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Dehydrozingerone, Chalcone, and Isoeugenol Analogs as *In Vitro* Anticancer Agents[#]

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Abstract

Twenty-eight compounds related to dehydrozingerone (**1**), isoeugenol (**3**), and 2-hydroxychalcone (**4**) were synthesized and evaluated *in vitro* against human tumor cell replication. Except for isoeugenol analogs **27–35**, most compounds exhibited moderate or strong cytotoxic activity against KB, KB-VCR (a multi-drug resistant derivative), and A549 cell lines. In particular, chalcone **15** showed significant cytotoxic activity against the A549 cell line with an IC₅₀ value of 0.6 µg/mL. Furthermore, dehydrozingerone analog **11** and chalcones **16** and **17** showed significant and similar cytotoxic activity against both KB (IC₅₀ values of 2.0, 1.0, and 2.0 µg/mL, respectively) and KB-VCR (IC₅₀ values of 1.9, 1.0, and 2.0 µg/mL, respectively) cells, suggesting that they are not substrates for the p-glycoprotein drug efflux pump.

Dehydrozingerone (**1**), isolated from ginger (*Zingiber officinale*),^{1,2} is a well known phenolic natural product with anti-inflammatory, antioxidant, and antitumor promoting activities.³ It is the structural half analog, as well as biosynthetic intermediate,⁴ of curcumin (**2**), which possesses various remarkable bioactivities such as cytotoxic effects on cancer cell lines^{5–8} and induction of apoptotic cell death in human promyelocytic leukemia HL-60 and human oral squamous carcinoma HSC-4 cells.⁹ Dehydrozingerone (**1**), isoeugenol (**3**) and 2-hydroxychalcone (**4**) (Figure 1) have similar structures (acetyl, methyl and benzoyl, respectively, attached to a styrene skeleton). Both **3** and **4** are also prominent bioactive compounds.^{10,11} Thus, we expected that **1**-analogs should show a wide range of pharmacological activities. In spite of the interesting and simple structure of **1**, we found only a few literature reports on analog syntheses and structure-activity relationships (SAR),^{12,13} although we recently reported the cytotoxic effects of curcumin analogs.^{7,14,15}

It is known that the presence of a prenyl or geranyl group on flavonoids, including chalcones, can lead to a remarkable increase in bioactivity.^{16,17} As dehydrozingerone (**1**) is structurally related to chalcones, the introduction of a prenyl or geranyl group on any position of **1** might also increase activity. In fact, most prenylflavonoids and geranylflavonoids as well as related compounds are known to have potent cytotoxic effects.^{18–21} Furthermore, with respect to cancer research, a prenyl moiety has been demonstrated to be essential for chemopreventive

[#]Antitumor Agents 249. For part 248, see L. Wei, A. Brossi, S. L. Morris-Natschke, K. F. Bastow, and K.H. Lee, “Chemistry and Antitumor Activity of Tylophorine-related Alkaloids,” *Studies in Natural Product Chemistry: Bioactive Natural Products*, Vol. 34, in press.

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activity in many compound types.²²⁻²⁴ Therefore, we herein report the syntheses and cytotoxic activities of dehydrozingerone analogs, including investigation of prenyl substitution.

Results and Discussion

As shown in Scheme 1, dehydrozingerone (**6–13**) and chalcone (**14–17**) analogs with various substitution patterns on the benzylidene ring were easily obtained from substituted benzaldehydes (**5**) reacted with the appropriate ketone (acetone for **6–13**, acetophenone for **14–17**) using an aldol condensation.¹² Alkylation/allylation of the phenol of **1** or **3** was achieved by a standard procedure²⁵ using an alkyl/allyl halide to give the corresponding phenoxy analogs **18–26** and **27–35**, respectively, as shown in Scheme 2.

Derivatives **6–35** were evaluated as inhibitors of human tumor cell replication using three human tumor cell lines, nasopharyngeal carcinoma KB, multi-drug resistant expressing p-glycoprotein KB-VCR, and lung carcinoma A549.^{26, 27} Curcumin (**2**) and doxorubicin were used as positive controls. The results are shown in Table 1.

Most analogs, except isoeugenol analogs **27–35**, showed moderate or strong cytotoxic activity against all three cell lines. Comparison of the corresponding dehydrozingerone (**1, 6–8**) and chalcone (**14–17**) analogs showed that the latter compounds were more potent in each case. Because the chalcones have a phenyl rather than methyl in the unsaturated ketone, these data suggest that this phenyl group is important for optimal activity. Chalcone **15** showed significant activity against the A549 cell line with an IC₅₀ value of 0.6 µg/mL. Furthermore, the dehydrozingerone analog **11** and chalcones **16** and **17** showed significant cytotoxic activity against both KB (IC₅₀ values of 2.0, 1.0, and 2.0 µg/mL, respectively) and KB-VCR (IC₅₀ values of 1.9, 1.0, and 2.0 µg/mL, respectively) cells. The similar potencies against the parental and drug-resistant cell lines suggested that these compounds were not substrates for the p-glycoprotein drug efflux pump.

