBS solution (pH 7.4), filtered and stored at 4 °C. The MTT lysis buffer was prepared by dissolving 25 g SDS in 100 mL of 50% DMF in water, and the pH was adjusted to 4.7 with a solution of 2.5% HCl in 80% acetic acid.

For the cell viability MTT assay, HepG2 cells were seeded at 30,000 cells/well on a 96 well plate for 4 h before the treatment with AFB1 and cis-terpenones. The treatments included medium only, AFB1 only, AFB1 plus *cis*-terpenones **1-3**, and *cis*-terpenones only. The final concentration for AFB1 was 2 μM (25), and those for *cis*-terpenones **1-3** were 10, 20, and 40 μM. The final solution of each well contained 9% serum with a volume of 200 μL and DMSO less than 0.18%. After incubation for 72 h, the MTT assay was carried out as reported (26). Briefly, the MTT solution (10 μL) was added to each well followed by incubation for 10 h. The medium was then removed, and lysis buffer (100 μL each) was added. After 10 h, the absorbance of each well at 570 nm (background correction at 690 nm) was obtained with a plate reader (μQuant, BioTek Instruments, VT). The statistical analyses (one-way ANOVAwith Dunnett's test) were performed by GraphPad Prism (version 4.00, GraphPad Software, San Diego, CA). Each treatment was performed in quadruplicate, and all of the experiments were repeated at least three times independently.

For the apoptosis assay, HepG2 cells were plated at 50,000 cells/well and treated with AFB1 and/or *cis*-terpenone **2** as described in the viability assay. Apoptosis induced by AFB1 treatments was determined with Annexin V fluorescein isothiocyanate (FITC) in conjunction with propidium iodide (PI), according to the procedures provided by the manufacturer (BD Biosciences, San Jose, CA). Labeled cells were quantitatively resolved by a Coulter EPICS

XL-MCL flow cytometer (Beckman Coulter, Inc., Fullerton, CA). Apoptotic cells (Annexin V-FITC positive and PI negative) were distinguished from cells that were either at the end of apoptosis, undergoing necrosis, or already dead (Annexin V-FITC positive and PI positive) (27,28).

For the EROD assay, HepG2 cells were seeded at 50,000 cells/well on a 96 well plate. After plating for 4 h, various treatments included DMSO only, TCDD only, TCDD plus *cis*-terpenones **1-3**, and *cis*-terpenones only. The final concentration of TCDD was 1 nM, and the concentrations of *cis*-terpenones **1-3** were 0.1, 1, 10, and 20 μM. The final volume of each well was 200 μL containing 0.1% DMSO. After 24 h, the EROD assay was performed as reported (23,24). Briefly, fresh medium (175 μL per well) containing 2% serum, 8 μM 7-ethoxyresorufin and 10 μM dicumarol was added to each well, and then incubated for 1 h. The resulting medium (150 μL each) was transferred to a black plate, and ethanol (150 μL each) was added. The fluorescence intensity between 580 and 600 nm of each well was obtained by a Cary Eclipse fluorescence spectrometer (Varian Instruments, CA) with an exciting wavelength at 550 nm. The fluorescent intensity at 590 nm (λ_{max}) was used as the index for the EROD activity, and the statistical analyses (one-way ANOVAwith Dunnett's test) were performed by GraphPad Prism. All the experiments were repeated at least three times independently. Also, the cell viability MTT assay was carried out similarly as described above. No cytotoxicity in HepG2 cells was observed in the treatment of TCDD and/or *cis*-terpenones after 24 h.

