Structure−Antimutagenic Activity Relationship Study of Plicatin B[1,2]

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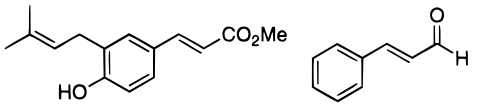
A systematic structure−activity relationship study of plicatin B (**1**), an antimutagenic constituent of *Psoralea juncea*, was undertaken with a view toward elucidating its chemical mode of action and possibly optimizing its antimutagenic activity during the process. Compound **1** and its related analogues were examined for their antimutagenic activity against mutations induced by ethyl methanesulfonate, a direct acting mutagen and alkylating agent, in *Salmonella typhimurium* strain TA100, utilizing the modified Ames test protocol. The dihydro analogue **3** resulting from saturation of the conjugated alkene double bond of **1** was found to exhibit reduced cytotoxicity and enhanced efficacy relative to the parent compound. This result serves preliminarily to exclude a Michael acceptor role of the α,β-unsaturated carbonyl moiety in connection with its antimutagenic activity.

Cancer is one of the leading causes of death today, at present estimated to claim annually about 550 000 lives in the United States and approximately 6 million lives worldwide.[3,4] Epidemiological studies suggest that about 60% of cancer mortality in the United States is potentially preventable, since it can be attributed to environmental causes alone.[5] Although early detection and subsequent treatment of cancer constitutes an effective stratagem for combating the disease once it has established itself, primary prevention of cancer is optimal and desirable for quite obvious reasons.[6] The finding that the dietary consumption of certain foodstuffs such as fruits, vegetables, and tea beverages is associated with a reduced risk of carcinogenesis eventually led to the concept of cancer chemoprevention that encompasses the use of natural (e.g., phytochemicals) and synthetic compounds for preventing the development of cancer.[7−11] Some of these chemical agents exert their cancer chemopreventive action via their ability to operate as antimutagens, i.e., by preventing or reversing the genetic damage caused to the organism by environmental mutagens/carcinogens and even mutagens arising from normal physiological processes.[12,13] Depending on their particular mode of action, antimutagenic agents can be classified into two broad categories: desmutagens, which operate by inactivating mutagens before they can cause damage to cellular constituents such as DNA, and bioantimutagens, which modulate mutagenesis at the cellular level (i.e., within the cell), one mechanism being the induction of cellular repair mechanisms in response to mutagenic damage.[14] It is well established that antimutagenic agents may operate via a combination of these different mechanisms.[15]

Some novel antimutagens from higher plants have been identified previously in this laboratory with the help of a systematic bioassay-directed fractionation methodology and modified screening procedures.[16,17] One of these agents, plicatin B (**1**), a substituted cinnamyl ester isolated from the active extract of the stem and leaf parts of *Psoralea juncea* (Eastw.) Rydb. (Leguminosae), was observed to be active in the modified Ames test against mutations induced in *Salmonella typhimurium* strain TA100 by the direct-acting mutagen, ethyl methanesulfonate (EMS).[17] Several members of this genus are folklorically reputed to be associated with a variety of general medicinal properties such as diuretic, stomachic, stimulant, and tonic.[18,19] Cinnamaldehyde (**2**), a structurally related compound, has long been known to be antimutagenic.[20] The antimutagenic activity of cinnamaldehyde was confirmed by us previously, and in our hands, under conditions slightly different from this study, cinnamaldehyde was shown to be antimutagenic against EMS induced mutations in *S. typhimurium* TA100 strain.[21] Although much of the focus of the scientific literature on cinnamaldehyde-related antimutagenesis concerns the biological mechanisms by which the antimutagenic effects of this compound are produced,[21−24] some effort has been directed toward establishing a correlation between the chemical structure and antimutagenic activity of this agent. Chemical studies with cinnamaldehyde (**2**) and other structurally related antimutagens with regard to their activity against UV-induced mutations in *Escherichia coli* have indicated that the antimutagenic activity of these agents may be correlated with the ability of the conjugated α,β-unsaturated carbonyl moiety to add cellular nucleophiles such as thiol groups on proteins or possibly DNA in a Michael acceptor sense.[25−27] In light of this evidence, we wished to examine if the same was also true in the case of the antimutagenic activity of plicatin B (**1**), particularly relating to its activity in our assay system utilizing EMS, a DNA alkylating agent,[16] as the mutagenic insult. For this purpose, we undertook a systematic structure−activity relationship (SAR) analysis of plicatin B (**1**), attempting to elucidate its chemical mode of action and possibly optimize the antimutagenic activity of this natural product during the process.