The substitution pattern on the benzylidene ring had some influence on potency. In particular, among the dehydrozingerone analogs, compounds with an *ortho*-hydroxy group were more active than compounds with *meta*- or *para*-hydroxy substituents [compare **6** (2'-OH, 3'-OMe), **8** (2'-OH, 4'-OMe), and **11** (2'-OH, 3'-OEt) with **1** (3'-OMe, 4'-OH), **7** (3'-OH, 4'-OMe), and **10** (3'-OEt, 4'-OH)]. Compound **12** (2'-OH, 3'-F) also exhibited significant activity, showing that fluorine could be substituted for an alkoxy group. In addition, substitution of 3'-fluoro for 3'-hydroxy (compare **13** with **7**) was beneficial.

The dehydrozingerone derivatives **18–26** showed cytotoxic activity, while the corresponding isoeugenol derivatives **27–35** were inactive. This result supported the supposition that the ketone on C-3 (numbering for dehydrozingerone) is important for anticancer properties.²⁸ Phenoxydehydrozingerone analogs **18** (C-4'-prenyloxy) and **21** (C-4'-geranyloxy) showed higher activity than dehydrozingerone itself and were as or more active than the other alkylated compounds **19, 20, 22–26**. With a longer farnesyl group, analog **22** showed decreased potency relative to **18** and **21**. This result supported those of prior investigations showing that prenylated and/or geranylated flavonoids were more active than farnesylated compounds.^{17,19} Derivative **26**, which has a saturated isoamyl rather than unsaturated prenyl group, showed equal and significant activity against KB and KB-VCR cells with IC₅₀ values of 3.6 and 3.2 µg/mL, respectively. However, the prenylated analog (**18**) was about twice as potent against the drug-resistant cell line (3.8 µg/mL, KB versus 2.0 µg/mL, KB-VCR).

In conclusion, chalcone **15** showed the highest *in vitro* potencies with IC₅₀ values ranging from 0.6 to 2.4 µg/mL. The ketone at the C-3 and phenyl at the C-4 positions are necessary for optimal cytotoxic activity. Compounds with hydroxyl at the *ortho* position of the benzylidene

moiety generally showed increased activity. Among alkylated **1**-analogs, alkylation of the phenolic OH with prenyl or geranyl resulted in the highest potency.

Experimental Section

Melting points were measured with a Fisher Johns melting point apparatus without correction. The proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were measured on a Varian Gemini 2000 (300 MHz) NMR spectrometer with TMS as the internal standard. All chemical shifts are reported in ppm. Mass spectra were obtained on a TRIO 1000 mass spectrometer. Analytical thin layer chromatography (TLC) was performed on Merck pre-coated aluminum silica gel sheets (Kieselgel 60 F 254). Column chromatography was performed on a CombiFlash Companion system using RediSep normal phase silica columns (ISCO, Inc., Lincoln, NE). In case, according to the circumstances, Inc. Silica gel (200–400 mesh) from Natland (Durham, NC) was used for column chromatography. All other chemicals were obtained from Aldrich, Inc. unless otherwise noted.

General Procedure for Aldol Reaction¹²

The appropriately substituted benzaldehyde **5** was dissolved in acetone, and 1 N sodium hydroxide was added to the solution with continuous stirring. Stirring was continued overnight. Excess acetone was removed under reduced pressure. Upon acidification with 1 N HCl, the reaction mixture was extracted with CHCl_3 or CH_2Cl_2 , and then solution was dried over anhydrous Na_2SO_4 . Solvent was removed and the yellow solid obtained was recrystallized from EtOAc to give **6–13**. Similarly, for the preparation of **14–17**, benzaldehyde **5** was dissolved in MeOH with acetophenone, and 5 N potassium hydride was added to the solution with continuous stirring. Stirring was continued overnight. MeOH was removed under reduced pressure. After acidification with 1 N HCl, the crude mixture was extracted with CH_2Cl_2 , and the solution was dried over anhydrous Na_2SO_4 . Solvent was removed and the solid obtained was purified with CombiFlash chromatography (EtOAc-hexane) to provide **14–17**.

***E*-1-(2-Hydroxy-3-methoxy-phenyl)-but-1-en-3-one (6)**

Starting with *o*-vanillin (5 g, 0.03 mol), acetone (40 mL), and 1 N NaOH (50 mL); yield 2.0 g (30%); powder, mp 79–80 °C; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.84 (d, 1H, $J = 16.5$ Hz, 1-H), 7.12 (dd, 1H, $J = 6.6, 2.7$ Hz, 5'-H), 6.90–6.84 (m, 2H, 4'- and 6'-H) 6.81 (d, 1H, $J = 16.5$, 2-H) 6.16 (s, 1H, OH) 3.92 (s, 3H, OCH_3), 2.40 (s, 3H, 4-H); MS m/z 277 $[\text{M}+\text{Na}]^+$