Results and Discussion

Synthesis of *cis*-Terpenones

cis-Terpenones **1-3** were synthesized with a modified conjugated polyene cyclization as reported previously (17). For the synthesis, methoxylbenzyl chloride **4** was first coupled with 1,3-dithiol protected citral (1:1 cis/trans isomers) at -40°C in 40% yield (Scheme 2). The thiol groups of the resulting **5** were removed with $HgCl_2$ and HgO in 71% yield. The obtained polyene **6** was cyclized in the presence of $BF_3{\cdot}OEt_2$, affording *cis*-terpenone **1** diastereomeric selectively. The cis-conformation of terpenone **1** was confirmed by 2-D NMR ROESY analysis (see Supporting Information), and was consistent with that of reported cis-conformers (17). Removal of the methyl ether group of **1** was achieved with BBr_3 to the desired *cis*-terpenone **2** in 57% yield, which was acetylated to *cis*-terpenone **3** in 84% yield.

Chemo-protection with *cis*-Terpenones against AFB1 Induced Cytotoxicity

The chemo-protection against AFB1 with *cis*-terpenones **1-3** was confirmed by a cell viability assay in HepG2 cells, and no cytotoxicity was observed by *cis*-terpenones alone. The cell viability was determined after the co-treatment with AFB1 (2 μM) and *cis*-terpenones **1-3** at various concentrations (10-40 μM) for 72 h. As shown in Figure 1, 40 μM *cis*-terpenones exhibited no cytotoxicity in HepG2 cells, while AFB1 at 2 μM resulted in more than 50% cell death. The co-treatment of AFB1 and *cis*-terpenones showed a gradual increase of cell viability with increasing concentrations of *cis*-terpenones (up to 80% at 40 μM), and all of three *cis*-terpenones showed similar chemo-protection at the concentrations investigated. These results indicated that novel *cis*-terpenones **1-3** effectively reduce the AFB1-induced cytotoxicity in HepG2 cells, and suggested that *cis*-terpenones could be a promising candidate as a chemopreventive agent against AFB1.

Chemo-protection with *cis*-Terpenones against AFB1 Induced Apoptosis

The chemo-protection with *cis*-terpenone **2** at various concentrations (10-40 μM) against AFB1 was further validated with an Annexin V-apoptosis assay by flow cytometry. Annexin V has a high affinity for the membrane phospholipid phosphatidylserine, an early marker when cells start to undergo apoptosis, and thus is a sensitive probe for identifying apoptotic cells (27,

28). When cell death eventually occurs, apoptotic cells will lose the membrane integrity and expose the nucleus to the vital dye PI, besides being stained positive for Annexin V-FITC. As shown in Figure 2, each graph (Figure 2a-d) is divided into four boxes, which are PI positive (at top left), both PI and annexin V positive (at top right), both PI and annexin V negative (at lower left), and annexin V positive (at lower right). The numbers within each box indicate the percent distribution of the cell population. The treatment with AFB1 alone (Figure 2b) resulted in only 41.7% viable HepG2 cells, while 11.8% were at early stage apoptosis and 41.8% at late stage apoptosis or dead. In contrast, co-treatment with 40 μM *cis*-terpenone **2** (Figure 2d) protected 84% of cells from the induced apoptosis by AFB1. Collectively, with the results from the cell viability assay, these data fully confirmed the chemo-protective role of *cis*-terpenones against AFB1 induced toxicity in HepG2 cells.

Chemoprevention of Induced P450 1A/B Activity in HepG2 Cells with *cis*-Terpenones

The chemopreventive agent resveratrol is a potent inhibitor of P450 1A/B activity, which is involved in the activation of pro-carcinogens including benzopyrenes, estrogens and even AFB1 (20,21). In liver cells, the levels of P450 1A/B are low yet highly inducible by poly aromatic compounds, such as TCDD and benzopyrenes (20-24). Because the hypothesized hydroxylated *cis*-terpenone metabolite resembles the structure of resveratrol partially, it is important to evaluate potential effects of the *cis*-terpenones on P450 1A/B activity, which would demonstrate chemopreventive actions in addition to anti-AFB1 activity.

