Pergamon

Bioorganic & Medicinal Chemistry 9 (2001) 199–209

BIOORGANIC & MEDICINAL CHEMISTRY

Mechanism of Toxicity of Esters of Caffeic and Dihydrocaffeic Acids

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Received 25 July 2000; accepted 28 August 2000

Abstract—Ten esters each of caffeic acid and dihydrocaffeic acid have recently been synthesized. Cytotoxicity evaluations of these esters versus L1210 leukemia and MCF-7 breast cancer cells in culture have led to the delineation of substantially different QSAR for each series. The L1210 QSAR for dihydrocaffeic acid esters resembles the QSAR obtained for simple phenols and estrogenic phenols. However, the QSAR pertaining to the caffeic acid esters differs considerably from its sister QSAR. This difference may be attributed to the presence of the olefinic linkage in the side chain. The octyl ester of caffeic acid is nearly ten times as toxic to the leukemia cells than the widely studied phenethyl ester, CAPE.

Introduction

Caffeic acid phenethyl ester (CAPE) has been identified as one of the major components of honeybee propolis.[1] This phenolic compound elicits several interesting and varied biological responses; it wields antimicrobial, antiinflammatory, antineoplastic and antioxidant activity.[2–5] It also inhibits the development of azoxymethane-induced tumors in the colon of rats and phorbol induced tumors in mouse skin.[6,7] CAPE is also a potent inhibitor of ornithine decarboxylase, cyclooxygenase, lipoxygenases and HIV-1 integrase.[8–11]

The ability of CAPE to alter oxidative processes is well documented.[12] It can inhibit the formation of intracellular H_2O_2 and oxidized bases in DNA of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) treated human cells.[13] CAPE has also demonstrated an ability to induce apoptosis in certain transformed cells but not in parental normal cells.[14] This was one of the first indications that CAPE could act via a redox mechanism as a pro-oxidant since its induced apoptosis could be reduced by treatment with the antioxidant *N*-acetyl-L-cysteine. In addition, the reduced glutathione inhibitor, buthionine sulfoximine, induced apoptosis after CAPE treatment in nontumorigenic cells indicating that these cells have an enhanced oxidant defense system in comparison to the tumor cells. Recently, the ability of Michael reaction acceptors, diphenols and phenols to generate reactive intermediates by redox cycling has been linked to their ability to modulate gene expression and to inhibit nuclear transcription regulators.[15]

In recent years a great deal of attention has focused on the role and mechanism of action of polyphenolic compounds as inhibitors of oxidative processes.[16] However, little attention has focused on simple phenols, some of which have been shown to be carcinogenic[17] and estrogenic.[18] The dearth of data pertaining to the effects of various substituted phenols on mammalian cytotoxicity led to our examination of the growth inhibitory effects of a series of simple and complex X-phenols on a rapidly growing murine cell line and the development of appropriate quantitative structure–activity relationships (QSAR).[19]

Chemical reactivity and biological activity have been well correlated by Brown's variation of the Hammett electronic constant, sigma-plus (σ^+). The sigma plus parameter has found great success in delineating radical reactions involving phenols.[20] Thus this variable was utilized in the study of the cytotoxic profiles of X-Phenols versus L1210 murine cells in culture. Eqs. 1 and 2 were formulated for electron-releasing and electron-withdrawing substituents respectively.[19]

$$\log 1/C = -1.58(\pm 0.26)\sigma^+ + 0.21(\pm 0.06)\log P + 3.10(\pm 0.24)$$
$$n = 23, \quad r^2 = 0.898, \quad s = 0.191, \quad q^2 = 0.868 \qquad (1)$$

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0968-0896/01/$ - see front matter
PII: S0968-0896(00)00238-8

$$\log\ 1/C = 0.62(\pm 0.16)\log P + 2.35(\pm 0.31)$$
$$n = 15, \quad r^2 = 0.845, \quad s = 0.232, \quad q^2 = 0.800 \qquad (2)$$

In these equations C is the molar concentration of X-phenol that induces 50% inhibition of growth after 48 h. Sigma-plus (σ^+) represents the through-resonance electronic attributes of the substituents while logP is the octanol–water partition coefficient of the phenol. The number of data points in the study is represented by n, the correlation coefficient by r, the standard deviation by s, and the cross-validated r^2 by q^2. Eq. 1 correlates a heterogeneous set of substituted phenols including complex ones such as bisphenol A, diethylstilbesterol, estradiol, equilin and equilenin. A larger data set of electron-releasing X-phenols, which include ortho substituted phenols, led to the development of Eqs. 3 and 4.[21]

$$\log\ 1/C = -1.35(\pm 0.15)\sigma^+ + 0.18(\pm 0.04)\log P + 3.31(\pm 0.11)$$
$$n = 51, \quad r^2 = 0.895, \quad s = 0.227, \quad q^2 = 0.882 \qquad (3)$$

$$\log 1/C = -0.19(\pm 0.02)\mathrm{BDE} + 0.21(\pm 0.03)\log P + 3.11(\pm 0.10)$$
$$n = 52, \quad r^2 = 0.920, \quad s = 0.202, \quad q^2 = 0.909 \qquad (4)$$

In QSAR 4, BDE represents the calculated homolytic bond dissociation energy for each phenol. In the analysis of only *ortho* substituted congeners, logP was set to zero when it became clear that hydrophobic contact was not important in the interactions. Cytotoxicities were then modelled by Eq. 5.[21]

$$\log\ 1/C = -0.17(\pm 0.03)\mathrm{BDE} + 3.18(\pm 0.16)$$
$$n = 14, \quad r^2 = 0.936, \quad s = 0.191, \quad q^2 = 0.915 \qquad (5)$$

Note that the slope and intercept of QSAR 5 are essentially the same as QSAR 4. The addition of a steric term for *ortho* substituted phenols did not improve Eq. 5. Thus, *ortho* substitued phenols appear to undergo radicalization via a similar mechanism that prevails for *meta* and *para* substituted phenols, but do not appear to make hydrophobic contact with a receptor.

Eqs. 1, 3, and 4 with their marked dependence on σ^+ or BDE suggest that homolytic cleavage of the O-H bond is critical to the development of cytotoxicity. The small coefficient with the hydrophobic term suggests that minimal binding on the surface of a specific receptor also mediates this toxicity. Note that the estrogenic phenols like Bisphenol A, β-estradiol, nonylphenol and estriol are well predicted by these models. Preliminary results with a few antioxidants such as quercetin indicate that they fit this model well; they are cytotoxic albeit at a lower level. In order to gain further understanding of this ambivalent behavior of phenolic compounds we have synthesized and evaluated 20 ester analogues of caffeic acid and dihydrocaffeic acid (Fig. 1).

Results

From the results in Table 1, we have derived Eq. 6 for the inhibition of growth of L1210 cells by esters of caffeic acid.

$$\log\ 1/C = 0.46(\pm 0.12)\mathrm{ClogP} + 3.84 \pm 0.37$$
$$n = 9, \quad r^2 = 0.915, \quad s = 0.165, \quad q^2 = 0.881 \qquad (6)$$
$$\textit{outliers}: \mathrm{R} = \mathrm{C_6H_{13}}$$

In this equation, C represents the concentration of the analogue that induces 50% inhibition of growth (IC_{50}) while ClogP is the calculated partition coefficient of each compound. In this equation, the absence of an electronic term such as σ^+ or BDE is to be expected since the catecholic moiety is present and constant in all the analogues. The coefficient with the hydrophobic term is larger than what is normally seen with the phenolic compounds; in fact it is twice as large as that in Eqs. 3 and 4. One data point was not included in the analysis: the hexyl ester of caffeic acid, which is significantly less active than predicted. The reduced solubility of this analogue may contribute to its reduced cytotoxicity. Although the *t*-Butyl ester is not as well

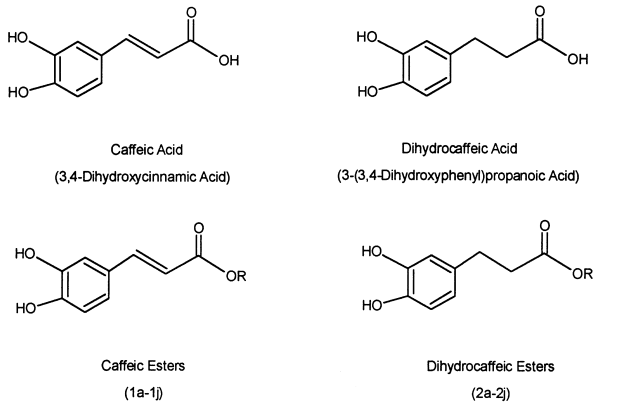

Figure 1. Structures of caffeic acid analogues.