***E*-1-(3-Hydroxy-4-methoxy-phenyl)-but-1-en-3-one (7)**

Starting with 3-hydroxy-4-methoxybenzaldehyde (1 g, 6.6 mmol), acetone (8 mL) and 1 N NaOH (10 mL); yield 1.2 g (94%); powder, mp 96–97 °C; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.42 (d, 1H, $J = 16.2$ Hz, 1-H), 7.15 (d, 1H, $J = 1.5$ Hz, 2'-H), 7.06 (dd, 1H, $J = 8.3, 2.1$ Hz, 5'-H), 6.86 (d, 1H, $J = 8.3$ Hz, 6'-H), 6.59 (d, 1H, $J = 16.2$ Hz, 2-H), 5.70 (s, 1H, OH), 3.93 (s, 3H, OCH_3), 3.36 (s, 3H, 4-H); MS m/z 193 $[\text{M}+\text{H}]^+$

***E*-1-(2-Hydroxy-4-methoxy-phenyl)-but-1-en-3-one (8)**

Starting with 2-hydroxy-4-methoxybenzaldehyde (1 g, 6.6 mmol), acetone (8 mL), and 1 N NaOH (10 mL); yield 497 mg (39%); powder, mp 129–130 °C; $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.78 (d, 1H, $J = 16.3$ Hz, 1-H), 7.34 (d, 1H, $J = 8.5$ Hz, 6'-H), 7.01 (d, 1H, $J = 16.3$ Hz, 2-H) 6.50 (dd, 1H, $J = 6.48, 2.1$ Hz, 3'-H), 6.16 (s, 1H, OH) 3.82 (s, 3H, OCH_3), 2.41 (s, 3H, 4-H); MS m/z 215 $[\text{M}+\text{Na}]^+$

E-1-Phenyl-but-1-en-3-one (9)

Starting with benzaldehyde (500 mL, 4.9 mmol), acetone (4 mL), and 1 _N NaOH (5 mL); yield 241 mg (34%); yellow oil; ¹H NMR (300 MHz, CDCl₃) δ 7.58–7.53 (m, 2H, 2×Ar-H), 7.52 (d, 1H, *J* = 16.2 Hz, 1-H), 7.42–7.40 (m, 3H, 3×Ar-H), 6.73 (d, 1H, *J* = 16.2 Hz, 2-H), 2.39 (s, 3H, 4-H); MS *m/z* 169 [M+Na]⁺

E-1-(3-Ethoxy-4-hydroxy-phenyl)-but-1-en-3-one (10)

Starting with 3-ethoxy-4-hydroxybenzaldehyde (1 g, 6.0 mmol), acetone (8 mL), and 1 _N NaOH (10 mL); yield 1.1 g (42%); needles, mp 104–105 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.44 (d, 1H, *J* = 16.0 Hz, 1-H), 7.08 (br d, 1H, *J* = 8.4 Hz, 6'-H), 7.05 (br s, 1H, 2'-H), 6.93 (d, 1H, *J* = 8.4 Hz, 5'-H), 6.57 (d, 1H, *J* = 16.0 Hz, 2-H), 5.85 (s, 1H, OH), 4.16 (q, 2H, *J* = 7.0 Hz, 1'-H), 2.36 (s, 3H, 4-H), 1.48 (t, 3H, *J* = 7.0 Hz, 2'-H); MS *m/z* 229 [M+Na]⁺

E-1-(3-Ethoxy-2-hydroxy-phenyl)-but-1-en-3-one (11)

Starting with 3-ethoxysalicyl-aldehyde (1 g, 6.0 mmol), acetone (8 mL), and 1 _N NaOH (10 mL); yield 1.0 g (84%); powder, mp 77–78 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.84 (d, 1H, *J* = 16.5 Hz, 1-H), 7.11 (dd, 1H, *J* = 4.8, 2.1 Hz, 5'-H), 6.88–6.81 (m, 2H, 4'- and 6'-H) 6.21 (s, 1H, OH), 6.81 (d, 1H, *J* = 16.5 Hz, 2-H), 4.14 (q, 2H, *J* = 6.9 Hz, 1'-H), 2.40 (s, 3H, 1-H), 1.47 (t, 3H, *J* = 6.9 Hz, 2'-H); MS *m/z* 207 [M+H]⁺

E-1-(3-Fluoro-2-hydroxy-phenyl)-but-1-en-3-one (12)

Starting with 3-fluorosalicyl-aldehyde (200 mg, 1.4 mmol), acetone (1.6 mL), and 1 _N NaOH (2 mL); yield 239 mg (93%); prisms, mp 167–168 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.79 (d, 1H, *J* = 16.5 Hz, 1-H), 7.29 (br d, 1H, *J* = 8.1 Hz, 6'-H), 7.12 (br t, 1H, *J* = 8.1 Hz, 5'-H), 6.91–6.82 (m, 2H, 4'- and 2-H), 5.96 (br s, 1H, OH), 2.41 (s, 3H, 4-H); MS *m/z* 203 [M+Na]⁺

E-1-(3-Fluoro-4-methoxy-phenyl)-but-1-en-3-one (13)