Indeed, we found that *cis*-terpenones effectively inhibited TCDD-induced P450 1A/B activity in HepG2 cells (23,24). The activity of P450 1A/B was determined by the EROD assay with either TCDD alone (1 nM) or the co-treatment of TCDD and *cis*-terpenones after 24 h (Figure 3). As shown in Figure 3, the basal level of P450 1A/B activity in the absence of the inducer TCDD is negligible, while TCDD (1 nM) significantly induced the enzymatic activity. In the co-treatment of *cis*-terpenones and TCDD, the EROD activity showed a decreasing trend in a concentration-dependent manner. At 10 μM concentration, *cis*-terpenones **1-3** were able to reduce the induced enzyme activity by approximately 50%. In addition, no cytotoxicity in HepG2 cells under these conditions was observed with the MTT assay. These results implied that *cis*-terpenones **1-3** could be used as a generic chemopreventive agent that suppresses activity of P450 1A/B, besides the protective effect against AFB1. The inhibitory effect with *cis*-terpenones may be due to either the inhibition of enzyme activity or the suppression of the expression level of enzyme. Therefore, the detailed study on the inhibitory mechanism with *cis*-terpenones **1-3** is currently under investigation.

In conclusion, we have demonstrated that *cis*-terpenones **1-3** have effective chemoprevention against both AFB1 and carcinogenic stimuli TCDD in liver cells, and thus are promising candidates for novel mechanism-based preventive agents. Future research efforts are focusing on the detailed mechanism by which *cis*-terpenones effectively prevent the cytotoxicity induced by AFB1 and potentially impact other metabolic P450 enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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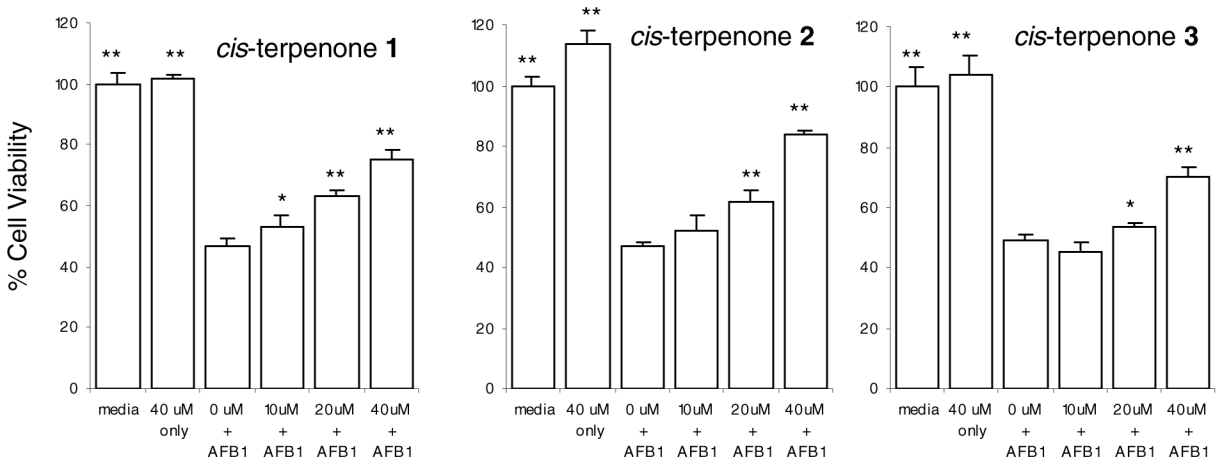


Figure 1.

Chemo-protection with *cis*-terpenones against AFB1 induced cytotoxicity. HepG2 cells were co-treated with AFB1 (2 μM) and *cis*-terpenones **1-3** at various concentrations (10-40 μM) and incubated for 72 h. Cell viability was measured with the MTT assay. The percentage of viable cells was based on cells treated with medium only. Each bar represents the mean ± SD of 4 replicates. The data are representative of three independent experiments. * P<0.05, ** P<0.001 compared to treatment with AFB1 by one way ANOVA and Dunnett's test.