Table 1. Biological and physicochemical constants used to derive QSAR 6 for the inhibition of growth of L1210 cells by caffeic acid esters

No.	Substituent	Obsd log 1/C	Calcd log 1/C	Δ log 1/C	ClogP
1a	CH_3	4.46	4.39	0.07	1.20
1b	C_2H_5	4.62	4.63	−0.01	1.73
1c	C_4H_9	4.99	5.12	−0.13	2.79
1d	C_6H_{13}[a]	5.20	5.60	−0.40	3.85
1e	C_8H_{17}	6.10	6.08	0.02	4.90
1f	$CHMe_2$	4.70	4.77	−0.07	2.04
1g	$CH_2C_6H_5$	5.01	5.20	−0.19	2.97
1h	$(CH_2)_2C_6H_5$	5.20	5.35	−0.15	3.30
1i	$(CH_2)_3C_6H_5$	5.74	5.52	0.22	3.68
1j	CMe_3	5.20	4.96	0.24	2.44

[a]Not used to derive Eq. 6.

predicted by this model due to its added bulk and substantial branching at the α-carbon, its activity still falls within an acceptable range.

Equation 7 is derived from data in Table 2, enumerating the cytotoxicities of esters of dihydrocaffeic acids.

$$\log\ 1/C = 0.15(\pm0.05)\mathrm{ClogP} + 0.14(\pm0.13)E_S + 4.62(\pm0.25)$$
$$n = 9, \quad r^2 = 0.906, \quad s = 0.067, \quad q^2 = 0.793 \tag{7}$$
$$\textit{outlier}: \quad R = CH_2C_6H_5$$

In QSAR 7, ClogP once again represents the calculated partition coefficient of each analogue while ES represents Taft's steric parameter for each alkyl group of the alkoxy moiety of each ester. The low coefficient with the ES term indicates that the cytotoxicity is not markedly attenuated by bulky esters. The positive coefficient with the ES term indicates that cytotoxicity is enhanced by a decrease in size of the substituents. However, the low magnitude of this coefficient and its relatively large confidence interval suggests that size plays a minor role in inhibiting cell growth. In this analysis the benzylic ester was a statistical outlier. A reason for its slightly anomalous behavior is not apparent. The coefficient with the hydrophobic term in QSAR 7 is less than that of the caffeic acid esters in QSAR 6 but similar to that seen in the case of the phenols.

Table 2. Biological and physicochemical constants used to derive QSAR 7 for the inhibition of growth of L1210 cells by dihydrocaffeic acid esters

No.	Substituent	Obsd log 1/C	Calcd log 1/C	Δ log 1/C	ClogP	ES
2a	Me	4.62	4.60	0.02	1.02	−1.24
2b	C_2H_5	4.63	4.67	−0.04	1.54	−1.31
2c	C_4H_9	4.70	4.79	−0.09	2.60	−1.63
2d	C_6H_{13}	5.06	4.96	0.10	3.66	−1.54
2e	C_8H_{17}	5.05	5.12	−0.07	4.72	−1.57
2f	$CHMe_2$	4.72	4.70	0.02	2.07	−1.71
2g	$CH_2C_6H_5$[a]	4.66	4.84	−0.18	2.79	−1.51
2h	$(CH_2)_2C_6H_5$	4.92	4.89	0.03	3.11	−1.47
2i	$(CH_2)_3C_6H_5$	4.98	4.95	0.03	3.49	−1.46
2j	CMe_3	4.62	4.61	0.01	2.47	−2.78

[a]Not used to derive Eq. 7.

Table 3. Biological and physicochemical constants used to derive QSAR 8 for the inhibition of growth of MCF-7 cells by caffeic acid esters

No.	Substituent	Obsd log 1/C	Calcd log 1/C	Δ log 1/C	ClogP[a]
1a	CH_3	3.02	3.07	−0.05	1.20
1b	C_2H_5	3.30	3.27	0.03	1.73
1c	$CH_2(CH_2)_2CH_3$	3.60	3.65	−0.05	2.79
1d	$CH_2(CH_2)_4CH_3$	4.08	4.04	0.04	3.85
1e	$CH_2(CH_2)_6CH_3$[a]	4.10	4.43	−0.33	4.90
1f	$CH(CH_3)_2$	3.40	3.38	0.02	2.04
1g	$CH_2C_6H_5$	3.80	3.72	0.08	2.97
1h	$(CH_2)_2C_6H_5$	3.70	3.84	−0.14	3.30
1i	$(CH_2)_3C_6H_5$	3.98	3.98	0.00	3.68
1j	$C(CH_3)_3$	3.59	3.53	−0.06	2.44

[a]Not used in the derivation of QSAR 8.

The growth inhibitory profiles of these two sets of esters versus MCF-7 (ER+) cells were also determined and the following QSAR models were developed. Eqs. 8 and 9 describe the QSAR for the inhibition of growth of MCF-7 cells by caffeic esters. See Table 3.

$$\log\ 1/C = 0.37(\pm0.07)\mathrm{ClogP} + 2.64(\pm0.20)$$
$$n = 9, \quad r^2 = 0.956, \quad s = 0.075, \quad q^2 = 0.931 \tag{8}$$

$$\log\ 1/C = 0.64(\pm0.30)\mathrm{ClogP} - 0.06(\pm0.05)\mathrm{ClogP}^2 + 2.34(\pm0.42) \tag{9}$$
$$n = 10, \quad r^2 = 0.958, \quad s = 0.081, \quad q^2 = 0.882,$$
$$\mathrm{ClogP}_o = 5.75$$

The linear model explains 95% of the variance in the data. Although the parabolic model (Eq. 9) is a marginal improvement it is able to establish an ideal hydrophobicity for optimal inhibitory activity. The optimum hydrophobicity 5.75 as delineated by ClogP is high and suggests that hydrophobic caffeic esters excel at inhibiting cell growth in this breast cancer line. It is difficult to ascertain whether this toxicity is due to increased permeability or enhanced interaction with a molecular target.

Eq. 10 was obtained for the inhibition of growth of MCF-7 cells by esters of dihydrocaffeic acid. See Table 4.

$$\log\ 1/C = 0.36(\pm0.12)B_1 + 3.40(\pm0.20)$$
$$n = 9, \quad r^2 = 0.885, \quad s = 0.050, \quad q^2 = 0.367 \tag{10}$$

B_1 represents Verloop's width parameter; its importance in equation 10 suggests that shape is an important constraint in the interaction between these dihydrocaffeic acid esters and a potential receptor. One point was poorly fit by this equation: the *n*-octyl ester. Perhaps the ease of flexibility of the octyl chain combined with its ability to fold on itself is not adequately represented by

Table 4. Biological and physiocochemical constants used to derive QSAR 10 for the inhibition of growth of MCF-7 cells by dihydrocaffeic acid esters

No.	Substituent	Obsd log 1/C	Calcd log 1/C	Δ log 1/C	B_1[a]
2a	CH_3	3.94	3.94	0.00	1.52
2b	C_2H_5	3.98	3.94	0.04	1.52
2c	$CH_2(CH_2)_2CH_3$	3.96	3.94	0.02	1.52
2d	$CH_2(CH_2)_4CH_3$	3.98	3.94	0.04	1.52
2e	$CH_2(CH_2)_6CH_3$[b]	4.22	3.94	0.28	1.52
2f	$CH(CH_3)_2$	4.16	4.08	0.08	1.90
2g	$CH_2C_6H_5$	3.92	3.94	−0.02	1.52
2h	$CH_2CH_2C_6H_5$	3.88	3.94	−0.06	1.52
2i	$CH_2(CH_2)_2C_6H_5$	3.89	3.94	−0.05	1.52
2j	$C(CH_3)_3$	4.30	4.33	−0.03	2.60

[a]Verloop's parameter.
[b]Not included in the derivation of QSAR 10.

Verloop's B_1 descriptor. Hence the large discrepancy between the observed and predicted value. Overall, the lack of diversity in biological activity of these analogues is striking. The average activity of all ten analogues falls within the following range: 4.02 ± 0.15. This suggests that the binding receptor in the case of these esters must have shape constraints that are non-restrictive.