1, Plicatin B **2**

Results and Discussion

As mentioned above, inspection of the chemical structure of cinnamaldehyde (**2**) and certain other structurally related compounds in relation to their antimutagenic activity has led to the reasonable speculation that the

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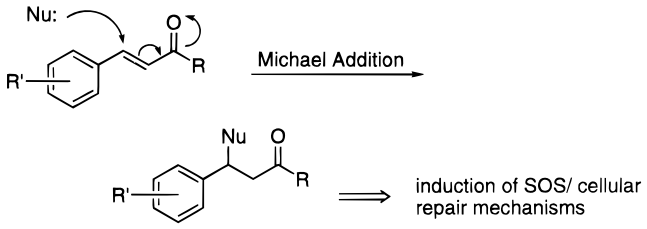


Figure 1. Putative chemical mechanism underlying the antimutagenic activity of plicatin B and structurally related antimutagens. Nu: denotes cellular nucleophiles, possibly proteins or DNA.

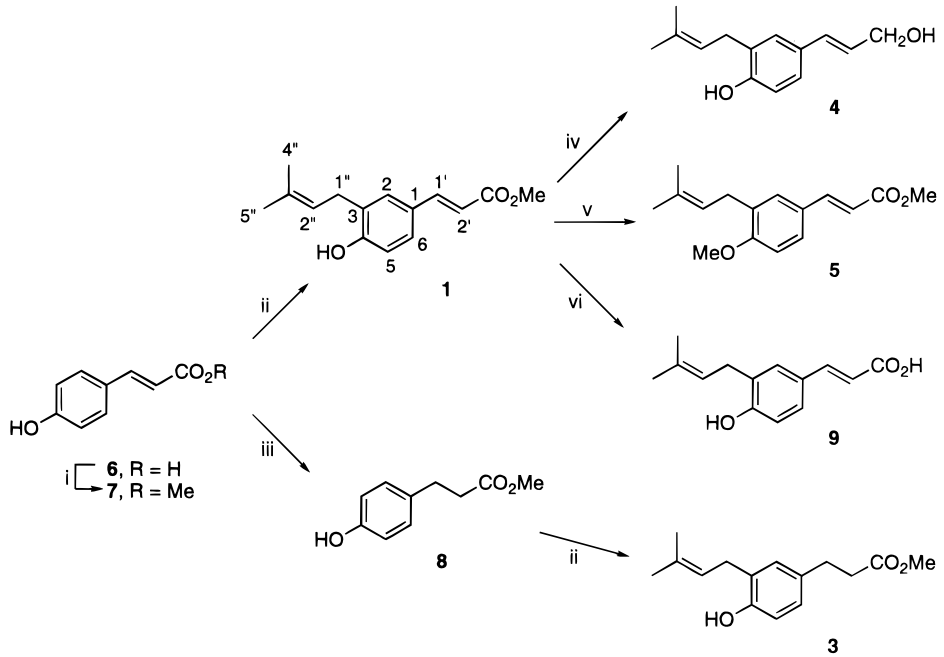
antimutagenic activity of these compounds may be associated with their ability to operate as Michael acceptors within the cell, where they could possibly effect the alkylation of cellular nucleophiles such as DNA and/or cellular proteins. The initial DNA damage caused by these agents may trigger the induction of a SOS response that can prime the cell to future exposure to other mutagens such as EMS (Figure 1). Thus, these compounds may be thought to be operating primarily via a bioantimutagenic mechanism rather than a desmutagenic mechanism. Due to the structural similarity between cinnamaldehyde (**2**) and plicatin B (**1**), one of the obvious issues we sought to investigate with regard to the latter was the effect of saturation of the conjugated alkene double bond on its antimutagenic activity. Such a modification would render the resulting dihydro analogue **3** incapable of functioning as a Michael acceptor, and its antimutagenic activity, or lack thereof, could shed some light on the relevance of the proposed chemical mode of action of cinnamaldehyde (**2**) and other structurally related antimutagens to plicatin B (**1**). A second, somewhat convergent approach to this problem would be to leave the conjugated alkene double bond in **1** intact but reduce the ester functionality. The resulting alcohol **4**, like compound **3**, would be far less capable of reacting in a Michael acceptor sense with cellular nucleophiles such as DNA and proteins than plicatin B (**1**) itself. Compound **7**, lacking the C-3 alkenyl substituent of plicatin B (**1**), was evaluated in the present study to determine the contribution of this substituent to the overall antimutagenic activity of **1**. Since an aromatic hydroxyl group para to an α,β-unsaturated carbonyl moiety has been observed to be essential for the antimutagenic activity of chalcones and certain other structurally related antimutagens, we sought to examine the effect of alkylation of the phenolic group in plicatin B (**1**) on its antimutagenic activity as well.[28,29] For this purpose, we synthesized and evaluated the methyl ether analogue (**5**) of plicatin B (**1**) for its antimutagenic activity.