Starting with 3-fluoro-4-methoxybenzaldehyde (500 mg, 3.24 mmol), acetone (4 mL), and 1 _N NaOH (5 mL); yield 139 mg (22%); needles, mp 96–97 °C; ¹H NMR (300 MHz, CDCl₃) δ 7.42 (d, 1H, *J* = 16.2 Hz, 1-H), 7.34–7.25 (m, 2H, 2×Ar-H), 6.97 (t, 1H, *J* = 8.7 Hz, 5'-H), 6.59 (d, 1H, *J* = 16.2 Hz, 2-H), 5.85 (s, 1H, OH), 3.93 (s, 3H, OCH₃), 2.37 (s, 3H, 4-H); MS *m/z* 217 [M+Na]⁺

E-1-(2-Hydroxy-3-methoxy-phenyl)-3-phenyl-propenone (15)

Starting with o-vanillin (128 mg, 0.85 mmol), acetophenone (100 μL, 0.85 mmol), MeOH (1 mL), and 5 _N NaOH (1 mL); yield 80 mg (37%); powder, mp 115 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.05–8.02 (m, 2H, 2×Ar-H), 8.04 (d, 1H, *J* = 15.9 Hz, 1-H), 7.75 (d, 1H, *J* = 15.9 Hz, 2-H), 7.61–7.47 (m, 3H, 3×Ar-H), 7.20 (dd, 1H, *J* = 3.3, 2.7 Hz, Ar-H), 6.92–6.86 (m, 2H, 2×Ar-H), 6.27 (s, 1H, OH), 3.94 (s, 3H, OCH₃); MS *m/z* 277 [M+Na]⁺

E-1-(3-Hydroxy-4-methoxy-phenyl)-3-phenyl-propenone (16)

Starting with 3-hydroxy-4-methoxybenzaldehyde (128 mg, 0.85 mmol), acetophenone (100 μL, 0.85 mmol), MeOH (1 mL), and 5 _N NaOH (1 mL); yield 194 mg (90%); powder, 97–98 °C; ¹H NMR (300 MHz, CDCl₃) δ 8.04–8.00 (m, 2H, 2×Ar-H), 7.74 (d, 1H, *J* = 15.7 Hz, 1-H), 7.61–7.47 (m, 3H, 3×Ar-H), 7.41 (d, 1H, *J* = 15.7 Hz, 2-H), 7.29 (d, 1H, *J* = 2.0 Hz, Ar-H), 7.15 (dd, 1H, *J* = 8.2, 2.0 Hz, Ar-H), 6.88 (d, 1H, *J* = 8.2 Hz, Ar-H), 5.69 (br s, 1H, OH), 3.95 (s, 3H, OCH₃); MS *m/z* 255 [M+H]⁺

E-1-(2-Hydroxy-4-methoxy-phenyl)-3-phenyl-propenone (17)

Starting with 2-hydroxy-4-methoxybenzaldehyde (128 mg, 0.85 mmol), acetophenone (100 μ L, 0.85 mmol), MeOH (1 mL), and 5 *N* NaOH (1 mL); yield 65 mg (30%); amorphous; ^1H NMR (300 MHz, CDCl_3) δ 8.14 (d, 1H, $J = 15.9$ Hz, 1-H), 8.04–8.01 (m, 2H, 2 \times Ar-H), 7.61 (d, 1H, $J = 15.9$ Hz, 2-H), 7.61–7.47 (m, 4H, 4 \times Ar-H), 6.53 (dd, 1H, $J = 8.7, 2.4$ Hz, Ar-H), 6.49 (d, 1H, Ar-H), 3.82 (s, 3H, OCH_3); MS m/z 277 $[\text{M}+\text{Na}]^+$

General Procedure for Alkylation²⁵

The mixture of dehydrozingerone (**2**) or isoeugenol (**3**), an appropriate alkyl halide, and K_2CO_3 in acetone was heated to reflux overnight. The reaction mixture was evaporated under vacuum. The crude mixture was extracted with CH_2Cl_2 , and the organic phase was washed with brine and dried over NaSO_4 and concentrated to obtain the product as a solid. The crude solid was purified with CombiFlash chromatography (EtOAc-hexane gradient) to obtain the target materials (**18–35**).²⁵

E-1-[3-Methoxy-4-(3-methyl-but-2-enyloxy)-phenyl]-but-1-en-3-one (18)

Starting with **1** (50 mg, 0.26 mmol), 4-bromo-2-methyl-2-butene (48 μ L, 0.39 mmol), K_2CO_3 (252 mg, 1.82 mmol); yield 57 mg (87%); powder, mp 89–90 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.46 (d, 1H, $J = 16.2$ Hz, 1-H), 7.10 (dd, 1H, $J = 8.2, 2.1$ Hz, 6'-H), 7.07 (d, 1H, $J = 2.1$ Hz, 2'-H) 6.88 (d, 1H, $J = 8.2$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.2$ Hz, 2-H), 5.51 (tt, 1H, $J = 6.6, 1.2$ Hz, 2''-H), 4.63 (d, 1H, $J = 6.6$ Hz, 1''-H), 3.90 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 1.78 (br s, 3H, CH_3), 1.75 (br s, 3H, CH_3); MS m/z 261 $[\text{M}+\text{H}]^+$