The overall results versus MCF-7 cells closely parallel those obtained versus L1210 cells. QSAR 8 demonstrates a strong dependence on hydrophobicity akin to QSAR 6. The coefficients with the hydrophobic term are very similar: 0.46 (L1210) versus 0.37 (MCF-7) for the caffeic acid esters. In the case of the dihydrocaffeic acid esters, steric effects once again come into play although they are more pronounced in the case of the MCF-7 cells, which lack a hydrophobic component. Both QSAR 7 and QSAR 10 contain steric terms such as ES and B_1 and their coefficients vary slightly (0.14 for L1210 and 0.35 for MCF-7) in magnitude but differ in effect. QSAR 7 also shows a marginal dependence on hydrophobicity. Nevertheless, the emphasis on steric parameterization in both cells is of great interest and suggests the involvement of a receptor with specific spatial constraints. QSAR 7 suggests that small substituents enhance cytotoxicity while QSAR 10 indicates that an increase in size enhances toxicity. This apparent difference could be attributed to differences in cell type and/or variations in receptor steric requirements. Overall, it appears that rapidly dividing cells such as L1210 are more susceptible to the cytotoxic effects of both the caffeic acid and dihydrocaffeic acid esters.

Discussion

Eqs. 1, 3, and 4 clearly establish the importance of electronic effects in the toxicity of phenols to fast growing leukemia cells. Since catechol is well fit by these equations, one can presume that caffeic and dihydrocaffeic esters would act via the same mechanism. The alkoxy groups of these esters will have essentially little or no effect on the electron density of the catechol ring. Thus, we are left to consider two of the three main structural features that generally affect biological response: hydrophobicity and steric bulk.

In examining QSAR 6, we find that the data are well correlated by hydrophobicity alone, while QSAR 7 yields a much different model. The most important term in QSAR 7 is hydrophobicity as represented by ClogP. Using the hydrophobic parameter alone, $r^2 = 0.800$ with slope of 0.15, while using ES alone, $r^2 = 0.067$. Thus 80% of the variance in the data in QSAR 7 can be explained by hydrophobicity alone while 10% of the variance in the data can be attributed to steric effects. Except for the small contribution by ES the coefficient with ClogP in QSAR 7 is similar to that in QSAR 3 and 4.

However, QSAR 6 suggests an entirely different mechanism. Unfortunately there is no electronic reference point, but caffeic acid analogues can be considered to be derivatives of cinnamic acid which have been implicated as antioxidants. A recent review shows that there has been considerable interest in the radical scavenging activity of cinnamic acids.[22] The following equation illustrates the mechanism of radical-mediated oxidation of substituted cinnamic acids.[23]

Oxidation of X-$C_6H_4CH=CHCOOH$ in 30% acetic acid by quinolinium dichromate

$$\log k_2 = -0.66(\pm 0.16)\sigma^+ + 0.95(\pm 0.08)$$
$$n = 7, \quad r^2 = 0.959, \quad s = 0.084, \quad q^2 = 0.897 \tag{11}$$

QSAR 12 deals with inhibition of lipid peroxidation by substituted cinnamic acids. There is a strong dependence on the electron-releasing capabilities of the substituents as delineated by the Hammett σ^+ term.

ED_{50} for reducing accumulation of malonaldehyde in mice by X-cinnamic acids[24]

$$\log 1/C = -0.64(\pm 0.25)\sigma^+ + 0.41(\pm 0.25)B5_3 + 2.41(\pm 0.48)$$
$$n = 10, \quad r^2 = 0.867, \quad s = 0.219, \quad q^2 = 0.798, \tag{12}$$
$$\textit{outlier}: \ 3, 4\text{-di-OMe}$$

The 3-OCH_2COOH and 4-OCH_2COOH derivatives were omitted from the analysis for lack of adequate sigma-plus values. Once again, a pronounced dependence on σ^+ is observed.

The striking similarity in dependence on σ^+ for the radical oxidation by dichromate in Eq. 11, and the prevention of the oxidation of polyunsaturated fatty acids (PUFAs) to malonaldehyde (QSAR 12) indicates that both are radical mediated processes in which the π electrons of the double bond are involved. Thus, caffeic acids have two possible reaction centers-the phenolic OH or π electrons of the side chain. The results in QSAR 6 tend to discount the involvement of the catecholic OH group, because of the unusual strong dependence on ClogP and the subsequent marked deviation of QSAR 6 from QSARs 3 and 4.

One can also look for similarity with styrenes and their associated biological activities. In QSAR 13, C is the concentration that induces an elevation of serum alanine transaminase, a measure of hepatotoxicity.[25] Thus the more electron releasing the substituents on the styrenes, the greater the resulting hepatoxicity.

$$\log 1/C = -0.46(\pm 0.26)\sigma^+ + 3.22(\pm 0.18)$$
$$n = 6, \quad r^2 = 0.862, \quad s = 0.118, \quad q^2 = 0.738 \tag{13}$$
$$\textit{outlier}: X = H$$

It is also conceivable that both reaction centers may be involved in cytotoxicity. A comparison of growth inhibitory values (cytotoxic potencies) in Tables 1 and 2,

reveal that the compounds with relatively low hydrophobicities have parallel activity in the two series. With increased hydrophobicity, the caffeic esters are more potent, which suggests that oxidation of the olefinic linkage may be enhanced in a more lipophilic milieu. See Figure 2.

Extensive studies by Fukaya et al.[26,27] on various caffeic acid derivatives have revealed that they are excellent inhibitors of arachidonate 5-lipoxygenase (5-LO), 12-lipoxygenase (12-LO) and prostaglandin (PG) synthase. The *n*-butyl ester showed potent inhibitory activity towards 5-LO as well as selectivity versus this enzyme. Various caffeic acid derivatives such as caffeic acid amides, 1-(3,4-dihydroxyphenyl)-1-alken-3-ones and 1-(3,4-dihydroxylphenyl)-1-alkenes were also investigated as 5-LO inhibitors. It was determined that the catecholic moiety and a hydrophobic alkyl side chain were critical for strong binding to 5-LO. Rigorous QSAR studies of an extensive set of catechol derivatives by Fujita et al.[27] delineated the structural requirements for maximal binding to 5-LO. These include an optimal hydrophobicity of 4.3–4.7, high electron density of the catechol moiety and a lack of thickness of the lipophilic αβ side chain close to the catechol benzene ring. There are some similarities between our QSAR for the caffeic acid esters (QSAR 7 and 9) and the ones elaborated by Fujita et al. There is a strong possibility that the inhibition of cell growth by the caffeic acid esters results from an interrupted synthesis of leukotrienes mediated by inhibition of 5-LO.

In undertaking this research, we hoped to learn about the mechanism of action of the caffeic esters as well as to find a compound more potent than CAPE. Compound **1e**, the *n*-octyl-caffeate, is almost ten times as potent as CAPE versus L1210 cells in culture and three times more active than CAPE in MCF-7 cells.

Guarini et al.,[28] examined the cytotoxic potential of CAPE versus a human melanoma cell line HO-1 and human glioblastoma cells GBM-18. From their data, it can be ascertained that the log 1/C of CAPE in the melanoma line would fall between 4.45 to 5.05, while the value in the astrocytoma line would be closer to 4.45. They deduced that HO-1 cells were more susceptible to CAPE induced growth suppression than GBM-18 cells. Our results for CAPE versus a rapidly growing cell line versus slow growing breast cancer cells are in agreement. The log 1/C values for L1210 and MCF-7 are 5.20 and 3.70, respectively. This suggests that CAPE and other caffeic acid esters should show promise versus aggressively growing tumor cells such as melanomas.[1]

Conclusion

A comparison of the cytotoxicities of caffeic and dihydrocaffeic acid esters versus L1210 cells reveals that there are subtle differences in potency between similar analogues. The olefinic linkage in caffeic esters appears to play a critical role in overall toxicity. Its susceptibility to peroxidation may be the underlying reason for its toxicity. Studies with similar analogues suggests that these esters may be inhibitors of 5-LO. *N*-octylcaffeate is a more potent inhibitor of cell growth versus L1210 and MCF-7 cells than CAPE. In the dihydrocaffeic acid ester series, *n*-octyldihydrocaffeate maintains its cytotoxic superiority over the corresponding hydrogenated CAPE analogue.