The synthesis of plicatin B and its related analogues is outlined in Scheme 1. Plicatin B (**1**) itself was conveniently prepared by the C-prenylation of readily accessible *p*-hydroxycinnamic acid methyl ester (**7**) utilizing the method of Fatope and Okogun.[30] Selective reduction of the conjugated ester functionality in plicatin B (**1**) using diisobutylaluminum hydride (DIBAL-H) resulted in the allylic alcohol **4**.[31] Catalytic hydrogenation of **7** over palladium catalyst[32] followed by C-prenylation of the resulting dihydro compound **8** yielded the differentially partially saturated analogue **3**. The methyl ether analogue **5** was prepared from plicatin B (**1**) using standard alkylation procedures.

Initially, cytotoxicity assays were conducted on all the compounds in this study to establish concentrations that were nontoxic to the tester strain since dead cells obviously cannot mutate. The true antimutagenic activities of these compounds were then determined at 2-fold concentration intervals each, ranging up to half the maximum nontoxic dose, utilizing the modified Ames test protocol and the direct-acting mutagen, ethyl methanesulfonate (EMS).[33–35] The results of these assays, denoted by the percent inhibition of EMS-induced mutations in the tester strain *S. typhimurium* TA100, by plicatin B (**1**) and its related analogues are listed in Table 1.

Notably, examination of these results for plicatin B (**1**) and its dihydro analogue **3** at the maximum comparative nontoxic concentration (7.8 μM) indicates that the reduction of the conjugated alkene double bond in plicatin B is not detrimental to its activity, implying that the antimutagenic activity of plicatin B is not likely to be associated with its ability to react with cellular nucleophiles in a Michael

Scheme 1[a]



[a] Key: (i) MeOH, concd H_2SO_4; (ii) NaH, toluene; then prenyl bromide; (iii) Pd/C, H_2, 1 atm, EtOH; (iv) DIBAL-H, toluene; (v) K_2CO_3, MeI; (vi) NaOH, H_2O.

Table 1. Percent Inhibition of EMS−Induced Mutations in *Salmonella Typhimurium* Strain TA100 by Plicatin B (1) and Its Analogues[a]

compd	concn per plate[b] (μM)	% inhibition[c]
plicatin B (**1**)	3.9	25.4 ± 0.95
	7.8	30.2 ± 1.56
3	3.9	25.2 ± 2.81
	7.8	38.5 ± 2.00
	31.3	46.8 ± 0.25
4	3.9	20.2 ± 0.65
	7.8	27.1 ± 0.56
	31.3	36.8 ± 0.61
5	3.9	13.2 ± 0.30
	7.8	21.5 ± 1.06
6	3.9	11.2 ± 0.89
	7.8	13.6 ± 2.97
	125	49.8 ± 2.10
7	3.9	0.0
	7.8	0.9 ± 0.9
	62.5	7.6 ± 6.0
8	3.9−125	0.0
9	3.9	0.3 ± 0.5
	7.8	0.6 ± 0.6
	15.6	12.0 ± 0.74

