The Mitochondrial Monoamine Oxidase−Aldehyde Dehydrogenase Pathway: A Potential Site of Action of Daidzin

Nadège Rooke, Dian-Jun Li, Junqing Li, and Wing Ming Keung*

Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, 250 Longwood Avenue, Boston, Massachusetts 02115

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Recent studies showed that daidzin suppresses ethanol intake in ethanol-preferring laboratory animals. In vitro, it potently and selectively inhibits the mitochondrial aldehyde dehydrogenase (ALDH-2). Further, it inhibits the conversion of monoamines such as serotonin (5-HT) and dopamine (DA) into their respective acid metabolites, 5-hydroxyindole-3-acetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in isolated hamster or rat liver mitochondria. Studies on the suppression of ethanol intake and inhibition of 5-HIAA (or DOPAC) formation by six structural analogues of daidzin suggested a potential link between these two activities. This, together with the finding that daidzin does not affect the rates of mitochondria-catalyzed oxidative deamination of these monoamines, raised the possibility that the ethanol intake-suppressive (antidipsotropic) action of daidzin is not mediated by the monoamines but rather by their reactive biogenic aldehyde intermediates such as 5-hydroxyindole-3-acetaldehyde (5-HIAL) and/or 3,4-dihydroxyphenylacetaldehyde (DOPAL) which accumulate in the presence of daidzin. To further evaluate this possibility, we synthesized more structural analogues of daidzin and tested and compared their antidipsotropic activities in Syrian golden hamsters with their effects on monoamine metabolism in isolated hamster liver mitochondria using 5-HT as the substrate. Effects of daidzin and its structural analogues on the activities of monoamine oxidase (MAO) and ALDH-2, the key enzymes involved in 5-HT metabolism in the mitochondria, were also examined. Results from these studies reveal a positive correlation between the antidipsotropic activities of these analogues and their abilities to increase 5-HIAL accumulation during 5-HT metabolism in isolated hamster liver mitochondria. Daidzin analogues that potently inhibit ALDH-2 but have no or little effect on MAO are most antidipsotropic, whereas those that also potently inhibit MAO exhibit little, if any, antidipsotropic activity. These results, although inconclusive, are consistent with the hypothesis that daidzin may act via the mitochondrial MAO/ALDH pathway and that a biogenic aldehyde such as 5-HIAL may be important in mediating its antidipsotropic action.

Introduction

For more than a millennium, herbalists practicing traditional Chinese medicine have used *Radix puerariae* (RP, root of kudzu)- or *Flos puerariae* (FP, flower of kudzu)-based herbal medicines for the treatment of "alcohol addiction." Recently, we showed that a crude extract of RP suppresses ethanol intake in ethanol-preferring Syrian golden hamsters (*Mesocricetus auratus*). Further, we isolated and identified daidzin (7-*O*-*β*-glucosyl-4′-hydroxyisoflavone) as its major active principle and demonstrated that pure daidzin synthesized in our laboratory also suppresses hamster ethanol intake.[1] Since then, we and others have confirmed the antidipsotropic (ethanol intake-suppressive) activity of RP extracts and daidzin in additional laboratory rodents such as Wistar rats, Fawn Hooded rats, and the genetically bred alcohol-preferring P rats under various experimental conditions including two-lever choice, two-bottle free-choice, limited access, and ethanol-deprived paradigms.[2−4]

The mechanism by which daidzin suppresses ethanol intake in laboratory rodents is still unknown. In an earlier study, we showed that daidzin potently and selectively inhibits human, rat, and hamster liver mitochondrial aldehyde dehydrogenase isozyme (ALDH-2), the major ALDH isozyme that catalyzes the oxidation of ethanol-derived acetaldehyde, and suggested that it may act as an alcohol sensitizing agent.[1,5] However, in an in vivo study, we demonstrated that daidzin at a dose that suppresses ethanol intake does not affect the overall acetaldehyde metabolism in golden hamsters.[6,7] These apparently conflicting results were later attributed to the presence of a cytosolic ALDH isozyme in hamster liver. This ALDH isozyme catalyzes acetaldehyde oxidation efficiently and with high capacity, and it is not inhibited by daidzin.[8] On the basis of these results, we concluded that the mechanism by which daidzin suppresses hamster ethanol intake is different from that proposed for the classic ALDH inhibitors such as disulfiram and calcium carbimide,[9] and we suggested that it may act by modulating the activity of an as-yet-undefined physiological pathway catalyzed by ALDH-2.

Recently, we showed that daidzin inhibits the conversion of monoamines such as serotonin (5-HT) and dopamine (DA) to their respective acid metabolites 5-hydroxyindole-3-acetic acid (5-HIAA) and 3,4-dihy-

* To whom correspondence should be addressed. Tel: (617) 432-4001. Fax: (617) 566-3137. E-mail: wingming_keung@hms.harvard.edu.

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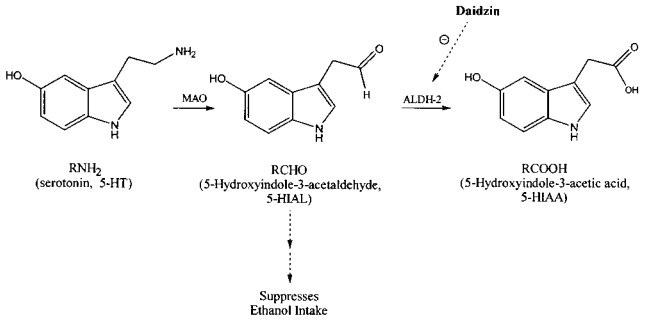


Figure 1. 5-HT metabolism in hamster liver mitochondria: a proposed site of action for daidzin.

droxyphenylacetic acid (DOPAC) in isolated hamster liver mitochondria, which is catalyzed by monoamine oxidase (MAO) and ALDH-2.[10] Further, we synthesized six structural analogues of daidzin and studied and compared their effects on 5-HT → 5-HIAA and DA → DOPAC conversion in isolated liver mitochondria and on ethanol intake in ethanol-preferring golden hamsters. Results of these studies suggested a potential link between these two activities: the stronger the inhibition of 5-HIAA (or DOPAC) formation, the greater the suppression of ethanol intake. Moreover, inhibition of 5-HIAA formation by daidzin was accompanied by a concomitant increase in 5-HIAL accumulation in the assay medium. This, together with the fact that daidzin does not affect the rate of 5-HT depletion in the same assay, raised the possibility that the antidipsotropic action of daidzin is not mediated by the monoamines but rather by their reactive metabolic intermediates such as 5-HIAL and DOPAL which accumulated in the presence of daidzin (Figure 1). To further evaluate this possibility, we here synthesized more structural analogues of daidzin and tested and compared their antidipsotropic activities in alcohol-drinking golden hamsters with their effects on mitochondria-catalyzed monoamine metabolism using 5-HT as the substrate. Effects of daidzin and its structural analogues on the activities of MAO and ALDH-2, the key enzymes involved in monoamine metabolism in the mitochondria, were also examined and compared with their antidipsotropic activities.