Experimental

General

All reagents and solvents were used as received, unless otherwise indicated. Caffeic acid (3,4-dihydroxycinnamic acid, 97%), dihydrocaffeic acid (3-(3,4-dihydroxyphenyl)-propanoic acid, 98%), and *tert*-Butyl bromoacetate (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Phenethyl caffeate

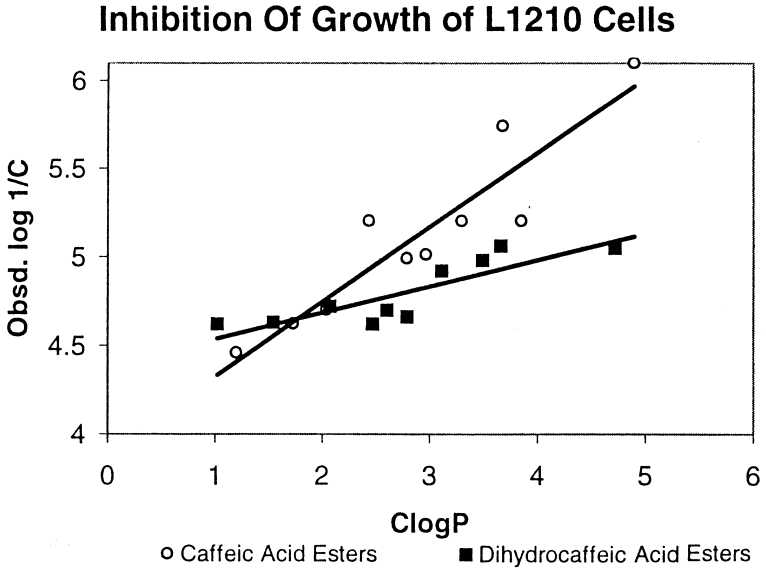

Figure 2. Cytotoxicity versus hydrophobicity of caffeic and dihydrocaffeic esters.

(Phenethyl-3,4-dihydroxycinnamate, CAPE) used for cell culture testing was purchased from LKT Laboratories, Inc. (St. Paul, MN). *tert*-Butoxycarbonylmethyl triphenylphosphonium chloride (98 + %) was purchased from Lancaster Chemical Company. Diethyl ether and hexanes (or petroleum ether) used for recrystallization were dried over CaH_2 and distilled before use. Column chromatography was carried out using 70–230 mesh silica gel (Aldrich). Silica gel IB TLC plates (Baker, precoated, plastic-backed) were used for thin layer chromatography. Melting points were determined on a Mel-Temp melting point apparatus with digital thermometer and are uncorrected. Elemental analyses (C, H) were conducted by Desert Analytics (Tucson, AZ). The acceptable range was ±0.4% of calculated values. Mass spectral analyses were performed by the UCR Mass Spectrometry Facility (University of California, Riverside). Proton (^{1}H) and ^{13}C FT-NMR spectra were obtained using either a General Electric QE-300 (300 MHz) or a Bruker Spectrospin 400 MHz spectrometer. NMR samples were prepared using deuterated acetone (acetone-d_6) or deuterated chloroform ($CDCl_3$) purchased from Aldrich Chemical Co. Either the protonated solvent impurity or TMS was used as the spectral reference peak. FT-IR spectra were obtained using a Perkin Elmer 1600 Series spectrophotometer. FT-IR samples were prepared as pressed KBr wafers or as thin films on single or between two NaCl plates.

Method 1: Fisher esterification of caffeic acid[29]

Synthesis of methyl-3,4-dihydroxycinnamate (methyl caffeate, 1a). Caffeic acid (1.8 g, 9.9 mmol), *p*-toluenesulfonic acid (PTSA, 0.10 g, 0.5 mmol), methyl alcohol (50 mL), and benzene (50 mL), were placed in a 250 mL 3-neck round bottom flask equipped with a magnetic stirring bar and a Dean–Stark trap. The mixture was stirred, heated to reflux, and monitored by thin layer chromatography (diethyl ether eluent; versus caffeic acid). As heating continued, the Dean–Stark trap was periodically emptied. The level of solvent in the reaction flask decreased gradually, so more methyl alcohol and benzene were added to maintain an azeotropic mixture. More PTSA was added (approx. 0.1 to 0.2 g) to the reaction mixture at 8–16 h intervals. The reaction was considered complete by TLC after about 48 h of refluxing. The reaction mixture was poured into a separatory funnel containing approximately 200 mL of distilled water. Diethyl ether (50–75 mL) was added and the mixture was shaken to extract the organic components. The organic layer was washed with distilled water (2–3×, 50–75 mL), 5% $NaHCO_3$ (2–3×, 50–75 mL), and saturated aqueous NaCl (1–2×,50–75 mL). The resulting yellow ether solution was dried over anhydrous $MgSO_4$, filtered, and concentrated on a rotary evaporator to leave a light to dark yellow solid residue (0.9 g, 47% crude yield). The crude solid was further purified by treating a diethyl ether solution of the solid with decolorizing carbon and filtering the recovered solution through a silica gel column (2×, diethyl ether eluent). For some uses, purification to this extent was sufficient. For cell culture and elemental analysis samples, recrystallization from diethyl ether and hexanes (or petroleum ether) was also carried out and repeated until the desired degree of purity was achieved. Pure (based on melting point, TLC, and proton NMR data) methyl ester was dried in an Aberhalden drying pistol (refluxing methanol, about 6 h) and submitted for elemental analysis and cell culture testing. Yield (pure) data reflects the amount of recovered product deemed pure enough for analytical testing. The final product was recovered as a white to very pale yellow, crystalline solid. Yield (pure): 0.26 g (14%). Melting point 161–163 °C. ^{1}H NMR (acetone-d_6) δ 7.52 (d, J = 16 Hz, 1H), 7.15 (d, J = 2 Hz, 1H), 7.04, 7.01 (dd, J = 2.0, 2.1, 8.2 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 6.27 (d, J = 15.9 Hz, 1H), 3.70 (s, 3H); ^{13}C NMR (acetone-d_6) δ 167.9, 148.6, 146.1, 145.7, 127.5, 122.5, 116.3, 115.2, 115.1, 115.1, 51.5. FT-IR (thin film, single NaCl plate) cm^{-1}: 3495, 3312, 1684, 1635, 1602. Anal. calcd ($C_{10}H_{10}O_4$): 61.86 %C, 5.20 %H; Found: 61.77 %C, 5.22 %H.

Synthesis of ethyl-3,4-dihydroxycinnamate (ethyl caffeate, 1b). Similar to the procedure for **1a**, except ethyl alcohol was used. The final product was recovered as a light-tan solid. Percent yield is based on 3.6 g (19.9 mmol) of caffeic acid starting material. Yield (pure): 0.56 g (13%). Melting point: 149–151 °C. ^{1}H NMR (acetone-d_6) δ 7.52 (d, J = 15.9 Hz, 1H), 7.15 (d, J = 1.8 Hz, 1H), 7.04, 7.01 (dd, J = 1.8, 1.9 Hz; 8.1 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 6.26 (d, J = 15.9 Hz, 1H), 4.17 (quartet, 2H), 1.26 (t, 3H); ^{13}C NMR (acetone-d_6) δ 167.5, 148.5, 146.1, 145.5, 127.5, 122.4, 116.3, 115.6, 115.1, 60.5, 14.6. FT-IR (KBr) cm^{-1}: 3442, 3249, 1670, 1598. Anal. calcd ($C_{11}H_{12}O_4$): 63.46 %C, 5.82 %H; Found: 63.32 %C, 5.99 %H.

Synthesis of *n*-butyl-3,4-dihydroxycinnamate (*n*-butyl caffeate, 1c). Similar to the procedure for **1a,** except *n*-butyl alcohol was used. Excess *n*-butyl alcohol and benzene were removed from the crude reaction mixture by steam distillation (insitu, atmospheric pressure). Subsequent work up and purification methods were similar to those for **1a**. The final product was recovered as a light-tan, crystalline solid. Percent yield is based on 3.6 g (19.9 mmol) of caffeic acid starting material. Yield (pure): 1.1 g (23%). Melting point 110–111 °C. ^{1}H NMR (acetone-d_6 = 2.04 ppm) δ 8.33 (broad singlet, 2H), 7.53 (d, J = 15.9 Hz, 1H), 7.16 (s, 1H), 7.03 (d, 8.1 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.28 (d, J = 15.9 Hz, 1H), 4.13 (t, 2H), 1.63 (quintet, 2H), 1.40 (sextet, 2H), 0.92 (t, 3H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 167.5, 148.6, 146.2, 145.5, 127.5, 122.4, 116.3, 115.6, 115.1, 64.3, 31.5, 19.8, 14.0. FT-IR (thin film, single NaCl plate) cm^{-1} 3486, 3342, 2952, 2868, 1683, 1638, 1602, 1536. Anal. calcd ($C_{13}H_{16}O_4$): 66.09 %C, 6.84 %H; Found: 66.07 %C, 6.90 %H.