[a] In an earlier set of experiments, cinnamaldehyde (**2**) at its maximum nontoxic concentration (25 μg/mL) produced approximately 30% inhibition of EMS induced mutations in *S. typhimurium* TA100 strain (see ref 21). [b] Results of all the examined test concentrations for each compound are not listed; rather, only the comparative and the half-maximum nontoxic test concentrations for each compound are given. [c] % inhibition = 100 − [(no. of induced revertants in the presence of EMS and test compound − no. of spontaneous revertants/no. of induced revertants in the presence of EMS − no. of spontaneous revertants) × 100].

acceptor sense. A similar conclusion has been arrived at previously regarding the antimutagenic activity of certain structurally related resin constituents (cinnamic acid, cinnamyl cinnamate, and cinnamyl ricinoleate) derived from the medicinal plant, *Liquidambar orientalis* and some synthetic analogues.[36] In fact, analogue **3** is observed to be slightly more potent than plicatin B itself; furthermore, this modification reduces the cytotoxicity of **3** toward the tester strain so that now analogue **3** at its maximum nontoxic dose (31.3 μM) exhibits significantly greater efficacy in inhibiting EMS-induced mutations in the tester strain than does plicatin B itself. Likewise, reduction of the ester functionality in plicatin B (**1**) to the alcohol moiety in **4** is also noted to have little effect on its antimutagenic activity at the maximum comparative nontoxic concentration (7.8 μM). O-Methylation of plicatin B results in a small reduction in the antimutagenic activity for the resulting ether analogue **5**. Since it is well-known that antimutagenic agents can operate via a combination of desmutagenic and bioantimutagenic mechanisms,[15] this decrease in antimutagenic activity for **5** in comparison to plicatin B could be attributed to its relative inabilty to inactivate EMS directly, possibly via the reactive phenolic hydroxyl group. An issue of some relevance to this matter is the observation made with certain antimutagens that phenolic hydroxyl groups are directly involved in the scavenging of mutagens, for example, the diol epoxide derived from benzo[*a*]pyrene activation.[37]

Significantly, compound **7**, which lacks the C-3 alkenyl moiety of plicatin B (**1**), is devoid of any antimutagenic activity in this assay. One could attribute this lack of activity for compound **7** relative to plicatin B to the absence of the C-3 alkenyl substituent, but further inspection of the data in Table 1 suggests a somewhat different explanation. Thus, a perusal of the data in Table 1 for compound **7** and its dihydro analogue **8** reveals that reduction of the alkene double bond in **7** does not produce an improvement in the antimutagenic activity of **8** in the present assay, an outcome that is in direct contrast to the trend observed with plicatin B and its dihydro analogue **3**. Similarly, comparison of the antimutagenic activities of compound **7** and its acid derivative **6** with plicatin B (**1**) and its acid derivative **9** implicates such a noncongruent trend as well. These results suggest that these two distinct sets of compounds in this study, i.e., the compounds bearing a C-3 alkenyl substituent and those lacking it, might be operating via different mechanisms, and thus, a direct comparison of their antimutagenic activities may not be fully justified. Thus, the actual contribution of the C-3 alkenyl substituent to the antimutagenic activity of plicatin B cannot be meaningfully ascertained from this study.

In summary, the modified Ames test results from this study, where comparable, indicate that structural modification of plicatin B (**1**) generally leads to a reduction in cytotoxicity but produces rather insignificant changes in its antimutagenic activity with one notable exception; reduction of the conjugated alkene double bond leads to a compound that exhibits reduced cytotoxicity and enhanced antimutagenic efficacy relative to plicatin B in the assay system employed. Although plicatin B might be operating via a combination of desmutagenic and bioantimutagenic mechanisms of action in the present assay, this significant finding serves to exclude the possibility of a Michael acceptor role of the conjugated α,β-unsaturated carbonyl moiety of plicatin B in connection with its antimutagenic activity. The actual mechanism of antimutagenesis must take place using different chemistry and evidence to this point will appear subsequently. This study serves to illustrate the point that an extension of the postulated chemical mechanism of action of one antimutagenic agent, such as cinnamaldehyde (**2**), to another, such as plicatin B (**1**), based solely on structural analogy is not always fully justifiable.