Results and Discussion

Synthesis of 7-O-Substituted Derivatives of Daidzein. In an earlier study,[5] we surveyed 41 commercially available compounds structurally related to daidzin for ALDH-2 inhibition and found that only a few of the isoflavones tested inhibit ALDH-2 potently (K_i < 1 μM) and selectively (K_i(ALDH-1)/K_i(ALDH-2) > 50). The characteristics of the isoflavones tested show that the substituents at the 4′, 5, and 7 positions are important for ALDH-2 inhibition. Isoflavones with a free 4′-hydroxyl group inhibit ALDH-2 more potently than those with a blocked 4′-hydroxyl group. Substituting the hydrogen with a hydroxyl group at position 5 decreases potency by at least 1 order of magnitude. Furthermore, blocking the 7 position hydroxyl group enhance ALDH-2 inhibition. In fact, the 7-*O*-glucosyl isoflavones are the most potent yet selective inhibitors of ALDH-2 among the naturally occurring isoflavones tested. On the basis of these results, we decided to synthesize a series of 7-O-substituted derivatives of the 4′,7-dihydroxyisoflavone, daidzein, for this study.

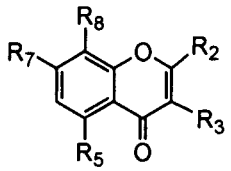
The target 7-O-substituted benzopyranone compounds were synthesized by selective 7-O-functionalization of daidzein. The pK_a's of the 4′- and 7-hydroxyl groups of daidzein are different enough (11.2 and 7.6, respectively) to allow selective ionization of the 7-OH group under appropriate reaction conditions, thus permitting regioselective functionalization.[11] The derivatives were prepared via Williamson synthesis, by reacting the 7-*O*-monoaroxide anion of daidzein with an appropriate alkyl halide RX (X = Br, I). The 7-*O*-monoaroxide anion could be obtained by reacting daidzein with 1 equiv of aqueous KOH in acetone (when X = Br) or with 1 equiv of powdered KOH in DMSO (when X = I).[12,13]

Structures of daidzin and its analogues included in this study are shown in Figure 2. Compounds **1**−**13** are the 7-O-substituted derivatives of daidzein synthesized in this laboratory, whereas compounds **14**−**18** are 7-hydroxyisoflavones and flavones purchased from commercial sources.

Effect of Daidzin and Its Structural Analogues on Hamster Ethanol Intake. The antidipsotropic activities of daidzin and its structural analogues were determined using ethanol-preferring Syrian golden hamsters, and the results are shown in Table 1. Daidzin and eight of its structural analogues suppress hamster ethanol intake when administered intraperitoneally at a dose of 0.07 mmol/hamster/day. Among them, daidzin (**1**) and the 7-*O*-ω-carboxyalkyl derivatives of daidzein (**3**−**6**) are most potent: they suppress daily ethanol intake by more than 60%. The aglycone daidzein (**2**) and its 7-[*O*-2-(1,3-dioxanyl)ethyl] (**10**) and 7-*O*-ω-bromohexyl (**9**) derivatives are moderately active: they suppress ethanol intake by about 22%, 32%, and 29%, respectively. At the same dose tested, the 7-*O*-ω-bromobutyl derivative of daidzein (**8**) is only marginally active, whereas the 7-*O*-ω-bromopropyl (**7**), 7-*O*-allyl (**11**), 7-*O*-(2,3-dihydroxypropyl) (**12**), and 7-*O*-ethyl (**13**), 5-hydroxy (**14**), and 5-hydroxy-4′-methyl (**17**) derivatives are not active at all. Puerarin (**18**), the 8-*C*-glucosyl derivative of daidzein, which has been shown to suppress ethanol intake in rats,[3] does not suppress ethanol intake in Syrian golden hamsters. None of the flavone analogues tested (**15**, **16**) are antidipsotropic.

Relative Bioavailability of Daidzin and Its Structural Analogues. The relative potencies of daidzin and its structural analogues in suppressing hamster ethanol intake depend not only on their intrinsic pharmacological activities but also on their relative bioavailabilities. To determine whether differences in bioavailabilities of these analogues contributed significantly to the observed differences in antidipsotropic activities, we evaluated and compared their basic pharmacokinetic properties under the same conditions employed in ethanol drinking experiments. The results, expressed as relative rate and extent of bioavailability, t_{max} and AUC (area under the curve), respectively, are listed in Table 2.

The t_{max} of daidzin and its structural analogues vary from 0.5 to 2 h. In the ethanol-drinking experiments, accumulated ethanol intake over a period of 24 h was measured. Therefore, it is unlikely that this relatively small variation in t_{max} will have any significant effect



Cpd	R_2	R_3	R_5	R_7	R_8
1	H	4-OH	H	OGlc	H
2	H	4-OH	H	OH	H
3	H	4-OH	H	$O(CH_2)_5CO_2H$	H
4	H	4-OH	H	$O(CH_2)_6CO_2H$	H
5	H	4-OH	H	$O(CH_2)_9CO_2H$	H
6	H	4-OH	H	$O(CH_2)_{10}CO_2H$	H
7	H	4-OH	H	$O(CH_2)_3Br$	H
8	H	4-OH	H	$O(CH_2)_4Br$	H
9	H	4-OH	H	$O(CH_2)_6Br$	H
10	H	4-OH	H	$O(CH_2)_2$-1,3-dioxolane	H
11	H	4-OH	H	$OCH_2CH{=}CH_2$	H
12	H	4-OH	H	$OCH_2CH(OH)CH_2OH$	H
13	H	4-OH	H	OCH_2CH_3	H
14	H	4-OH	OH	OH	H
15	phenyl	H	OH	OH	H
16	phenyl	H	H	OH	OH
17	H	4-OCH_3	OH	OH	H
18	H	4-OH	H	OH	CGlc

Figure 2. Structures of daidzin and its analogues.