E-1-(4-Allyloxy-3-methoxy-phenyl)-but-1-en-3-one (19)

Starting with **1** (100 mg, 0.52 mmol), allyl bromide (68.1 mL, 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 115 mg (94%); needles, mp 71–72 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.48 (d, 1H, $J = 16.3$ Hz, 1-H), 7.12–7.06 (m, 2H, 2'- and 6'-H), 6.88 (d, 1H, $J = 7.8$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.3$ Hz, 2-H), 6.08 (ddt, 2H, $J = 17.4, 10.5, 5.1$ Hz, 2''-H) 5.42 (br d, 1H, $J = 17.4$ Hz, 3''-H) 5.32 (br d, 1H, $J = 10.5$ Hz, 3''-H) 3.91 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H); MS m/z 233 $[\text{M}+\text{H}]^+$

E-1-(4-But-2-enyloxy-3-methoxy-phenyl)-but-1-en-3-one (20)

Starting with **1** (100mg, 0.52 mmol), crotyl bromide (95 μ L, 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 119 mg (93%); powder, mp 68–69 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.48 (d, 1H, $J = 16.2$ Hz, 1-H), 7.12–7.06 (d, 2H, 2'- and 6'-H), 6.88 (d, 1H, $J = 8.4$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.2$ Hz, 2-H), 5.94–5.72 (m, 2H, 2''- and 3''-H) 4.56 (d, 1H, $J = 6.0$ Hz, 1''-H), 3.91 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 1.75 (d, 3H, $J = 5.1$ Hz, 4''-H); MS m/z 247 $[\text{M}+\text{H}]^+$

E-1-[4-(3,7-Dimethyl-octa-2,6-dienyloxy)-3-methoxy-phenyl]-but-1-en-3-one (21)

Starting with **1** (100 mg, 0.52 mmol), crotyl bromide (149 μ L, 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 160 mg (93%); powder, mp 56–57 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.46 (d, 1H, $J = 16.2$ Hz, 1-H), 7.10 (dd, 1H, $J = 8.2, 1.9$ Hz, 6'-H), 7.07 (d, 1H, $J = 1.9$ Hz, 2'-H), 6.87 (d, 1H, $J = 8.2$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.2$ Hz, 2-H), 5.51 (br t, 1H, $J = 6.6$ Hz, 2''-H), 5.07 (br t, 1H, $J = 6.6$ Hz, 6''-H), 4.67 (d, 2H, $J = 6.6$ Hz, 1''-H), 3.91 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 2.16–2.05 (m, 4H, 4''- and 5''-H), 0.74 (br s, 3H, CH_3), 1.66 (br s, 3H, CH_3), 1.60 (br s, 3H, CH_3); MS m/z 351 $[\text{M}+\text{H}]^+$

E-1-[3-Methoxy-4-(3,7,11-trimethyl-dodeca-2,6,10-trienyloxy)-phenyl]-but-1-en-3-one (22)

Starting with **1** (100 mg, 0.52 mmol), farnesyl bromide (222.8 μ L, 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 182 mg (88%); powder, mp 66–67 $^\circ\text{C}$; ^1H NMR (300 MHz,

CDCl_3) δ 7.46 (d, 1H, $J = 16.2$ Hz, 1-H), 7.10 (dd, 2H, $J = 7.8, 2.0$ Hz, 6'-H), 7.07 (d, 1H, $J = 2.0$ Hz, 2'-H), 6.87 (d, 1H, $J = 7.8$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.2$ Hz, 2-H), 5.51 (br t, 1H, $J = 6.0$ Hz, 2''-H), 5.12–5.04 (m, 2H, 6''- and 10''-H), 4.67 (d, 2H, $J = 6.3$ Hz, 1''-H), 3.91 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 2.18–1.92 (m, 8H, 4''-, 5''-, 8''-, and 9''-H), 0.75 (br s, 3H, 3''- or 7''- CH_3), 1.68 (br s, 3H, 3''- or 7''- CH_3), 1.59 (br s, 6H, gem-diMe); MS m/z 397 $[\text{M}+\text{H}]^+$

E-1-(3,4-Dimethoxy-phenyl)-but-1-en-3-one (23)

Starting with **1** (100 mg, 0.52 mmol), bromomethane (50 μL , 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 72 mg (78%); needles, mp 87–88 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.47 (d, 1H, $J = 16.2$ Hz, 1-H), 7.13 (br d, 1H, 6'-H), 7.08 (br s, 1H, 2'-H), 6.88 (d, 1H, $J = 8.2$ Hz, 5'-H), 6.61 (d, 1H, $J = 16.2$ Hz, 2-H), 4.08 (t, 2H, $J = 6.6$ Hz, 1''-H), 3.92 (s, 3H, $\text{OCH}_3 \times 2$), 2.37 (s, 3H, 4-H); MS m/z 179 $[\text{M}+\text{H}]^+$

E-1-(4-Ethoxy-3-methoxy-phenyl)-but-1-en-3-one (24)