Experimental Section

General Experimental Procedures. Flash column chromatography was carried out using silica gel 32−63 (40 micron) purchased from Selecto, Inc., Kennesaw, GA. Proton nuclear magnetic resonance spectra (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded on either a GE QE-300 or a Bruker AM 500 spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (Me_4Si; 0.0 ppm) for ^{1}H NMR and deuterated chloroform ($CDCl_3$, 77.0 ppm) or deuterated dimethyl sulfoxide (DMSO-d_6, 39.5 ppm) for ^{13}C NMR. Infrared spectra (IR) were recorded on a Perkin-Elmer 1420 infrared spectrophotometer. Electron-impact mass spectra (EIMS), chemical-ionization mass spectra (CIMS), and high-resolution mass spectra (HRMS) were obtained on a ZAB HS or a Nermag R 10-10 mass spectrometer. The intensity of each peak in the mass spectrum relative to the base peak is reported in parentheses. Microanalyses were performed on a Perkin-Elmer 2400 CHN analyzer at the University of Kansas. Melting points were determined in open capillaries on a Thomas-Hoover capillary melting point apparatus and are uncorrected.

p-Hydroxycinnamic acid (**6**) was purchased from Aldrich Chemical Co.

Oxoid Nutrient Broth No. 2 was commercially obtained from Unipath, Ogdensburg, NY. Ethyl methanesulfonate (EMS) and the other biochemicals used in this study were purchased from Sigma.

4-Hydroxycinnamic Acid Methyl Ester (7). A solution of **6** (10.0 g, 60.9 mmol) in MeOH (100 mL) containing concentrated H_2SO_4 (3 mL) was refluxed overnight. Solvent was evaporated under reduced pressure, and the residual solid was partitioned between $CHCl_3$ (250 mL) and H_2O (100 mL).

The organic phase was separated, and the aqueous phase was reextracted with $CHCl_3$ (100 mL). The organic extracts were pooled, washed successively with saturated aqueous $NaHCO_3$ (75 mL), water (50 mL), and brine, and finally dried over anhydrous Na_2SO_4. Removal of solvent under reduced pressure followed by recrystallization of the residue from $CHCl_3$−hexane yielded 6.84 g (63%) of **7** as a colorless solid: mp 138−139 °C; IR (KBr) ν_{max} 3360, 1680, 1590, 1420, 1190, 1165 cm^{-1}; ^1H NMR ($CDCl_3$−DMSO-d_6, 300 MHz) δ 8.76−8.66 (1H, b, OH), 7.63 (1H, d, J = 16.0 Hz, H-1′), 7.39 (2H, d, J = 8.6 Hz, H-2, H-6), 6.86 (2H, d, J = 8.6 Hz, H-3, H-5), 6.27 (1H, d, J = 16.0 Hz, H-2′), 3.78 (3H, s, CO_2CH_3); ^{13}C NMR ($CDCl_3$−DMSO-d_6, 300 MHz) δ 168.4, 159.8, 145.4, 130.3, 126.5, 116.4, 114.8, 51.9; EIMS m/z 178 (M^+, 53), 147 (100), 119 (30), 91 (34), 65 (28); *anal*. C 67.29%, H 5.80%, calcd for $C_{10}H_{10}O_3$, C 67.41%, H 5.66%.