Table 1. Suppression of Hamster Ethanol Intake by Daidzin and Its Structural Analogues[a]

compd	% suppression	compd	% suppression
1	62 ± 4	**10**	32 ± 10
2	22 ± 5	**11**	0
3	69 ± 12	**12**	0
4	69 ± 8	**13**	0
5	84 ± 5	**14**	0
6	86 ± 7	**15**	0
7	0	**16**	0
8	14 ± 5	**17**	0
9	29 ± 5	**18**	0

[a] Ethanol intake suppression was measured as described in the Experimental Section. Dose = 0.07 mmol/hamster/day, ip. Values are mean ± SD of 4−6 hamsters.

Table 2. AUC and t_{max} of Daidzin and Its Structural Analogues[a]

compd	t_{max} h	AUC nmol·h·mL^{-1}	compd	t_{max} h	AUC nmol·h·mL^{-1}
1	1	120 ± 5	**10**	0.5	99 ± 22
2	1	130 ± 16	**11**	1	29 ± 1.6
3	0.5	77 ± 18.1	**12**	0.5	141 ± 11
4	0.5	68 ± 14	**13**	1	38 ± 1.5
5	0.5	73 ± 10	**14**	1	134 ± 25
6	0.5	56 ± 14	**15**	1	110 ± 44
7	2	73 ± 12.5	**16**	0.5	111 ± 22
8	2	68 ± 17	**17**	1	99 ± 5.5
9	2	62 ± 10	**18**	1	129 ± 7

[a] For details of the method, refer to the Experimental Section. Values are mean ± SD of 3 hamsters.

on the antidipsotropic activities determined for these compounds.

The AUC values of daidzin and its antidipsotropic analogues also are very similar. The AUC values estimated for the active analogues vary by less than 3-fold, ranging from 56 nmol·h·mL for **6** to 130 nmol·h·mL for **2**. In fact, except for **1** and **2**, which have relatively high AUC values (120 and 130 nmol·h·mL,

respectively), the AUC values estimated for all other active analogues are, within experimental error, virtually identical. These results indicate that differences in relative bioavailability contribute little, if any, to the differences in the antidipsotropic activities observed for the active analogues.

Except for analogues **11** and **13**, which have relatively low AUC values (29 and 38 nmol·h·mL, respectively), those estimated for the other inactive analogues (**7**, **12**, **14**−**18**) range from 73 to 141 nmol·h·mL, similar to or slightly higher than those for the active analogues. This result suggests that these analogues do not suppress hamster ethanol intake because they are intrinsically inactive, not because of poor bioavailability.

Effect of Daidzin and Its Structural Analogues on Mitochondria-Catalyzed 5-HT → 5-HIAA Conversion. Monoamine metabolism and its inhibition by daidzin and its structural analogues were studied in isolated hamster liver mitochondria using 5-HT as the substrate. Oxidative deamination of biogenic monoamines is catalyzed by the membrane-bound MAO on the outer surface of mitochondria. This reaction generates reactive aldehyde intermediates which are either oxidized to their corresponding acid metabolites by ALDH-2 present in the matrix of the mitochondria[14,15] or reduced to their corresponding alcohols by NADH-dependent alcohol dehydrogenase (ADH) and/or NADPH-dependent aldehyde reductase (AR).[16,17] In liver and brain, 5-HT is primarily converted to its acid product 5-HIAA, indicating that the mitochondrion is probably an important subcellular compartment in which 5-HT metabolism occurs in vivo. The mitochondria prepared for this study contained both MAO and ALDH-2 but not ADH or AR.[10] Therefore, they provide a simple yet physiologically relevant system in which the effects of daidzin and its structural analogues on 5-HT metabolism can be examined.

In an assay medium containing 10 μM 5-HT and 0.08 mg/mL of the isolated mitochondria, about 5% of the substrate is converted to 5-HIAA after incubation at 37 °C for 30 min. Under these conditions, daidzin potently inhibits the formation of 5-HIAA. Inhibition is concentration-dependent with an estimated IC_{50} of about 2 μM (Table 3), similar to that reported in a previous study.[10]

Among the daidzin analogues tested, all but analogue **18** inhibit 5-HT → 5-HIAA conversion. Analogues **5**−**7**, **13**, and **17** are the most potent with IC_{50} values <0.3 μM; followed by **3**, **4**, **8**−**12**, **14** (0.3 μM < IC_{50} < 0.9 μM); **1**, **2**, **15** (0.9 μM < IC_{50} < 3 μM); and **16** (3 μM < IC_{50} < 9 μM) (Table 3).

Figure 3A, a scatter plot of the antidipsotropic activities of daidzin and its structural analogues versus their abilities in inhibiting mitochondria-catalyzed 5-HIAA formation, shows that the two activities do not correlate with each other. It appears that daidzin analogues which suppress hamster ethanol intake (**1**−**6**, **8**−**10**) also inhibit 5-HIAA formation. However, not all daidzin analogues that inhibit 5-HIAA formation are antidipsotropic. In fact, among the most potent inhibitors of 5-HT → 5-HIAA conversion, six (**7**, **11**−**14**, **17**) do not suppress hamster ethanol intake (Figure 3A).

Effect of Daidzin and Its Structural Analogues on MAO and ALDH-2 Activity. MAO and ALDH-2 act in tandem in the catalytic conversion of 5-HT → 5-HIAA in the isolated mitochondria (Figure 1). Therefore, in principle, daidzin and its structural analogues can inhibit 5-HIAA formation by blocking either MAO and/or ALDH-2. To identify the target enzyme(s) of daidzin and its structural analogues, we studied their effects on MAO and ALDH-2 independently using the membrane and lysate of a density-gradient-purified mitochondria preparation as the respective enzyme sources. The results, expressed in IC_{50} values, are listed in Table 4.

Table 3. Inhibition of 5-HIAA Formation in Isolated Hamster Liver Mitochondria by Daidzin and Its Structural Analogues[a]