Synthesis of *n*-hexyl-3,4-dihydroxycinnamate (*n*-hexyl caffeate, 1d). Similar to the procedure for **1c**, except *n*-hexyl alcohol (30 mL/3.6 g of caffeic acid) was used. The final product was recovered as a white to very pale-yellow, finely crystalline solid. Percent yield is based on 3.6 g (19.9 mmol) of caffeic acid starting material. Yield (pure): 0.33 g (6%). Melting point 128–129 °C. ^{1}H NMR (acetone-d_6) δ 8.33 (broad singlet, 2H), 7.53 (d, J = 15.9,

1H), 7.15 (d, $J=2.0$ Hz, 1H), 7.04, 7.02 (dd, $J=2.1$, 8.2 Hz, 1H), 6.86 (d, $J=8.2$ Hz, 1H), 6.27 (d, $J=15.9$ Hz, 1H), 4.13 (t, $J=6.7$ Hz, 2H), 1.66 (quintet, 2H), 1.34 (mult., 6H), 0.88 (t, $J=6.8$, 6.7; 3H); ^{13}C NMR (acetone-$d_6=29.8$ ppm) δ 67.5, 148.6, 146.2, 145.5, 127.5, 122.4, 116.3, 115.6, 115.1, 64.6, 32.1, 29.4, 26.3, 23.1, 14.2. FT-IR (thin film, single NaCl plate) cm^{-1} 3491, 3335, 2854, 1686, 1639, 1602, 1535. Anal. calcd ($C_{15}H_{20}O_4$): 68.16 %C, 7.64%H; Found: 68.27 %C, 7.69%H.

Synthesis of isopropyl-3,4-dihydroxycinnamate (isopropyl caffeate, 1f). Similar to the procedure for **1a**, except isopropyl alcohol was used. The reaction appeared complete by TLC after about six days of refluxing. work up and purification methods were similar to those for **1a**. The final product was recovered as an off-white, crystalline solid. Percent yield is based on 1.8 g (9.9 mmol) of caffeic acid starting material. Yield (pure) 0.50 g (23%). Melting point 145–147 °C. ^{1}H NMR (acetone-d_6) δ 8.4 (broad singlet, 2H), 7.51 (d, $J=15.9$ Hz, 1H), 7.15 (d, $J=1.8$ Hz, 1H), 7.03, 7.01 (dd, $J=1.7$, 8.1 Hz, 1H), 6.85 (d, $J=8.1$ Hz, 1H), 6.23 (d, $J=15.9$ Hz, 1H), 5.04 (septet, 1H), 1.24 (d, 6H); ^{13}C NMR (acetone-d_6) δ 167.0, 148.5, 146.1, 145.3, 127.5, 122.4, 116.2, 116.1, 115.0, 67.7, 22.1. FT-IR (KBr) cm^{-1} 3466, 1679, 1633, 1601, 1534. Anal. calcd ($C_{12}H_{14}O_4$): 64.85 %C, 6.36%H; Found: 64.81 %C, 6.28 %H.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, methyl ester (methyl dihydrocaffeate, 2a). Similar to the procedure for **1a**, except dihydrocaffeic acid (1 g, 5.5 mmol) and 25 mL each of methyl alcohol and benzene were used. During work up, the aqueous layer was extracted two additional times with 50 mL of diethyl ether. Ether extracts were combined, and work up continued essentially as described for **1a**. After column chromatography and recrystallization, pure **2a** was recovered as a white to very-pale yellow, crystalline solid. Yield (pure): 0.28 g (26%). Melting point 73–75 °C. ^{1}H NMR ($CDCl_3=7.26$ ppm) δ 6.77 (d, $J=8.1$ Hz, 1H), 6.71 (d, $J=2.0$ Hz, 1H), 6.62, 6.60 (dd, $J=2.0$, 8.1 Hz, 1H), 5.73 broad singlet, 2H), 3.67 (s, 3H), 2.83 (t, $J=7.5$, 7.8 Hz, 2H), 2.59 (t, $J=7.8$, 7.5 Hz, 2H); ^{13}C NMR (acetone-$d_6=29.8$ ppm) δ 173.6, 145.6, 144.0, 133.2, 120.2, 116.1, 115.9, 51.5, 36.4, 30.8. FT-IR (KBr) cm^{-1} 3485, 3312, 2947, 1712, 1608, 1517. Anal. calcd ($C_{10}H_{12}O_4$): 61.22 %C, 6.18%H; Found: 61.50 %C, 6.13 %H.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, ethyl ester (ethyl dihydrocaffeate, 2b). Similar to the procedure for **1a**, except dihydrocaffeic acid (1 g, 5.5 mmol), 50 mL of ethyl alcohol, and 25 mL of benzene were used for the Fisher esterification reaction. Workup was similar to that described for **2a**. An oily product was recovered. The products of two separate reactions (5.5 mmol of dihydrocaffeic acid starting material each) were purified in combination. Purification to obtain analytical samples involved decolorization of a diethyl ether solution of the combined reaction products, two rounds of column chromatography (first round: 100% $CHCl_3$ followed by 2:1 diethyl ether/petroleum ether; second round: 4:1 $CHCl_3$/ethyl acetate), and Kugelrohr distillation (80–85 °C, 2 h) to remove any volatile impurities. A pale, champagne colored oil was recovered. Percent yield is based the sum of the theoretical yields of the combined reaction products (11 mmol). Yield (pure): 0.88 g (38%). ^{1}H NMR ($CDCl_3=7.26$ ppm) δ 6.75 (d, $J=8.1$ Hz, 1H), 6.69 (d, $J=1.5$ Hz, 1H), 6.59, 6.57 (dd, $J=1.4$, 8.1 Hz, 1H), 6.22 (broad singlet, 2H), 4.13 (quartet, 2H), 2.81 (t, $J=7.6$, 7.7 Hz; 2H), 2.58 (t, $J=7.7$, 7.6 Hz; 2H), 1.23 (t, 3H); ^{13}C NMR ($CDCl_3=77.0$ ppm) δ 174.3, 143.7, 142.1, 132.8, 120.2, 115.4 (2 overlapped peaks), 60.9, 36.0, 30.1, 13.9. FT-IR (neat, thin film between two NaCl plates) cm^{-1} 3368, 2981, 1706, 1606, 1519. Exact Mass Data (Electron Impact, 20 eV): For ($C_{11}H_{14}O_4$): calcd M^+: 210.089209; Found M^+: 210.089015.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, *n*-Butyl ester (*n*-Butyl dihydrocaffeate, 2c). Similar to the procedure for **1c**, except dihydrocaffeic acid (1 g, 5.5 mmol) was used and 0.3–0.5 g of PTSA were used initially, with no more added later. Oily products from two separate reactions (1 g, 5.5 mmol starting material each) were recovered and combined for purification. Purification to generate analytical samples consisted of two rounds of column chromatography (first round: 100% $CHCl_3$, followed by 2:1 diethyl ether/petroleum ether; second round: 4:1 $CHCl_3$/ethyl acetate). Kugelrohr distillation (80–85 °C, 2 h) to remove volatile impurities followed. A pale-orange oil was recovered. Yield (pure): 0.45 grams (17%). ^{1}H NMR ($CDCl_3=7.26$ ppm) δ 6.75 (d, $J=8.1$ Hz, 1H), 6.69 (d, $J=1.9$ Hz, 1H), 6.60, 6.58 (dd, $J=1.9$, 8.1 Hz, 1H), 6.15 (s, 1H), 5.99 (s, 1H), 4.07 (t, 2H), 2.82 (t, $J=7.5$, 7.8 Hz; 2H), 2.59 (t, $J=7.8$, 7.5 Hz, 2H), 1.58 (quintet, 2H), 1.33 (sextet, 2H), 0.91 (t, 3H); ^{13}C NMR ($CDCl_3=77.0$ ppm) δ 174.4, 143.6, 142.1, 132.7, 120.1, 115.4 (two overlapping peaks), 64.7, 36.0, 30.3, 30.0, 18.8, 13.4. FT-IR (neat, thin film between two NaCl plates) cm^{-1} 3390, 2960, 2935, 1707, 1607, 1519. Anal. calcd ($C_{13}H_{18}O_4$): 65.53 %C, 7.63 %H; Found: 65.15 %C, 7.71 %H. Exact Mass Data (Electron Impact, 20 eV): For ($C_{13}H_{18}O_4$): calcd M^+: 238.120509; Found M^+: 238.120999.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, *n*-octyl ester (*n*-octyl dihydrocaffeate, 2e). Similar to the procedure for **2c** except *n*-octyl alcohol (20 mL) was used. Excess *n*-octyl alcohol and benzene were removed by azeotropic steam distillation. Crystalline product recovered after workup was recrystallized one time (diethyl ether/hexanes) to yield product pure enough for analytical samples. Compound **2e** was recovered as a champagne colored, crystalline solid. Percent yield is based on 5.5 mmol of dihydrocaffeic acid starting material. Yield (pure): 0.89 g (55%). Melting point 56–58 °C. ^{1}H NMR ($CDCl_3=7.26$ ppm) δ 6.76 (d, $J=8.1$ Hz, 1H), 6.71 (d, $J=2.0$ Hz, 1H), 6.63, 6.61 (dd, $J=2.0$, 8.1 Hz, 1H), 5.53 (s, 1H, OH), 5.37 (s, 1H, OH), 4.06 (t, 2H), 2.83 (t, $J=7.5$, 7.8 Hz, 2H), 2.58 (t, $J=7.9$, 7.5 Hz, 2H), 1.59 (quintet, 2H), 1.32–1.23 (broad mult., 10H), 0.88 (t, 3H); ^{13}C NMR ($CDCl_3=77.0$ ppm) δ 174.5, 143.7, 142.2, 132.9, 120.3, 115.3 (two overlapping peaks), 65.2, 36.2, 31.7, 30.2, 29.1, 29.09, 28.4, 25.8, 22.6, 14.0. FT-IR