Plicatin B (1). To a suspension of **7** (3.31 g, 18.6 mmol) in dry toluene (50 mL) was added NaH (60%, 0.820 g, 20.5 mmol) in one portion at ambient temperature, and subsequently the reaction mixture was heated at 60−65 °C for 3.5 h. The reaction mixture was cooled to 35 °C, 4-bromo-2-methyl-2-butene (prenyl bromide; 2.95 mL, 25.6 mmol) was added dropwise (15−20 min) by syringe to the reaction mixture at 35 °C, and stirring was continued at this temperature for 72 h. At this time, the reaction mixture was quenched by pouring it into 300 mL of ice−water. The aqueous layer was neutralized, with ice cooling, with cold 2 N acetic acid and then extracted with Et_2O (3 6 200 mL). The organic extract was washed with H_2O (100 mL) and brine, and then dried (anhydrous Na_2SO_4). Evaporation of the solvent under reduced pressure and subsequent flash chromatography (300 g silica gel) on the residue utilizing hexanes−EtOAc (5:1) as the eluent yielded 1.84 g (40%) of **1**. An analytical sample of **1** was recrystallized from Et_2O−petroleum ether as a pale yellow solid: mp 78−82 °C (lit.[38] mp 86−87 °C, lit.[39] mp 70−72 °C); IR (KBr) ν_{max} 3220, 1660, 1580, 1260, 1230, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.63 (1H, d, J = 16.0 Hz, H-1′), 7.31−7.26 (2H, m, H-2, H-6), 6.82 (1H, d, J = 8.9 Hz, H-5), 6.29 (1H, d, J = 16.0 Hz, H-2′), 5.92 (1H, s, OH), 5.35−5.29 (1H, m, H-2″), 3.80 (3H, s, CO_2CH_3), 3.36 (2H, d, J = 7.2 Hz, H-1″), 1.81−1.77 (6H, m, H-4″, H-5″); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 168.4, 156.9, 145.4, 135.5, 130.5, 128.1, 128.0, 127.6, 121.6, 116.5, 115.3, 51.9, 29.8, 26.1, 18.2; EIMS m/z 246 (M^+, 87), 191 (100), 171 (26), 131 (31), 115 (25); *anal*. C 72.83%, H 7.75%, calcd for $C_{15}H_{18}O_3$, C 73.15%, H 7.36%.

4-Hydroxyhydrocinnamic Acid Methyl Ester (8). A mixture of **7** (3.25 g, 18.3 mmol) and 5% Pd/C catalyst (0.325 g) in EtOH (65 mL) was hydrogenated at room temperature and 1 atm for 24 h. The reaction mixture was filtered through Celite, and the filter bed was washed with MeOH. The filtrate was evaporated under reduced pressure, and the residual oil was distilled bulb to bulb (145 °C, 3.5 mmHg) to afford 3.27 g (99%) of **8** as a colorless oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.05 (2H, d, J = 8.5 Hz, H-2, H-6), 6.75 (2H, d, J = 8.5 Hz, H-3, H-5), 5.27 (1H, s, OH), 3.67 (3H, s, CO_2CH_3), 2.88 (2H, t, J = 7.7 Hz, H-1′), 2.60 (2H, t, J = 7.7 Hz, H-2′); CIMS (NH_3) m/z 180 (M^+, 70), 149 (14), 120 (65), 107 (100); *anal*. C 66.48%, H 7.00%, calcd for $C_{10}H_{12}O_3$, C 66.65%; H 6.71%.

4-Hydroxy-3-(3-methyl-2-butenyl)hydrocinnamic Acid Methyl Ester (3) (Dihydroplicatin B). To a solution of **8** (1.40 g, 7.78 mmol) in dry toluene (20 mL) was added NaH (60%, 0.343 g, 8.58 mmol) in one portion at room temperature. The reaction mixture was heated at 65 °C for 3.5 h and cooled to 35 °C, and then prenyl bromide (1.30 mL, 11.3 mmol) was added dropwise by syringe over a period of 10 min. The reaction mixture was stirred and heated at this temperature for 3 days and then quenched by pouring it into 150 mL of ice−water. After neutralization with cold 2 N acetic acid, the aqueous phase was extracted with Et_2O (3 × 100 mL). The organic extract was washed with H_2O (100 mL) and brine and then dried (anhydrous Na_2SO_4). Solvent was evaporated under reduced pressure and the residue was subjected to flash chromatography (200 g silica gel) with hexanes−EtOAc (10:1) as eluent to ultimately afford 0.638 g (33%) of **3** as an oil: IR (neat) ν_{max} 3420, 2940, 2900, 1715, 1500, 1430, 1340, 1250, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96−6.90 (2H, m, H-2, H-6), 6.75−6.70 (1H, m, H-5), 5.35−5.25 (1H, m, H-2″), 5.14−5.10 (1H, m, OH), 3.67 (3H, s, CO_2CH_3), 3.32 (2H, d, J = 7.2 Hz, H-1″), 2.86 (2H, t, J = 7.8 Hz, H-1′), 2.59 (2H, t, J = 7.8 Hz, H-2′), 1.77 (6H, s, H-4″, H-5″); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 173.5, 152.7, 134.6, 132.6, 129.8, 127.1, 126.8, 121.8, 115.7, 51.6, 36.1, 30.2, 29.8, 25.8, 17.8; EIMS m/z 248 (M^+, 87), 193 (100), 175 (80), 159 (65), 133 (64); *anal*. C 72.36%, H 8.22%, calcd for $C_{15}H_{20}O_3$, C 72.55%, H 8.12%.