	% inhibition			
compd	0.3 μM	0.9 μM	3 μM	9 μM
1	18.7 ± 2.6	43.7 ± 3.5	64.4 ± 2.2	76.3 ± 1.9
2	9.5 ± 1.6	29.2 ± 5.8	58.9 ± 0.9	80.6 ± 2.5
3	48.4 ± 1.3	72.7 ± 0.1	95.1 ± 6.9	98.9 ± 1.6
4	48.6 ± 3.2	71.2 ± 0.8	87.4 ± 6.7	94.6 ± 8.1
5	74.4 ± 0.9	85.1 ± 3.2	89.6 ± 3.8	95.0 ± 3.5
6	57.0 ± 5.9	72.7 ± 2.0	82.5 ± 6.6	96.7 ± 4.7
7	51.1 ± 2.9	67.7 ± 5.7	90.8 ± 4.7	100
8	19.2 ± 2.1	55.3 ± 10.9	84.7 ± 6.5	98.3 ± 2.5
9	47.2 ± 3.0	71.2 ± 2.5	88.5 ± 4.4	95.7 ± 4.3
10	41.0 ± 6.0	57.6 ± 4.7	75.3 ± 9.3	88.5 ± 5.4
11	37.9 ± 12.0	68.8 ± 7.6	89.3 ± 0.6	100
12	33.3 ± 0.3	63.8 ± 1.9	85.0 ± 0.1	95.1 ± 0.6
13	64.8 ± 11.0	86.9 ± 5.4	95.6 ± 24.4	100
14	31.3 ± 3.2	65.0 ± 5.6	77.6 ± 5.2	96.8 ± 5.3
15	20.7 ± 3.1	28.1 ± 3.0	64.7 ± 4.1	83.5 ± 6.8
16	4.6 ± 2.0	7.9 ± 2.5	25.4 ± 4.1	53.2 ± 1.6
17	74.1 ± 6.8	90.7 ± 6.2	96.8 ± 4.9	100
18	0	0	0	0

[a] See the Experimental Section for assay conditions. Values are mean ± SD of 3 separate determinations.

As we reported previously,[5] daidzin is a potent inhibitor of ALDH-2. The IC_{50} value, determined at a substrate concentration equal to K_m, is 0.04 μM. Further, at concentrations up to 10 μM, daidzin does not inhibit MAO activity. This result is consistent with our previous finding that daidzin at concentrations that significantly inhibit 5-HIAA formation does not affect the rate of 5-HT depletion catalyzed by isolated hamster liver mitochondria.[10]

The 7-*O*-ω-carboxyalkyl derivatives of daidzein (**3**−**6**) are by far the most potent ALDH-2 inhibitors synthesized thus far. The IC_{50} values determined under the same conditions range from 0.009 to 0.003 μM, 4.4 to 13.3 times lower than that of daidzin. Analogues **2** and **7**−**13** also inhibit ALDH-2 but are relatively less potent with IC_{50} values ranging from 0.04 to 9 μM. Further, unlike daidzin, these analogues also inhibit MAO with **7** (IC_{50} = 0.15 μM), **13** (0.3 μM), and **11** (0.45 μM) being the most potent, followed by **12** (1.7 μM), **9** (2 μM), **4** (2.1 μM), **3** (3 μM), **8** (4 μM), **10** (7 μM), **5** (10 μM), **6** (13 μM), and **2** (14 μM).

The isoflavone aglycones **14** and **17** and the flavone aglycones **15** and **16** do not inhibit ALDH-2. However, they all inhibit MAO with **17** being the most potent (IC_{50} = 0.4 μM) followed by **14** (0.9 μM), **15** (1.6 μM), and **16** (17 μM). Analogue **18**, at concentrations up to 10 μM, does not affect ALDH-2 or MAO.

Analysis of a scatter plot of ethanol intake suppression versus ALDH-2 inhibition (Figure 3B) shows that these two activities do not correlate with each other. Nevertheless, the data appear to show that (i) ALDH-2 inhibition is necessary for antidipsotropic activity be-

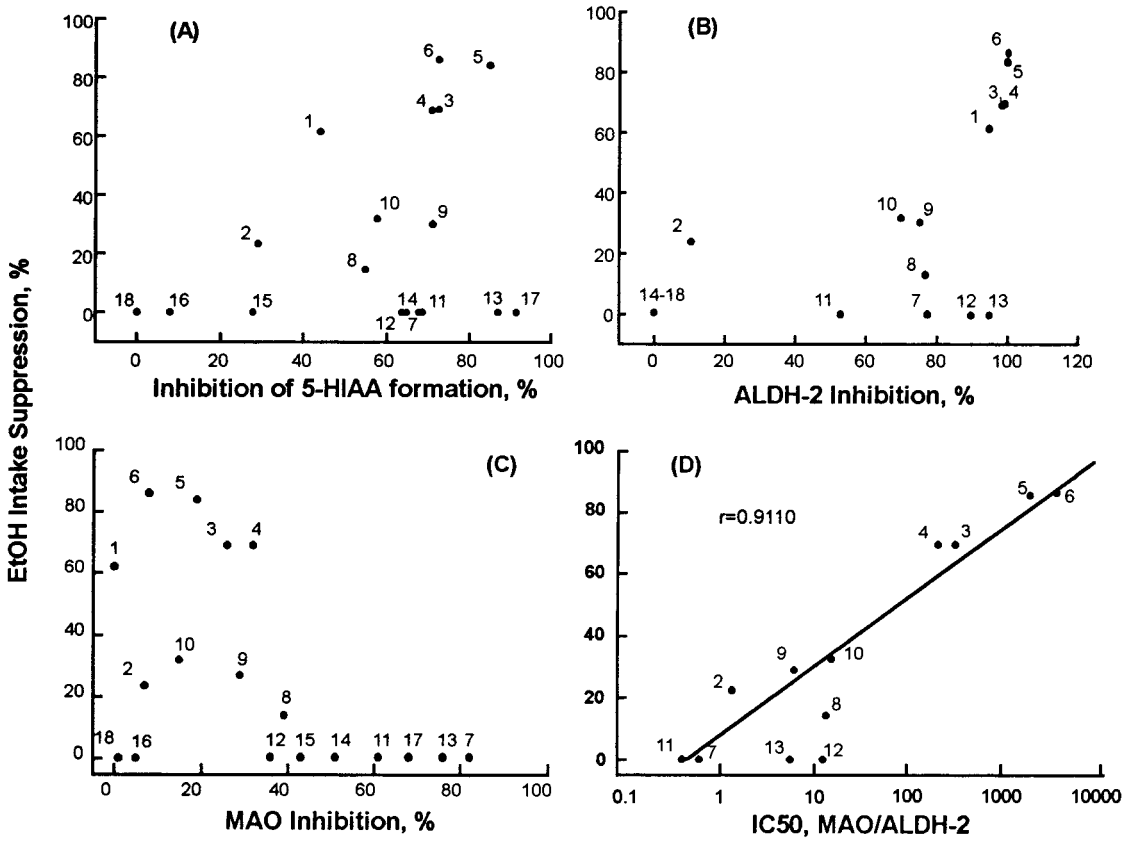


Figure 3. Scatter of ethanol intake suppression by daidzin and its structural analogues against their inhibition on (A) 5-HIAA formation in liver mitochondria-catalyzed 5-HT metabolism, (B) ALDH-2 activity, (C) MAO activity, and (D) IC_{50}(MAO)/IC_{50}(ALDH-2). Ethanol intake suppression were measured using ethanol-preferring golden hamsters at a dose of 0.07 mmol/hamster/day, ip. Effects of daidzin and analogues on ALDH-2, MAO, and 5-HIAA formation were determined at a concentration of 0.9 μM. IC_{50}(MAO)/IC_{50}(ALDH-2) ratios were calculated from data shown in Table 3. Analogues that do not inhibit both MAO and ALDH-2 are not included in plot D because their IC_{50}(MAO)/IC_{50}(ALDH-2) values are undefined.