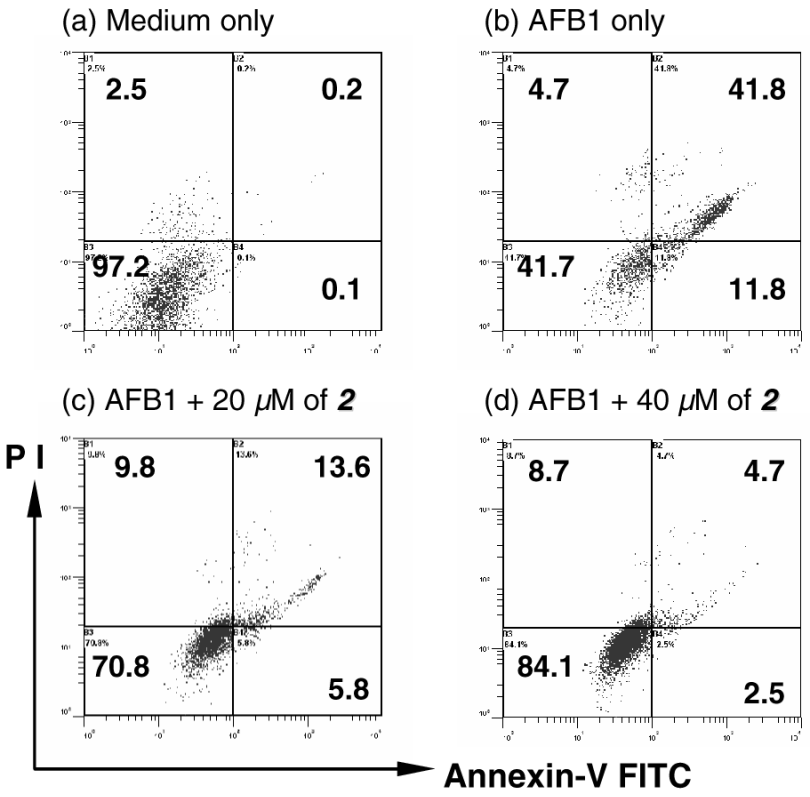


Figure 2.

Chemo-protection with *cis*-terpenone **2** against AFB1 induced apoptosis. HepG2 cells were co-treated with AFB1 (2 μM) and *cis*-terpenone **2** at various concentrations (10-40 μM) and incubated for 72 h. The cells were stained with PI (shown as the Y-axis of each graph) and Annexin-V FITC (shown as the X-axis of each graph), and analyzed with a flow cytometer. The numbers in each divided box represent the percent distribution of HepG2 cells within the gated areas. PI positive (at top left), both PI and annexin V positive - late apoptosis or dead (at top right), both PI and annexin V negative - viable (at lower left), and annexin V positive - early apoptosis (at lower right). The data are representative of three independent experiments.