4-Hydroxy-3-(3-methyl-2-butenyl)cinnamyl Alcohol (4). A solution of **1** (0.200 g, 0.813 mmol) in dry toluene (2 mL) was cooled to 0 °C, and then a 1.5 M solution of DIBAL-H in toluene (1.70 mL, 2.55 mmol) was added to it dropwise by syringe over a period of 0.5 h. The reaction mixture was stirred at 0 °C for an additional 4.5 h and then quenched at 0 °C by the dropwise addition of MeOH (over a period of 20−30 min). The reaction mixture was then stirred at 5 °C for 15 min and filtered through Celite, and the filter bed was washed thoroughly with hot MeOH. The filtrate was concentrated under reduced pressure to yield a yellow solid, which upon flash chromatography [20 g flash silica gel, hexanes−EtOAc (4:1)] afforded 0.130 g (73%) of **4**: mp 87−90 °C; IR (KBr) ν_{max} 3380, 3280−3060, 2920, 1600, 1490, 1250, 1220, 1100 cm^{-1}; ^1H NMR ($CDCl_3$−DMSO-d_6, 300 MHz) δ 8.17 (1H, s, ArOH), 7.12 (1H, d, J = 2.0 Hz, H-2), 7.06 (1H, dd, J = 8.2, 2.2 Hz, H-6), 6.77 (1H, d, J = 8.2 Hz, H-5), 6.50 (1H, d, J = 15.9 Hz, H-1′), 6.18 (1H, dt, J = 15.8, 5.9 Hz, H-2′), 5.38−5.29 (1H, m, H-2″), 4.25 (2H, m, H-3′), 3.31 (2H, d, J = 7.3 Hz, H-1″), 2.91 (1H, t, J = 5.7 Hz, CH_2OH), 1.75 (3H, s, H-4″ or H-5″), 1.72 (3H, s, H-4″ or H-5″); ^{13}C NMR ($CDCl_3$−DMSO-d_6, 500 MHz) δ 154.4, 132.2, 130.5, 128.1, 127.7, 127.3, 125.6, 124.7, 122.2, 115.0, 63.1, 28.1, 25.5, 17.5; CIMS (NH_3) m/z 218 (M^+, 49), 175 (89), 163 (100), 145 (30), 133 (37); *anal*. C 77.00%, H 8.00%, calcd for $C_{14}H_{18}O_2$, C 77.03%, H 8.31%.

4-Methoxy-3-(3-methyl-2-butenyl)cinnamic Acid Methyl Ester (5) (Plicatin B Methyl Ether). To a mixture of **1** (0.207 g, 0.841 mmol) and anhydrous K_2CO_3 (0.124 g, 0.899 mmol) in acetone (1.5 mL) was added iodomethane (0.11 mL, 1.77 mmol) in one portion at room temperature. The reaction mixture was stirred and heated at 60−65 °C for 4 h, and solvent was then evaporated under reduced pressure. Some water was added to the residue, and the mixture was neutralized with cold acetic acid (2 N). The aqueous phase was extracted with EtOAc (3 6 10 mL), the combined organic extract was dried (Na_2SO_4), and solvent was removed under reduced pressure. The crude residue was subjected to flash chromatography (7 g silica gel) with hexanes−EtOAc (5:1) as the eluent. Rechromatography of the product fractions on flash silica gel (20 g) using hexanes−EtOAc (7:1) as eluent afforded 0.139 g (63%) of **5** as a colorless oil: IR (neat) ν_{max} 2940−2820, 1710, 1630, 1590, 1490, 1240 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.64 (1H, d, J = 16.0 Hz, H-1′), 7.37−7.32 (2H, m, H-2, H-6), 6.83 (1H, d, J = 8.3 Hz, H-5), 6.30 (1H, d, J = 16.0 Hz, H-2′), 5.33−5.22 (1H, m, H-2″), 3.86 (3H, s, $ArOCH_3$ or CO_2CH_3), 3.79 (3H, s, $ArOCH3$ or CO_2CH_3), 3.30 (2H, d, J = 7.3 Hz, H-1″), 1.76 (3H, s, H-4″ or H-5″), 1.71 (3H, s, H-4″ or H-5″); ^{13}C NMR ($CDCl_3$, 500 MHz) δ 167.9, 159.2, 145.0, 133.2, 130.7, 128.9, 127.7, 126.7, 121.7, 114.8, 110.2, 55.5, 51.5, 28.2, 25.8, 17.7; EIMS m/z 260 (M^+, 100), 245 (15), 229 (16), 213 (48), 205 (26), 185 (46), 115 (35); *anal*. C 73.48%, H 8.00%, calcd for $C_{16}H_{20}O_3$, C 73.82%, H 7.74%.