Table 4. Inhibition of Hamster Liver Mitochondrial MAO and ALDH-2 Activities by Daidzin and Its Structural Analogues[a]

	IC_{50}, μM			IC_{50}, μM	
compd	MAO	ALDH-2	compd	MAO	ALDH-2
1	ni[b]	0.04	**10**	7	0.4
2	14	9	**11**	0.45	0.8
3	3	0.009	**12**	1.7	0.1
4	2.1	0.009	**13**	0.3	0.04
5	10	0.004	**14**	0.9	ni
6	13	0.003	**15**	1.6	ni
7	0.15	0.26	**16**	17	ni
8	4	0.27	**17**	0.4	ni
9	2	0.3	**18**	ni	ni

[a] MAO and ALDH-2 activity were assayed as described in the Experimental Section, using 10 μM 5-HT and 0.6 mM formaldehyde as the substrates, respectively. IC_{50} values were estimated graphically with inhibition data determined at more than five inhibitor concentrations. [b] ni, no inhibition up to 10 μM.

cause all structural analogues that do not inhibit ALDH-2 (**14**−**18**) are not antidipsotropic and (ii) ALDH-2 inhibition alone is not sufficient for ethanol intake suppression because not all daidzin analogues that inhibit ALDH-2 are antidipsotropic. In fact, four of the most potent ALDH-2 inhibitors (**7**, **11**−**13**) exhibit no effect on hamster ethanol intake.

Among the 18 compounds tested, 16 inhibit MAO. The scatter plot of MAO inhibition versus ethanol intake suppression by these compounds (Figure 3C) clearly shows no correlation between the two. In fact, the MAO inhibitory activity of some daidzin analogues may have contributed negatively to antidipsotropic activity. As shown in Figure 3B,C, daidzin analogues that inhibit ALDH-2 potently but which have little or no effect on MAO (**1**, **3**−**6**) are most antidipsotropic, whereas those which also inhibit MAO potently do not suppress ethanol intake (**7**, **11**−**13**). These results appear to show that the antidipsotropic activities of daidzin and its active analogues may stem from their abilities to increase the mitochondrial MAO:ALDH-2 activity ratio.

To evaluate this possibility, we constructed a scatter plot of suppression of ethanol intake by daidzin and its structural analogues versus their abilities to increase MAO:ALDH-2 activity ratio, IC_{50}(MAO)/IC_{50}(ALDH-2) (Figure 3D). This plot clearly shows a positive correlation between the two and suggests that the antidipsotropic isoflavones may indeed suppress hamster ethanol intake by increasing the mitochondrial MAO:ALDH activity ratio.

In this context, it is of interest to point out that in a previous study,[10] we showed that hamster liver mitochondria exhibit a lower MAO:ALDH-2 activity ratio (0.18) than that of the rat (1.6). As a consequence, the concentration of the metabolic intermediate 5-HIAL found in isolated hamster liver mitochondria during 5-HT metabolism is also much lower than that in the rat (0.2 μM vs 2.3 μM). We also pointed out that golden hamsters are by nature inclined to prefer and consume large quantities of ethanol,[18] whereas the randomly bred Wistar rats used in our studies avoid ethanol.[19] Epide-

miological studies also have associated a low MAO: ALDH-2 activity ratio with high ethanol consumption: (i) low platelet MAO activity correlates with type II alcoholism[20] and (ii) Asians who have inherited a low activity (or inactive) mutant form of ALDH-2 seldom have a problem with alcohol abuse.[21] These findings, although inconclusive, are consistent with the hypothesis that the antidipsotropic action of daidzin may be mediated by a biogenic aldehyde intermediate of the mitochondrial MAO/ALDH pathway such as 5-HIAL. To further evaluate this hypothesis, we studied the effects of daidzin and its structural analogues on the accumulation of 5-HIAL during 5-HT metabolism in hamster liver mitochondria.

Effects of Daidzin and Its Structural Analogues on 5-HIAL Accumulation during 5-HT Metabolism in Isolated Hamster Liver Mitochondria. All but analogue **18** affect 5-HIAL accumulation during 5-HT metabolism. On the basis of their effects, these compounds can be classified into four groups. Group I, which includes only daidzin, increases 5-HIAL accumulation during mitochondria-catalyzed 5-HT metabolism (Figure 4A). Within the concentration range studied, the effect is concentration-dependent and is accompanied by a concomitant decease in 5-HIAA formation. At 9 μM, the highest daidzin concentration studied, 5-HIAL accumulation in the assay medium increased by nearly 200% (Table 5). These results are consistent with the finding that daidzin is a potent inhibitor of ALDH-2 but has no effect on MAO (Table 4).

Group II analogues, which include compounds **2**−**6** and **8**−**10**, affect 5-HIAL accumulation in a biphasic manner: they increase 5-HIAL accumulation at low concentrations but decrease it at high concentrations. Figure 4B shows a typical biphasic concentration-dependent effect of this group of analogues on 5-HIAL accumulation. These results are consistent with the fact that this group of analogues are all more potent ALDH-2 inhibitors than they are MAO inhibitors (Table 4).

Group III comprises analogues **7** and **11**−**17**, which are strong MAO but relatively weak ALDH-2 inhibitors (Table 4). As a consequence, they inhibit 5-HT → 5-HIAL conversion more potently and, hence, decrease both 5-HIAL accumulation and 5-HIAA formation in the mitochondria (Figure 4C, Table 4). Group IV, represented solely by analogue **18**, affects neither 5-HIAL accumulation nor 5-HIAA formation during 5-HT metabolism in isolated hamster liver mitochondria (Table 5), consistent with the finding that analogue **18** has no effect on either ALDH-2 or MAO (Table 4).

Comparing the abilities of daidzin and its structural analogues in increasing 5-HIAL accumulation and suppressing hamster ethanol intake reveals a positive correlation (Figure 5). This result, together with that shown in Figure 3, suggests that the mitochondrial MAO/ALDH pathway is a potential target pathway of daidzin and that a biogenic aldehyde intermediate, such as 5-HIAL, derived from the action of MAO may be involved in the regulation of hamster ethanol intake and its suppression by daidzin.