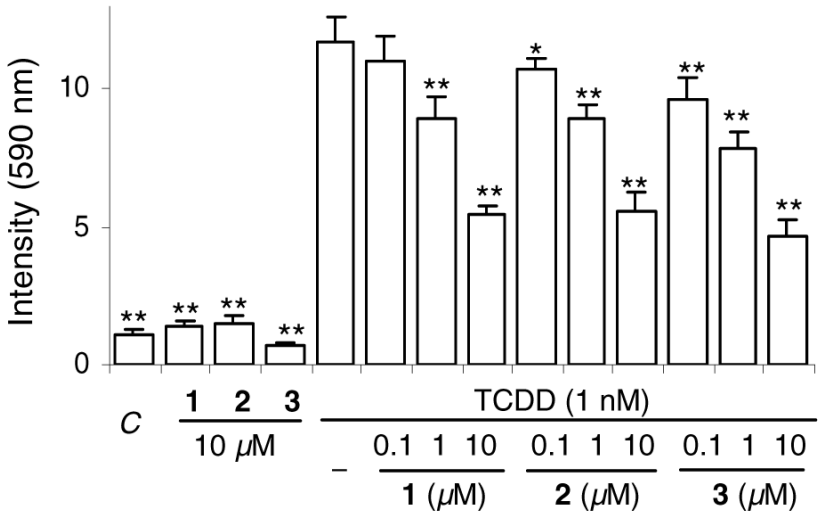
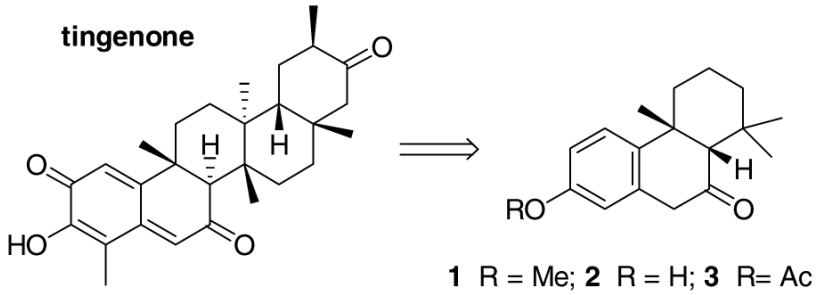
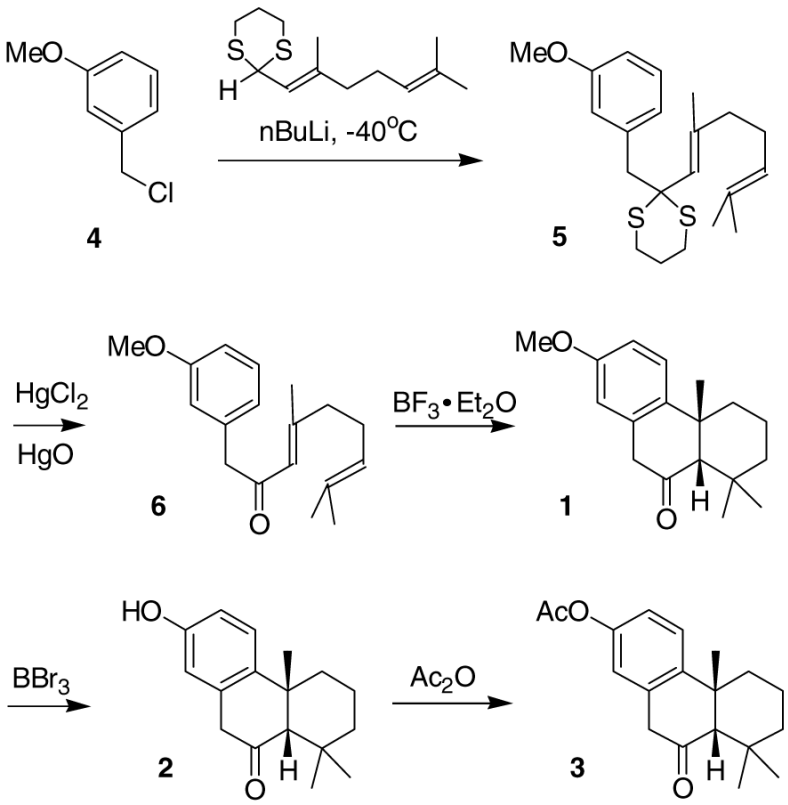


Figure 3.

Inhibition of TCDD-induced P450 1A/B activity with *cis*-terpenones. HepG2 cells were co-treated with TCDD (1 nM) and *cis*-terpenones **1-3** and incubated for 24 h. The activity of P450 1A/B was determined with the EROD assay. Bars represent the mean ± SD of EROD activity at 590 nm in 4 replicates. The data are representative of three independent experiments. *C*: DMSO only. * P<0.05, ** P<0.001 compared to TCDD only with one way ANOVA and Dunnett's test.



Scheme 1.
cis-Terpenones **1**-**3** as Precursors of Analogues of the Natural Bioactive Tingenone.



Scheme 2.
Synthesis of *cis*-Terpenones **1-3**.