Starting with **1** (100 μL , 0.65 mmol), bromoethane (59 μL , 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 70 mg (61%); needles, mp 108–109 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.46 (d, 1H, $J = 16.0$ Hz, 1-H), 7.10 (br d, 1H, $J = 8.1$, 6'-H), 7.08 (br s, 1H, 2'-H), 6.87 (d, 1H, $J = 8.1$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.0$ Hz, 2-H), 4.14 (q, 2H, $J = 6.9$ Hz, 1''-H), 3.91 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 1.48 (t, 2H, 7.0 Hz, 2''-H); MS m/z 221 $[\text{M}+\text{H}]^+$

E-1-(3-Methoxy-4-propoxy-phenyl)-but-1-en-3-one (25)

Starting with **1** (100 mg, 0.52 mmol), 1-iodopropane (77 μL , 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 57 mg (47%); needles, mp 101 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.46 (d, 1H, $J = 16.2$ Hz, 1-H), 7.10 (br d, 1H, 6'-H), 7.08 (br s, 1H, 2'-H), 6.87 (d, 1H, $J = 8.0$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.1$ Hz, 2-H), 4.02 (t, 2H, $J = 6.9$ Hz, 1''-H), 3.90 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 1.89 (qt, 2H, $J = 7.3, 6.9$ Hz, 2''-H), 1.05 (t, 3H, $J = 7.3$ Hz, 3''-H); MS m/z 207 $[\text{M}+\text{H}]^+$

E-1-[3-Methoxy-4-(3-methyl-butoxy)-phenyl]-but-1-en-3-one (26)

Starting with **1** (100 mg, 0.52 mmol), 1-methyl-3-bromobutane (102.3 μL , 0.78 mmol), and K_2CO_3 (503 mg, 3.64 mmol); yield 32 mg (23%); needles, mp 77–78 $^\circ\text{C}$; ^1H NMR (300 MHz, CDCl_3) δ 7.45 (d, 1H, $J = 16.1$ Hz, 1-H), 7.10 (br d, 1H, $J = 8.4$ Hz, 6'-H), 7.07 (br s, 1H, 2'-H), 6.88 (d, 1H, $J = 8.4$ Hz, 5'-H), 6.60 (d, 1H, $J = 16.1$ Hz, 2-H), 4.08 (t, 2H, $J = 6.6$ Hz, 1''-H), 3.90 (s, 3H, OCH_3), 2.37 (s, 3H, 4-H), 1.92–1.70 (m, 1H, 3''-H), 1.77 (br t, 2H, $J = 6.6$ Hz, 2''-H), 0.98 (br s, 3H, CH_3), 0.96 (br s, 3H, CH_3); MS m/z 263 $[\text{M}+\text{H}]^+$

2-Methoxy-1-(3-methyl-but-2-enyloxy)-4-propenyl-benzene (27)

Starting with **3** (100 μL , 0.65 mmol), 4-bromo-2-methyl-2-butene (118 μL , 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 45 mg (30%); yellow oil; ^1H NMR (300 MHz, CDCl_3) δ 6.89–6.78 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 15.6$ Hz, 1''-H), 6.16–6.04 (m, 1H, 2''-H), 5.89–5.71 (m, 2H, 2'- and 3'-H), 4.50 (d, 2H, $J = 5.4$ Hz, 1'-H), 3.87 (s, 3H, OCH_3), 1.86 (d, 3H, $J = 6.3$ Hz, 3''-H), 1.76 (s, 3H, CH_3), 1.72 (s, 3H, CH_3); MS m/z 233 $[\text{M}+\text{H}]^+$, 255 $[\text{M}+\text{Na}]^+$

1-Allyloxy-2-methoxy-4-propenyl-benzene (28)

Starting with **3** (100 μL , 0.65 mmol), allyl bromide (85 μL , 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 78 mg (59%); pale yellow oil; ^1H NMR (300 MHz, CDCl_3) δ 6.89–6.78 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 16.2$ Hz, 1''-H), 6.16–6.02 (m, 1H, 2''-H), 5.42 (br d, 1H, $J = 17.4$ Hz, 3'-H), 5.27 (br d, 1H, $J = 9.6$ Hz, 3'-H), 4.60 (d, 2H, $J = 5.7$ Hz, 1'-H), 3.88 (s, 3H, OCH_3), 1.86 (d, 3H, $J = 6.3$ Hz, 3''-H); MS m/z 205 $[\text{M}+\text{H}]^+$

1-But-2-enyloxy-2-methoxy-4-propenyl-benzene (29)

Starting with **3** (100 μ L, 0.65 mmol), crotyl bromide (118 μ L, 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 98 mg (69%); pale yellow oil; 1H NMR (300 MHz, $CDCl_3$) δ 6.89–6.78 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 15.3$ Hz, 1''-H) 6.16–6.04 (m, 1H, 2''-H), 5.51 (m, 1H, 2'-H), 4.55 (d, 2H, $J = 6.6$ Hz, 1'-H), 3.87 (s, 3H, OCH_3), 1.86 (d, 3H, $J = 6.3$ Hz, 3''-H), 1.71 (d, 3H, $J = 5.1$ Hz, 4'-H); MS m/z 219 $[M+H]^+$