While the correlation coefficients obtained from the scattered analyses (Figures 3 and 5) are good, we are aware of the fact that correlations do not prove a mechanism. Moreover, the mitochondrial MAO/ALDH pathway exists and operates not only in the liver but also in other organs and tissues as well. Therefore, our results provide no information on where daidzin act in vivo. Daidzin can either act on the mitochondrial MAO/ALDH pathway in the liver and transmit its antidipsotropic signal into the brain via a second messenger or act directly on a similar pathway that operates in the central nervous system.

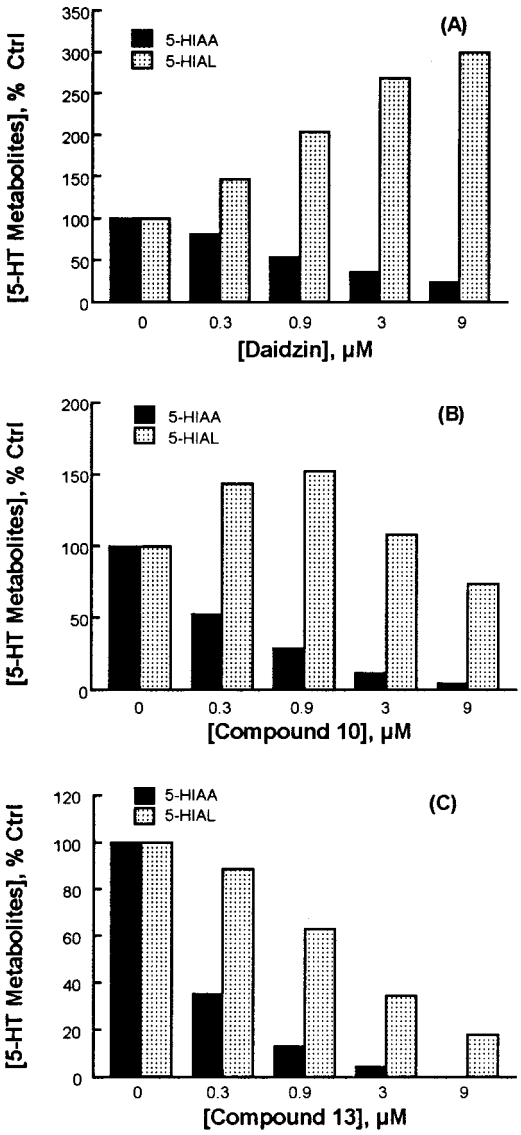


Figure 4. Concentration effect of (A) daidzin, (B) analogue **10**, and (C) analogue **13** on hamster liver mitochondria-catalyzed serotonin metabolsim: formation of 5-HIAL and 5-HIAA.

Concluding Remarks

Early interest in biogenic aldehydes in relation to alcohol research stems largely from the belief that acetaldehyde, the reactive intermediate of ethanol metabolism, interferes with their oxidative metabolism.[22] It was postulated that levels of biogenic aldehydes increase during ethanol metabolism because of competitive inhibition of ALDH-2 by acetaldehyde. Biogenic aldehydes, accumulated under such conditions, can be diverted to a reductive pathway leading to the formation of their alcohol metabolites[16,17] and/or undergo nonenzymatic condensation reactions forming

Table 5. Effect of Daidzin and Its Structural Analogues on 5-HIAL Accumulation in Isolated Hamster Liver Mitochondria[a]

	5-HIAL accumulation in isolated liver mitochondria, % of control			
compd	0.3 μM	0.9 μM	3 μM	9 μM
1	146.5 ± 21.0	203.5 ± 22.8	268.3 ± 53.0	299.0 ± 59.0
2	103.5 ± 2.0	110.5 ± 3.5	103.5 ± 9.2	74.0 ± 4.2
3	229.0 ± 6.4	182.5 ± 40.0	162.0 ± 35.0	130.5 ± 14.8
4	310.0 ± 21.2	265.0 ± 39.2	227.6 ± 12.6	192.0 ± 25.3
5	234.0 ± 5.7	233.0 ± 19.8	189.0 ± 4.2	129.0 ± 4.2
6	214.0 ± 69.5	292.0 ± 66.5	294.0 ± 55.2	205.5 ± 44.5
7	75.8 ± 5.0	57.8 ± 9.8	34.7 ± 6.0	20.5 ± 4.0
8	111.5 ± 5.6	115.7 ± 9.5	87.7 ± 26.0	42.9 ± 9.8
9	143.6 ± 8.6	112.5 ± 9.9	108.3 ± 13.7	74.0 ± 26.0
10	165.0 ± 24.4	175.8 ± 9.1	141.1 ± 22.0	86.1 ± 27.9
11	106.3 ± 5.2	91.0 ± 0	56.1 ± 0.2	26.7 ± 1.6
12	111.9 ± 7.5	97.5 ± 10.7	63.0 ± 4.9	30.5 ± 1.7
13	88.6 ± 12.1	62.9 ± 6.9	34.3 ± 9.8	17.8 ± 3.3
14	97.5 ± 13.2	66.7 ± 8.6	43.4 ± 6.2	23.5 ± 3.3
15	73.5 ± 12.3	55.6 ± 3.8	33.6 ± 4.6	17.2 ± 2.8
16	92.6 ± 3.9	92.3 ± 5.7	79.1 ± 2.5	59.9 ± 3.6
17	26.3 ± 1.8	12.9 ± 1.3	0	0
18	87.9 ± 6.9	94.9 ± 16.5	109.1 ± 2.5	130.3 ± 23.7

[a] For details of the assay, refer to the Experimental Section. Values are mean ± SD of 3 separate determinations.

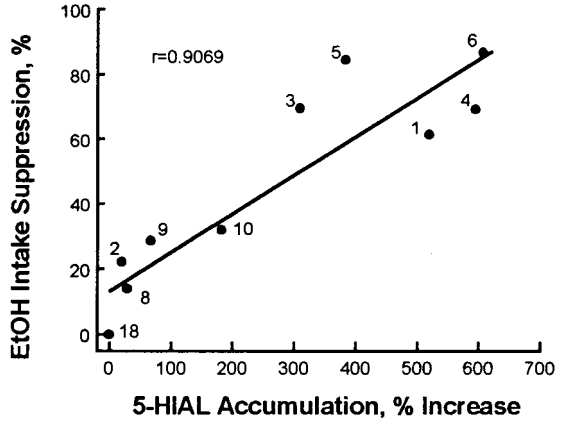


Figure 5. Scatter of suppression of ethanol intake versus increase in 5-HIAL accumulation by daidzin and its structural analogues. Data on ethanol intake suppression were taken from Table 1. Values of increase in 5-HIAL accumulation are the sum of 5-HIAL accumulation (% increase) caused by a test compound at 1−4 different concentrations (Table 5). Using 5-HIAL accumulation measured at a single drug concentration could lead to wrong conclusion because the effect of group II analogues on 5-HIAL accumulation is biphasic: increase accumulation at low concentration but decrease it at high concentration. Further, the sums of % increase in HIAL accumulation measured at a series of drug concentrations better approximate the real exposure (AUC) of a hamster to 5-HIAL in in vivo experiments (ethanol-drinking experiment). Group III analogues, which inhibit 5-HIAL accumulation at all concentrations studied, neither decrease nor increase hamster ethanol intake (Tables 1 and 5) and are, therefore, not included in this plot. For the same reason, the decrease in 5-HIAL accumulation caused by some group II analogues at high drug concentrations is not included in the calculation of the sum.