Drupanin (9). A mixture of **1** (0.333 g, 1.35 mmol) and NaOH (0.125 g, 3.12 mmol) in H_2O (0.5 mL) was heated at reflux for 2.5 h. The black solution was allowed to cool to room temperature and then extracted with Et_2O (3 × 1 mL). The aqueous phase was cooled (ice−water bath), acidified with cold AcOH (2 N), and then reextracted with Et_2O (3 × 5 mL). The combined organic extract was washed with H_2O (5 mL) and then brine and finally dried over anhydrous Na_2SO_4. Solvent was evaporated under reduced pressure to yield 0.274 g (87%) of **9**. An analytical sample of **9** was recrystallized from Et_2O−hexane: mp 145−147 °C (lit.[38] mp 146−147 °C); IR (KBr) ν_{max} 3300, 3040−2840, 1640, 1580, 1420, 1300, 1250, 1220, 1150, 1100 cm^{-1}; ^1H NMR ($CDCl_3$−DMSO-d_6, 300 MHz) δ 9.20−8.90 (1H, b, CO_2H), 7.58 (1H, d, J = 15.9 Hz, H-1′), 7.26 (1H, d, J

= 2.0 Hz, H-2), 7.21 (1H, dd, J = 8.3, 2.2 Hz, H-6), 6.84 (1H, d, J = 8.2 Hz, H-5), 6.22 (1H, d, J = 15.9 Hz, H-2′), 5.35−5.29 (1H, m, H-2″), 3.30 (2H, d, J = 7.3 Hz, H-1″), 1.76 (3H, s, H-4″ or H-5″), 1.71 (3H, s, H-4″ or H-5″); ^{13}C NMR ($CDCl_3$−DMSO-d_6, 500 MHz) δ 168.9, 157.0, 144.8, 132.4, 129.1, 128.2, 126.8, 125.4, 121.6, 115.0, 114.4, 27.7, 25.4, 17.4; CIMS (NH_3) m/z 233 (M + 1, 74), 215 (37), 189 (100), 177 (33), 133 (36); *anal.* C 72.30%, H 7.30%, calcd for $C_{14}H_{16}O_3$, C 72.39%, H 6.94%.

Cytotoxicity Assays. Cytotoxicity assays were performed using *S. typhimurium* strain TA100 grown in Oxoid Nutrient Broth No. 2 using a 96 well ELISA plate containing varying concentrations of test compounds incubated for 15 h at 37 °C. After incubation, the plates were subjected to readings at absorbance 570 nm using a Cambridge Technology, Inc., plate solver ver. 4.00.

Modified Ames Tests. The modified Ames tests were performed based on the protocol described by Maron and Ames[33] using *S. typhimurium* strain TA100. Each experiment was performed in triplicate. The cells were grown for 15 h in Oxoid Nutrient Broth No. 2 at 37 °C with shaking to an approximate density of (1−2) × 10^8 cells/mL prior to each assay.

To induce His$^+$ revertants, 0.1 mL of the culture was added to test tubes containing 0.5 μL/mL of EMS in the presence or absence of varying concentrations of the test compounds and incubated at 37 °C for 30 min. Following this incubation, 2 mL of Ames top agar supplemented with 50 μg/mL of L-histidine and 0.74 μg/mL of D-biotin was added to the above compound, and the mixture was then plated on VogelBonner E Medium using the top agar layer method as described by Maron and Ames.[33] After incubation at 37 °C for 2 days, the plates were scored for His$^+$ revertant colonies using a Dynatech Autocount colony counter.

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