2-Methoxy-4-propenyl-1-(3,7,11-trimethyl-dodeca-2,6,10-trienyloxy)-benzene (30)

Starting with **3** (100 μ L, 0.65 mmol), farnesyl bromide (277 μ L, 0.78 mmol), and K_2CO_3 (626 mg, 4.53 mmol); yield 215 mg (90%); yellow oil; 1H NMR (300 MHz, $CDCl_3$) δ 6.88–6.78 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 15.3$ Hz, 1''-H) 6.16–6.04 (m, 1H, 2''-H), 5.51 (m, 1H, 2'-H), 5.10 (m, 2H, 6'- and 10'-H), 4.60 (d, 2H, $J = 6.3$ Hz, 1'-H), 3.87 (s, 3H, OCH_3), 2.15–1.90 (m, 8H, 4'-, 5'-, 8'-, 9'-H) 1.86 (d, 3H, $J = 6.3$ Hz, 3''-H), 1.77 (s, 3H, CH_3), 1.72 (s, 3H, CH_3) 1.59 (s, 6H, gem-di CH_3); MS m/z 369 $[M+H]^+$

1,2-Dimethoxy-4-propenyl-benzene (32)

Starting with **3** (100 μ L, 0.65 mmol), bromomethane (61 μ L, 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 101 mg (88%); pale yellow oil; 1H NMR (300 MHz, $CDCl_3$) δ 6.89–6.78 (m, 3H, 3-, 5-, and 6-H), 6.34 (d, 1H, $J = 15.9$ Hz, 1''-H) 6.16–6.04 (m, 1H, 2''-H), 3.89 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3) 1.87 (d, 3H, $J = 6.3$ Hz, 3''-H); MS m/z 179 $[M+H]^+$

2-Methoxy-4-propenyl-1-propoxy-benzene (34)

Starting with **3** (100 μ L, 0.65 mmol), 1-iodopropane (96 μ L, 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 129 mg (96%); pale yellow oil; 1H NMR (300 MHz, $CDCl_3$) δ 6.89–6.80 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 1.56$ Hz, 1''-H) 6.15–6.04 (m, 1H, 2''-H), 3.96 (t, 3H, $J = 6.7$ Hz, 1'-H), 3.87 (s, 3H, OCH_3) 1.91–1.79 (m, 5H, 3''- and 2'-H), 1.03 (t, 3H, $J = 7.5$ Hz, 3'-H); MS m/z 235 $[M+H]^+$

2-Methoxy-1-(3-methyl-butoxy)-4-propenyl-benzene (35)

Starting with **3** (100 μ L, 0.65 mmol), 1-methyl-3-bromobutane (127 μ L, 0.97 mmol), K_2CO_3 (626 mg, 4.53 mmol); yield 91 mg (59%); pale yellow oil; 1H NMR (300 MHz, $CDCl_3$) δ 6.89–6.78 (m, 3H, 3-, 5-, and 6-H), 6.33 (d, 1H, $J = 15.9$ Hz, 1''-H) 6.16–6.04 (m, 1H, 2''-H), 4.03 (t, 2H, $J = 6.9$ Hz, 1'-H), 3.87 (s, 3H, OCH_3), 1.86 (d, 3H, $J = 6.6$ Hz, 3''-H), 1.92–1.70 (m, 3H, 2'- and 3'-H), 0.97 (s, 3H, CH_3), 0.95 (s, 3H, CH_3); MS m/z 263 $[M+H]^+$

Cytotoxic Activity Assay

The *in vitro* cytotoxicity assay was carried out according to procedures described in Rubinstein et al.²⁶ Drug stock solutions were prepared in DMSO, and the final solvent concentration was <1% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel consisted of epidermoid carcinoma of the nasopharynx (KB), lung carcinoma (A-549). The drug resistant cell line was KB-VCR, an MDR variant selected for growth in vincristine. It is cross-resistant to doxorubicin (Table 1). Detailed characterization of this cell line is described elsewhere.²⁷ Cells were cultured at 37 °C in RPMI-1640 with 100 μ g/mL kanamycin and 10% (v/v) fetal bovine serum in a humidified atmosphere containing 5% CO_2 . Initial seeding densities varied among the cell lines to ensure a final absorbance of 1–2.5 A_{562} units. Drug exposure was for 2 days and the IC_{50} value, the drug concentration that reduced the absorbance by 50%, was interpolated from dose-response data. Each test was performed in duplicate, and absorbance readings varied no more than 5% between replicates.