adducts with biogenic amines, proteins, and phospholipids.[22,23] While the physiological implication of shifting from an oxidative to a reductive metabolic pathway is completely unknown at this time, the condensation products have been shown to affect ethanol-drinking behavior in laboratory animals.[24] Moreover, biogenic aldehydes themselves could be physiologically active[25] and may play a more direct role in regulating ethanol intake. Our results suggest that daidzin, and its active antidipsotropic analogues, could act by mimicking the effect of acetaldehyde on biogenic aldehyde metabolism and provided a strong case for further pursuit of the role biogenic aldehydes in the regulation of alcohol use and abuse.

Experimental Section

General chemicals were either purchased from Aldrich Chemical Co. (Milwaukee, WI) or Lancaster Synthesis Inc. (Windham, NH). All organic solvents used were of AR grade and were supplied by J. P. Baker (Phillipsburg, NJ) or Fisher Scientific Co. (Pittsburgh, PA). Daidzin (**1**) was purchased from L. C. Laboratories (Woburn, MA). Genistein (**14**), chrysin (**15**), 7,8-dihydroxyflavone (**16**), and biochanin A (**17**) were purchased from Indofine Chemical Co. (Somerville, NJ). Daidzein (**2**) was prepared either in this laboratory or by Tyger Scientific Inc., Princeton, NJ. Puerarin (**18**) and 5-hydroxytryptophol (5-HTOL) were purchased from Sigma Chemical Co. (St. Louis, MO). Serotonin (5-HT) and its metabolite 5-HIAA were products of Research Biochemical International (Natick, MA). The metabolic intermediate 5-HIAL was produced in this laboratory by MAO-catalyzed oxidative deamination of 5-HT using rat liver mitochondrial membrane as a source of MAO.[23] All other reagents used were best grade available.

Animal Experiments. 1. Ethanol-Drinking Experiments. Mature male Syrian golden hamsters (80−120 g) were obtained from Harlan Sprague−Dawley Inc. (Indianapolis, IN). Animals were housed in a room maintained at 23 °C on a 12/12-h light/dark cycle with ad libitum access to Purina rodent laboratory chow (5001), water, and a 15% ethanol solution prepared in tap water. After 1 week, hamsters were transferred to and housed in individual stainless steel metabolic cages (26 × 18 × 17.5 cm^3). Two 50-mL drinking bottles, one containing tap water and the other a 15% ethanol solution, were provided. The drinking bottles were placed in holders equipped with tilted platforms which collect spillage in tubes placed outside of the cages. The positions of the two drinking bottles on each cage were alternated daily to prevent development of positional preference. Fluid intake was measured between 3:00 and 4:00 p.m. each day. Hamsters that drank significant (>8 mL/day) and consistent (daily variance less than ±10%) amounts of ethanol solution were selected for drug testing. To establish baseline fluid intake, 1 mL saline with 0.5% DMSO was administered (ip) to each hamster between 5:00 and 5:30 p.m. each day for 6 consecutive days. They were then divided into groups of six animals each and given daily doses (ip) of 0.07 mmol of daidzin or one of its structural analogues for 6 consecutive days. All test compounds were administered in 1 mL saline containing 0.5% DMSO. Food, water and a 15% ethanol solution were available continuously. The response of each hamster to daidzin or its analogues is expressed as: percent (%) ethanol intake suppression (% suppression) = $(V_o - V_e) \times 100/V_o$, where V_o and V_e are the average daily intakes of 15% ethanol solution during the baseline and treatment periods, respectively.

2. Time Course of Plasma Daidzin (or Analogue) Concentration and Area Under the Curve (AUC). Golden hamsters used for this study were selected according to the same criteria as those for ethanol drinking experiments. Selected animals were also housed under the same conditions with ad libitum access to Purina rodent laboratory chow (5001), water, and a 15% ethanol solution throughout the study. Three hamsters were used for each compound tested. Before the administration of a test compound, the hamster was anesthetized with diethyl ether and a 0-time blood sample (50 μL) was drawn from the orbital venous plexus into a tube containing 5 μL of 9% K_3EDTA. Animals were then given (ip) 0.07 mmol of a test compound in 1 mL saline containing 0.5% DMSO. Blood samples (50 μL) were taken 0.5, 1, 2, 4, 8, 16 h after drug administration. Plasma samples, obtained by centrifugation, were extracted with 10 volumes of methanol/85% perchloric acid (8:2). Protein precipitates were removed

by centrifugation. Supernatants were concentrated 10 times (Speedvac, Savant) and were analyzed for the test compound on a Waters HPLC system according to conditions described in the Synthesis section. Plasma drug concentration−time curves were constructed from these data and the AUCs between the 0th and 16th hours were estimated by the trapezoid method. Calibration curves were prepared using solutions of standard compounds (0.5−10 μM) in methanol. Stock solutions were stored at −20 °C for 1 month, and working standard solutions were freshly prepared. Standards were analyzed as control during the analysis of plasma samples. The recoveries of test compounds from blood were determined in control blood samples spiked with a single concentration of one compound each time. The intra-assay coefficient of variation determined at 1 and 10 μM for all tested compounds was ≤17.3% and 10.6%, respectively. The calibration curves were linear in all cases.