(KBr) cm^{-1} 3509, 3390, 2926, 2849, 1710, 1608, 1537. Anal. calcd ($C_{17}H_{26}O_4$): 69.35 %C, 8.92%H; Found: 69.35 %C, 9.16 %H.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, isopropyl ester (isopropyl dihydrocaffeate, 2f). Procedure was similar to that described for **2a**, except that isopropyl alcohol was used. The products of two separate reactions (5.5 mmol starting material each) were combined for purification by column chromatography (2:1 diethyl ether/petroleum ether). The recovered oil was subjected to Kugelrohr distillation (80–85 °C, 2 h) to remove volatile impurities. The final product was recovered as a pale brown oil. Percent yield is based on the theoretical yields of the combined reaction products (2×5.5 mmol = 11 mmol theoretical yield). Yield (pure): 1.2 g (48%). ^{1}H NMR ($CDCl_3$ = 7.26 ppm) δ 6.75 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 1.6 Hz, 1H), 6.60, 6.58 (dd, J = 1.7, 8.1 Hz, 1H), 6.23 (s, 1H, OH), 6.08 (s, 1H, OH), 5.00 (septet, J = 6.2 Hz, 1H), 2.81 (t, J = 7.5, 7.7 Hz, 2H), 2.56 (t, J = 7.7, 7.5 Hz, 2H), 1.21 (d, J = 6.2 Hz, 6H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 173.9, 143.7, 142.2, 132.7, 120.2, 115.4, 115.3, 68.4, 36.4, 30.2, 21.6. FT-IR (neat, thin film between two NaCl plates) cm^{-1} 3380, 2981, 2935, 1703, 1606, 1520. Exact Mass Data (Electron Impact, 20 eV): M^+ for ($C_{12}H_{16}O_4$): calcd 224.104859; Found: 224.104339.

Method 2: Alkylation of the caffeic acid carboxylate salt[30]

Synthesis of *n*-octyl-3,4-dihydroxycinnamate (*n*-octyl caffeate, 1e). Caffeic acid (1.0 g, 5.5 mmol) was dissolved in 40–50 mL of *N*,*N*-Dimethylformamide (DMF). A dark-brown solution resulted. 1.14 mL of aqueous NaOH (2.8 g NaOH in 10 mL of H_2O) was added slowly. The mixture was stirred for 2.5 h after which, a precipitate was observed. A solution of 1-bromooctane (3.4 mL, 24 mmol) in DMF was added dropwise. The mixture was allowed to stir for 7 days. Work up consisted of pouring the reaction mixture into ice water (1200 mL, distilled), extracting the aqueous mixture with diethyl ether (until extract had very little color), and washing the ether extract successively with 1 N HCl (3×150 mL), H_2O (3×150 mL), and brine (2×75 mL). The extract was dried over anhydrous $MgSO_4$, filtered, and concentrated on a rotary evaporator. When oily residues were obtained after concentration, trituration with petroleum ether or hexanes yielded a solid product. Purification proceeded similarly to that described for compound **1a**, except additional column chromatography (2:1 diethyl ether/hexanes) and recrystallization (diethyl ether/hexanes) steps were necessary. Pure *n*-octyl caffeate was recovered as a canary yellow, crystalline solid. Percent yield is based on the sum of the theoretical yields of two individual reactions (5.5 mmol + 11 mmol = 16.5 mmol total theoretical yield) that were combined for late stage purification. Yield (pure): 0.32 g (7%), melting point 111–112 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) 8.39, (broad singlet, 2H), 7.54 (d, J = 15.9 Hz, 1H), 7.17 (d, J = 2.0 Hz, 1H), 7.06, 7.04 (dd, J = 2.0 Hz, 8.1 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.29 (d, J = 15.9 Hz, 1H), 4.14 (t, 2H), 1.68 (quintet, 2H), 1.34 (mult., 10H), 0.89 (t, 3H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 167.4, 148.6, 146.2, 145.5, 127.8, 127.5, 122.4, 116.2, 115.6, 115.0, 64.6, 32.5, 29.92, 29.89, 29.5, 26.6, 23.2, 14.3. FT-IR (KBr) cm^{-1} 3489, 3316, 2919, 2854, 1684, 1604, 1533. Anal. calcd ($C_{17}H_{24}O_4$): 69.83 %C, 8.29%H; Found 69.78 %C, 8.16 %H.

Synthesis of benzyl-3,4-dihydroxycinnamate (benzyl caffeate, 1g). The procedure is similar to that described for **1e**, except that benzyl bromide was used. Purification involved an initial decolorization (carbon) and round of chromatography (100% diethyl ether) followed by three separate recrystallizations (diethyl ether/hexanes). The recrystallization product was chromatographed again (2:1 diethyl ether/petroleum ether) and recrystallized a final time (diethyl ether/petroleum ether). Pure **1g** was recovered as a fluffy, white to pale-yellow solid. Percent yield is based on 5.5 mmol of caffeic acid starting material. Yield (pure): 0.05 g (3%). Melting point 151–154 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) δ 8.49 (broad s, 2H), 7.59 (d, 1H, J = 15.9 Hz), 7.47–7.30 (m, 5H), 7.18 (d, J = 2.0 Hz, 1H,), 7.07, 7.05 (dd, J = 2.1, 8.2 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.34 (d, J = 16.0 Hz, 1H), 5.22 (s, 2H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 167.3, 148.8, 146.1, 146.05, 137.7, 129.2 (two equivalent carbon atoms), 128.9 (two equivalent carbon atoms), 128.7, 127.5, 122.6, 116.3, 115.3, 115.1, 66.2. FT-IR (KBr) cm^{-1} 3465, 1688, 1636, 1601, 1533. Anal. calcd ($C_{16}H_{14}O_4$): 71.10 %C, 5.23 %H; Found 71.23 %C, 5.10 %H.

Synthesis of phenethyl-3,4-dihydroxycinnamate (phenethyl caffeate, CAPE, 1h). This compound was synthesized to serve as the hydrogenation substrate for formation of the saturated side chain analogue. The procedure was similar to that for **1e,** except 2.0 g (11 mmol) of caffeic acid and 150 mL of DMF (reagent grade) were used. 2.3 mL of aq NaOH (2.7 g NaOH in 10 mL H_2O) was added. This mixture was allowed to stir for 2.5 h. A solution of 5.6 mL (41 mmol) 1-bromo-2-phenylethane in 10 mL of DMF was added dropwise to the stirring mixture. Stirring was continued for 7 days. Workup was essentially the same as done for **1e** except the reaction mixture was initially poured into 1600 mL of distilled water. Purification consisted of treating a diethyl ether solution of the crude compound with decolorizing carbon, followed by filtration through a short, small diameter silica gel column (Et_2O eluant). Compound **1h** was recovered as a tan to off-white solid after initial purification and judged pure enough to use for hydrogenation reactions. Yield: 1.45 g (46%), melting point 116–123 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) δ 8.39 (broad s, 2H), 7.56 (d, J = 15.9 Hz, 1H), 7.30 (d, 3H), 7.22 (t, 2H), 7.18 (d, J = 1.9 Hz, 1H), 7.05, 7.03 (dd J = 1.9, 8.2 Hz, 1H), 6.89 (d, J = 8.2 Hz, 1H), 6.29 (d, J = 15.9 Hz, 1H), 4.36 (t, J = 7.0 Hz, 2H), 2.99 (t, J = 7.0 Hz, 2H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 167.3, 148.6, 146.1, 145.7, 139.1, 129.7 (two equivalent carbon atoms), 129.1 (two equivalent carbon atoms), 127.5, 127.1, 122.5, 116.3, 115.4, 115.1, 65.3, 35.7. FT-IR (KBr) cm^{-1} 3480, 3327, 1684, 1636, 1602.