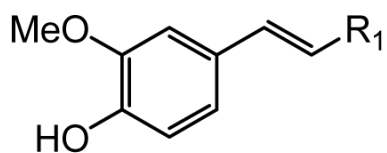
Acknowledgment

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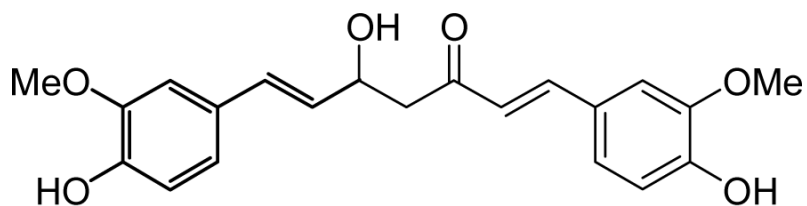
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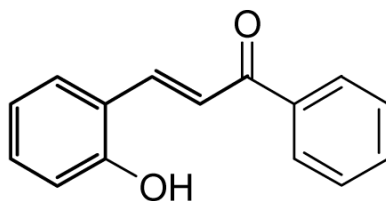
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- 1**, Dehydrozingerone: $R_1 = \text{COMe}$
3, Isoeugenol: $R_1 = \text{Me}$

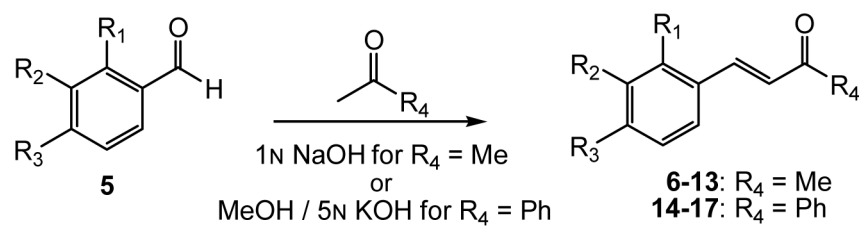


2, Curcumin



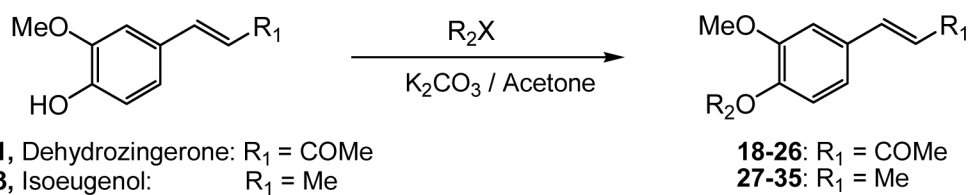
4, 2-Hydroxychalcone

Figure 1. Structures of Dehydrozingerone (**1**), Curcumin (**2**), Isoeugenol (**3**) & 2-Hydroxychalcone (**4**).



	R_1	R_2	R_3	R_4
1	H	OMe	OH	Me
6	OH	OMe	H	Me
7	H	OH	OMe	Me
8	OH	H	OMe	Me
9	H	H	H	Me
10	H	OEt	OH	Me
11	OH	OEt	H	Me
12	OH	F	H	Me
13	H	F	OMe	Me
14	H	OMe	OH	Ph
15	OH	OMe	H	Ph
16	H	OH	OMe	Ph
17	OH	H	OMe	Ph

Scheme 1.
Syntheses of Dehydrozingerone (**6–13**) and Chalcone (**14–17**) Analogs



Dehydrozingerone $R_1 = \text{COMe}$	Isoeugenol $R_1 = \text{Me}$	R_2
18	27	
19	28	
20	29	
21	30	
22	31	
23	32	Me
24	33	
25	34	
26	35	

Scheme 2.
C-4' Alkylated Dehydrozingerone (18–26) and Isoeugenol (27–35) Analogs

Table 1
Activities of Analogs Against Human Tumor Cell Replication

compound	cell line/IC ₅₀ (μg/mL) ^a		
	KB ^b	KB-VCR ^b	A549 ^b
Dehydrozingerone Analogs			
1	>10	>10	>10
6	3.8	2.0	2.5
7	10.0	8.2	>10
8	3.8	4.0	3.5
9	5.3	5.6	2.8
10	7.7	7.8	8.5
11	2.0	1.9	2.3
12	2.1	3.0	3.0
13	4.3	5.0	5.5
Chalcone Analogs			
14	3.5	1.9	2.3
15	2.4	1.3	0.6
16	1.0	1.0	3.5
17	2.0	2.0	3.8
C-4' Alkylated Dehydrozingerone Analogs			
18	5.7	3.5	3.8
19	6.8	5.0	5.8
20	4.8	3.4	6.8
21	2.2	3.3	3.4
22	6.5	7.4	7.4
23	8.6	7.8	8.0
24	5.5	8.0	>10
25	6.5	4.2	5.8
26	3.6	3.2	7.2
C-4' Alkylated Isoeugenol Analogs			
27–35^c	>10	>10	ND ^d
Controls			
Curcumin (2)	5.5	3.1	5.2
Doxorubicin	0.1	2.7	0.1

^aCytotoxicity as IC₅₀ values for each cell line, the concentration of compound that caused 50% reduction in absorbance at 562 nm relative to untreated cells using the sulforhodamine B assay. The average value is from two independent determinations and variation (SEM) was no greater than 10%.

^bHuman epidermoid carcinoma of the nasopharynx (KB), multi-drug resistant expression P-glycoprotein (KB-VCR), human lung carcinoma (A549).

^c**30** and **33** were not tested.

^dND = Not determined.