Synthesis of 3-phenylpropyl-3,4-dihydroxycinnamate (3-phenylpropyl caffeate, 1i). Procedure was similar to that for **1e** except that 4.0 g (22 mmol) of caffeic acid were used. Other reagents were scaled up accordingly. Only a portion of the initially purified (decolorization (carbon), column chromatography (100% diethyl ether)) reaction product was carried through a final purification process (column chromatography (1:1 diethyl ether/hexanes), second decolorization, recrystallization (diethyl ether/hexanes). The final product was recovered as a white to very pale yellow, finely crystalline solid. Yield (pure): 0.63 g (10%, based on 22 mmol of caffeic acid starting material). More was available in a less pure form. Melting point 123–124 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) δ 8.39 (broad s, 2H), 7.55 (d, J = 15.9 Hz, 1H) 7.31–7.19 (m, 5H) 7.17 (d, J = 2.1 Hz, 1H), 7.07, 7.05 (dd, J = 2.1, 8.2 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 6.31 (d, J = 16.0 Hz, 1H), 4.16 (t, 2H), 2.75 (t, 2H), 2.01 (quintet, 2H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 167.4, 148.6, 146.1, 145.6, 142.3, 129.1, 127.5, 126.6, 122.5, 116.3, 115.6, 115.1, 64.0, 32.7, 31.1. FT-IR (KBr) cm^{-1} 3488, 1684, 1637, 1602. Anal. calcd ($C_{18}H_{18}O_4$): 72.47 %C, 6.09 %H; Found 72.48 %C, 6.06 %H.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, benzyl ester (benzyl dihydrocaffeate, 2g). Similar to the procedure for **1e**, except that 0.8 g (4.4 mmol) of dihydrocaffeic acid, 0.91 mL of aq NaOH (approx. 22% w/w) and benzyl bromide (1.05 mL, 8.8 mmol) were used. A smaller excess of benzyl bromide was used to facilitate easier removal of unreacted reagent, while still driving the reaction forward. After about 2.5 days of stirring, TLC (diethyl ether eluant; versus benzyl bromide and dihydrocaffeic acid) indicated the reaction to be reasonably complete. Workup was similar to that for **1e**. The oily reaction product was chromatographed (100% $CHCl_3$ followed by 100% diethyl ether) and fractions containing a faster moving component (likely benzyl bromide) were discarded. After a second round of column chromatography (2:1 diethyl ether/petroleum ether), the product was combined with the product of a second reaction. The combination was chromatographed again (4:1 $CHCl_3$/ethyl acetate; gravity elution). TLC of the combined fractions still showed the presence of a slightly faster moving fluorescent component. Nevertheless, the oily, orange product was subjected to Kugelrohr distillation (80–85 °C, 2 h) to remove any volatile impurities and samples were prepared for cell culture analysis, mass spectrometry, and elemental analysis. The percent yield is based on the sum of the theoretical yields of the combined reaction products (4.4 mmol + 5.5 mmol = 9.9 mmol). Yield (pure) 0.91g (34%). ^{1}H NMR ($CDCl_3$ = 7.26 ppm) δ 7.4–7.3 (mult., 5H), 6.74 (d, J = 8.1 Hz, 1H), 6.65 (d, J = 1.9 Hz, 1H), 6.60, 6.58 (dd, J = 1.9, 8.1 Hz, 1H), 5.57 (s, 1H, OH), 5.48 (s, 1H, OH), 5.11 (s, 2H), 2.85 (t, J = 7.5, 7.7 Hz, 2H), 2.64 (t, J = 7.7, 7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 173.9, 143.6, 142.2, 135.4, 132.8, 128.5, 128.2 (two equivalent carbon atoms), 128.1 (two equivalent carbon atoms), 120.4, 115.4 (two overlapping signals), 66.6, 36.0, 30.1. FT-IR (neat, thin film on one NaCl plate) cm^{-1}: 3394, 3033, 2953, 1710, 1606, 1519. Anal. calcd ($C_{16}H_{16}O_4$) 70.57 %C, 5.94 %H; Found: 70.71 %C, 5.86%H. Exact Mass Data (Electron Impact, 20 eV): M^+ for ($C_{16}H_{16}O_4$): Calcd: 272.104859; Found: 272.104524.

Method 3: Hydrogenation of the unsaturated analogue[29]

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, *n*-Hexyl ester (*n*-hexyl dihydrocaffeate, 2d). One gram (3.8 mmol) of *n*-hexyl caffeate (**1d**) was combined with 0.5 g of 10% Pd/C and absolute ethanol (approx. 125 mL). Using a Parr hydrogenator, the mixture was placed under a hydrogen atmosphere (40 psi) and shaken for about 3 h. The mixture was filtered through Celite, concentrated, and analyzed by proton NMR, which revealed that conversion was complete. The residue was taken up in acetone and filtered with air pressure through a short silica gel column to remove traces of catalyst. Upon rotary evaporation, the product solidified to become a soft green-grey residue. The solid was dissolved and the solution treated with decolorizing carbon. Concentration, followed by trituration with hexanes yielded a yellow solid. An initial recrystallization (diethyl ether/hexanes) was followed by an additional decolorization step (carbon) and column chromatography (1:1 diethyl ether/hexanes). A final recrystallization (diethyl ether/hexanes) resulted in product pure enough for analytical samples. *n*-Hexyl dihydrocaffeate (**2d**) was recovered as a white crystalline solid. Yield (pure) 0.23 g (23%). Melting point 67–68 °C. ^{1}H NMR ($CDCl_3$ = 7.26 ppm) δ 6.76 (d, J = 8.1 Hz, 1H), 6.70 (d, J = 1.9 Hz, 1H), 6.62, 6.60 (dd, J = 1.9, 8.1 Hz, 1H), 4.06 (t, 2H), 2.83 (t, J = 7.5, 7.8 Hz, 2H), 2.58 (t, J = 7.9, 7.5 Hz, 2H), 1.59 (quartet, 2H), 1.34–1.26 (multiplet, 6H), 0.88 (t, 2H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 174.4, 143.7, 142.2, 132.9, 120.4, 115.3 (two overlapping peaks), 65.2, 36.2, 31.3, 30.3, 28.4, 25.5, 22.4,13.9. FT-IR (KBr) cm^{-1} 3507, 2955, 1708, 1608, 1540. Anal. calcd ($C_{15}H_{22}O_4$): 67.64 %C, 8.34 %H; Found: 68.03 %C, 8.71 %H.

Compound **2d** was also successfully prepared by Fisher esterification of dihydrocaffeic acid with *n*-hexyl alcohol.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, *t*-butyl ester (*t*-butyl dihydrocaffeate, 2j). *t*-Butyl caffeate (**1j**) (0.72 g, 3 mmol) was combined with 10% Pd/C catalyst (0.39 g) and absolute ethanol (125 mL). Parr hydrogenation (40 psi, 6 h) followed. The mixture was filtered through Celite and solvent removed. The oily residue was chromatographed twice (first round: 3:1 diethyl ether/petroleum ether; second round: 4:1 $CHCl_3$/ethyl acetate), followed by Kugelrohr distillation (80–85 °C, 2 h) to remove volatile impurities. A pale orange oil was recovered. Yield (pure): 0.19 g (26%). ^{1}H NMR ($CDCl_3$ = 7.26 ppm) δ 6.75 (d, J = 8.1 Hz, 1H), 6.69 (d, J = 1.9 Hz, 1H), 6.61, 6.59 (dd, J = 1.9, 8.1 Hz, 1H), 5.90 (s, 1H), 5.70 (s, 1H), 2.78 (t, J = 7.5, 7.8 Hz, 2H), 2.50 (t, J = 7.9, 7.5 Hz, 2H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 173.7, 143.7, 142.2, 132.9, 120.2, 115.4, 115.2, 81.1, 37.3, 30.4, 27.9. FT-IR (neat, thin

film between two NaCl plates) cm^{-1} 3390, 2978, 2932, 1699, 1606, 1520. Anal. calcd ($C_{13}H_{18}O_4$) 65.53 %C, 7.63 %H; Found: 65.28 %C, 7.69 %H. Exact Mass Data (Electron Impact, 20 eV): M^+ for ($C_{13}H_{18}O_4$): Calcd: 238.120509; Found: 238.119568.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, phenethyl ester (phenethyl dihydrocaffeate, 2h). Similar to the procedure for **2d**, except 2.0 g (7 mmol) of phenethyl caffeate, 0.7 g of 10% Pd/C catalyst, and 100 mL of absolute ethanol were combined. Parr hydrogenation (40 psi, 4 h) followed. After filtering the ethanol mixture through Celite, the solvent was removed and the residue taken up in acetone. This mixture was filtered with air pressure through silica gel to remove traces of catalyst. The oily reaction product was chromatographed two times (first round: 2:1 diethyl ether/petroleum ether; second round: 100% $CHCl_3$, followed by 100% diethyl ether or 2:1 diethyl ether/petroleum ether). Upon standing, the combined fractions solidified. Recrystallization (diethyl ether/petroleum ether) led to recovery of a white to pale champagne colored, crystalline solid. Yield (pure): 0.5 g (25%), melting point 73–74 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) δ 7.74 (broad singlet, 2H), 7.31–7.19 (multiplet, 5H), 6.72 (d (upfield half of doublet overlapped by down field half of 6.70 ppm doublet), J = 8.1 Hz, 1H), 6.70 (d, J = 1.9 Hz, 1H), 6.53, 6.51 (dd, J = 2.0, 8.0 Hz, 1H), 4.24 (t, J = 7.0 Hz, 2H), 2.90 (t, J = 7.0 Hz, 2H), 2.74 (t, J = 7.5, 7.7 Hz, 2H), 2.52 (t,J = 7.8, 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 174.1, 143.6, 142.1, 137.5, 132.9, 128.8, 128.5, 126.5, 120.4, 115.3 (two overlapping peaks), 65.3, 36.1, 34.8, 30.1. FT-IR (KBr) cm^{-1} 3330, 3027, 2958, 1707, 1604, 1524. Anal. calcd ($C_{17}H_{18}O_4$): 71.31 %C, 6.35 %H; Found: 71.41 %C, 6.52 %H.

Synthesis of 3-(3,4-dihydroxyphenyl)propanoic acid, 3-phenylpropyl ester (3-phenylpropyl dihydrocaffeate, 2i). One-half gram (1.7 mmol) of 3-phenylpropyl caffeate (**1i**) was combined with 0.3 g of 10% Pd/C and 120 mL of absolute ethanol. Parr hydrogenation (40 psi, 4.5 h) followed. The mixture was filtered two times (through Celite as an ethanol solution, then through silica gel as an acetone solution) to remove excess catalyst, then chromatographed (2:1 diethyl ether/petroleum ether). Crystals evolved from the combined fractions after adding more petroleum ether and cooling. A second recrystallization (diethyl ether/petroleum ether) resulted in a white to pale-yellow crystalline product. Yield (pure): 0.11 g (22%), melting point 69–71 °C. ^{1}H NMR (acetone-d_6 = 2.04 ppm) δ 7.74 (broad singlet, 2H), 7.28–7.14 (multiplet, 5H), 6.709 (d, J = 8.0 Hz, 1H), 6.71 (d, J = 2 Hz, 1H), 6.56, 6.54 (dd, J = 2.0, 8.0 Hz, 1H), 4.02 (t, 2H), 2.76 (t, J = 7.5, 7.7 Hz, 2H), 2.64 (t, 2H), 2.54 (t, J = 7.7, 7.5 Hz, 2H), 1.89 (quintet, 2H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 174.3, 143.7, 142.1, 140.9, 132.9, 128.3 (two overlapping peaks representing two sets of equivalent carbon atoms), 125.9, 120.4, 115.3 (two overlapping peaks) 64.2, 36.1, 31.9, 30.2, 29.9. FT-IR (KBr): cm^{-1} 3496, 3284, 2937, 1701, 1611. Anal. calcd ($C_{18}H_{20}O_4$) 71.98 %C, 6.73 %H; Found: 72.26 %C, 6.71 %H.

Method 4: Wittig Reaction[31a]

Synthesis of *tert*-butoxycarbonylmethyl triphenylphosphonium bromide (3).[31a,b,c] Triphenylphosphine (6.0 g, 23 mmol) was dissolved in benzene or toluene (10–20 mL). *tert*-Butyl bromoacetate (3.8 mL, 26 mmol) was added to the solution. The mixture was swirled well and almost immediately became cloudy. White precipitate formed within 10 min. The mixture was allowed to stand in the refrigerator for at least 24 h before collecting the precipitate. The solid was filtered, washed with benzene or toluene, then with hexanes, and allowed to dry over vacuum. The product was not purified further but used directly for Wittig reactions. The product was stored in the refrigerator. Yield: 10.2 g (97%), melting point 169–173 °C, dec. ^{1}H NMR ($CDCl_3$ = 7.26 ppm) 8.00–7.60 (mult. 15H), 5.32 (d, J = 14 Hz, 2H), 1.19 (s, 9H). FT-IR (KBr) cm^{-1} 2876, 1721, 1586, 1436, 1273, 1138, 1112, 757.

Synthesis of *t*-butyl-3,4-dihydroxycinnamate (*t*-butyl caffeate, 1j).[31a] Although the above reference utilizes sonication as the main energy source for the Wittig reaction, preliminary reactions during this study showed that refluxing was indeed more convenient, if not just as effective. 3,4-Dihydroxybenzaldehyde (1.0 g, 7.2 mmol) was dissolved in distilled 1,4-dioxane (20 mL). 4.5 grams of Wittig reagent **3** (9.8 mmol) was dissolved in reagent grade $CHCl_3$ (20 mL). The solutions were added to a flask containing 2.10 g (21 mmol) of $KHCO_3$ and a stir bar. A reflux condenser was attached, and the mixture was heated to reflux temperature. The resulting mixture was filtered and concentrated on a rotary evaporator. Column chromatography (2:1 ethyl acetate/petroleum ether) and recrystallization (diethyl ether/petroleum ether) led to recovery of a champagne colored, crystalline solid. Yield (pure): 0.30 g (18%), melting point 130–134 °C. ^{1}H NMR (acetone-d_6, TMS = 0.00 ppm) δ 8.38 (broad singlet, 2H), 7.46 (d, J = 15.9 Hz, 1H), 7.14 (d, J = 2.1 Hz, 1H), 7.03, 7.01 (dd, J = 2.0, 8.2 Hz, 1H), 6.86 (d, J = 8.2 Hz, 1H), 6.20 (d, J = 15.8 Hz, 1H), 1.50 (s, 9H); ^{13}C NMR (acetone-d_6 = 29.8 ppm) δ 166.8, 148.3, 146.1, 144.6, 127.6, 122.2, 117.5, 116.2, 115.0, 80.1, 28.3. FT-IR (KBr) cm^{-1} 3488, 3061, 2976, 1680, 1606, 1531. Anal. calcd ($C_{13}H_{16}O_4$) 66.09 %C, 6.84 %H; Found 66.05 %C, 7.04 %H.

Cytotoxicity assays

L1210 cells. The IC_{50} values in the L1210 cell line were determined according to previously published protocols.[19] The IC_{50} is defined as the concentration of a compound that inhibits cell growth by 50%.

MCF-7 cells. Human Breast cancer cells were maintained in asynchronous logarithmic growth at 37 °C in Phenol red free Iscoves modified Dulbeccos medium with L-glutamine supplemented with 10% (v/v) FBS. The population doubling time was 24–36 h. Every 48 h, the old media was replaced by the fresh media. All stock solutions and dilutions were made in unsupplemented Dulbeccos medium.

Cell cultures were seeded at 2–5×10^4 cells/mL in duplicate for each inhibitor concentration in a 24-well microtiter plate (0.9 mL/well). The test compounds (0.1 mL) were then added to the cell cultures in 1:10 dilution in order to achieve the desired concentration. Each inhibitor was tested at a minimum of 8 concentrations. After 4 days of continuous drug exposure the cells were counted by using the CyQUANT GR assay kit from Molecular Probes. For this purpose, the media was removed from the plates which were then frozen at −80 °C for a minimum of 1 h. The cells were thawed at 37 °C and 200 uL of CyQUANT GR dye/cell lysis buffer added to each well. The plates were incubated for 5 min at 37 °C and fluorescence was measured using Cytofluor II multiwell fluorescence plate reader. The excitation maximum was 485 nM and the emission maximum was 530 nM.

From the data, a dose-response curve was drawn and the IC_{50} determined. Physicochemical constants were then utilized to formulate a quantitative structure activity relationship (QSAR) for these compounds.[32] Calculated logP values were obtained using ClogP program.[33] (Biobyte Corp. version 4.1).

Acknowledgement

This research was supported by a grant from R. J. Reynolds for fundamental studies in toxicology and NIEHS grant R01 ES 07595.

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