Metabolic and Enzyme Assays. 1. Serotonin Metabolism in Isolated Liver Mitochondria. Ethanol-naive hamsters were sacrificed in a CO_2 chamber. Livers were removed immediately and isolated mitochondria were prepared as described previously.[23] The integrity of these mitochondrial preparations, evaluated by measuring their latent glutamate dehydrogenase activity before and after the metabolic study, were over 97% and 93%, respectively. Mitochondria-catalyzed 5-HT metabolism was carried out in a 0.5-mL standard assay medium containing 10 mM Tris-HCl (pH 7.4), 0.3 M mannitol, 2.5 mM $MgCl_2$, 10 mM K_2HPO_4, 10 mM KCl, 0.1% DMSO, 0.01% ethanol, 10 μM 5-HT, and specified concentrations of test compounds and freshly prepared isolated mitochondria. Most test compounds are not readily soluble in aqueous solution. Therefore, stock solutions of the test compounds were prepared in 90% DMSO and 10% ethanol. The final concentrations of DMSO and ethanol in all assays, including controls, were 0.1% and 0.01%, respectively, and in which all tested compounds were soluble at the highest concentration tested, i.e., 9 μM. Reactions were initiated by the addition of mitochondria and allowed to proceed in a 37 °C shaking water bath for 30 min. Reactions were terminated by the addition of 0.05 mL each of ice-cold 1 M $HClO_4$ and 10 mM EDTA. Samples were kept on ice for 30 min and then centrifuged at 12 000 rpm in a microcentrifuge (Microspin 24S, Sorvall) for 15 min. The metabolic intermediate 5-HIAL and product 5-HIAA in the supernatant were analyzed by HPLC. In the presence of $HClO_4$ (final concentration = 0.1 M), both 5-HIAL and 5-HIAA are stable for at least 12 h at 4 °C. The calibration curves obtained with standard 5-HIAA and 5-HIAL solutions (range from 0.05−10 μM) are linear. At E_{app} = 650 mV and I = 20 nA, on column detection limit for both 5-HIAA and 5-HIAL was 0.2 ng with a signal-to-noise ratio of 3. The overall recovery of 5-HIAL and 5-HIAA in the mitochondrial assay samples spiked with standard compounds was 0.65 and 0.84, and the intra-assay coefficient of variation of the analytical methods determined with samples spiked with 2 μM of the respective analytes was 12.5% and 8.6%. Effect of daidzin and its analogues on 5-HIAA formation during the assay is expressed as: percent (%) inhibition = (5-$HIAA_o$− 5-$HIAA_e$) × 100/5-$HIAA_o$, where 5-$HIAA_o$ and 5-$HIAA_e$ are 5-HIAA formation measured in the absence and presence of a test compound, respectively. Effect of daidzin and its analogues on 5-HIAL accumulation during the assay is expressed as: percent (%) of control = (5-$HIAL_e$/5-$HIAL_o$) × 100, where 5-$HIAL_e$ and 5-$HIAL_o$ are concentrations of 5-HIAL found in assay media with and without a test compound, respectively.

2. MAO and ALDH-2 Assays. The mitochondrial pellet obtained from 5 g of hamster liver was resuspended in 10 mL of 10 mM sodium phosphate buffer (pH 7.4), kept on ice, and sonicated for 3 × 15 s at 90 W of power with a Branson Sonifier cell disruptor. This suspension was centrifuged at 105000*g* for 70 min in a Beckman L8 ultracentrifuge and the supernatant, which contained ALDH-2 activity, was used for ALDH-2 assay. The pellet, which contained mainly mitochondrial membrane, was washed 3 times in 30 mL TKK buffer (10 mM Tris, 10 mM KCl, and 10 mM KPi, pH 7.4). The final pellet, which contained only MAO but not ALDH-2 activity, was used for MAO assay. ALDH-2 activity was assayed in 0.1 M $NaPP_i$, pH 9.5, containing 0.15 M KCl, 1.2 mM NAD^+, 0.6 mM formaldehyde, and specified concentrations of daidzin or its structural analogues. Activity was determined by following the increase in absorbance at 340 nm with a Varian Cary 1 spectrophotometer at 25 °C.[23] MAO activity was assayed in TKK buffer containing 10 μM 5-HT, 0.4 mM sodium bisulfite, specified concentrations of daidzin or its structural analogues, and MAO. Enzyme reaction was initiated by the addition of enzyme and was allowed to proceed at 37 °C for 30 min. The reaction was terminated by centrifugation at 4 °C in a Sorvall Microspin at top speed for 15 min. The reaction product 5-HIAL, present in the supernatant as its stable bisulfite complex, was liberated by diluting the supernatant 10−100-fold in 50 mM $NaPP_i$, pH 8.8 and analyzed by HPLC. Since 5-HIAL is relatively unstable at alkaline pH, 5-HIAL was liberated not more than 4 h before HPLC analysis. The overall recovery of 5-HIAL and 5-HIAA in assay samples spiked with standard analytes were 0.78 and 0.86, and the intra-assay coefficient of variation of the analytical methods determined with samples spiked with 2 μM of the respective analytes are 11.2% and 7.5%. Effect of daidzin and its analogues on ALDH-2 and MAO activities is expressed as: percent (%) inhibition = $(A_o - A_e) \times 100/A_o$, where A_o and A_e are enzyme activities measured in the absence and presence of a test compound, respectively.

3. HPLC Analysis. The HPLC system for 5-HIAL and 5-HIAA analyses consisted of a BAS Sample Sentinel autosampler with refrigerated sample compartment (set at 4 °C for all analyses), a PM80 solvent delivery system, and a LC-26 on-line degasser. The detector is a LC-4C amperometric controller with a CC-5 cross-flow thin-layer (0.005 in.) electrochemical cell composed of glassy carbon and silver/silver chloride reference electrode (Bioanlytical Systems, Inc., West Lafayette, IN). For routine analysis, the potential and sensitivity were set at 650 mV and 20 nA full scale, respectively. Column temperature was maintained with a Waters temperature control module (Waters, Milford, MA). Metabolites 5-HIAL and 5-HIAA were analyzed on a Beckman ultrasphere ODS, 5-μm, 4.6- × 250-mm column. The column was developed at 37 °C at 1 mL/min in a mobile phase containing 3% methanol (v/v), 1% acetonitrile (v/v), 0.2 mM EDTA, and 0.1% TFA (v/v). The retention times for 5-HIAL and 5-HIAA were 14.2, and 21.4 min, respectively. Data were collected and analyzed with a Waters 740 data module.

Synthesis. General Methods. ^1H and ^{13}C NMR spectra were obtained on a Bruker AMX500 BQ spectrometer at 500 MHz and Bruker AM-500 spectrometer at 126 MHz (NuMega Resonance Labs Inc., San Diego, CA) respectively, using DMSO as solvent and as internal standard (2.50 and 39.51 ppm for ^1H and ^{13}C, respectively), unless otherwise indicated. Mass spectra were obtained from NuMega Resonance Labs Inc. on a Perkin-Elmer PE-SCIEX API 100 mass spectrometer by infusion. Samples were ionized by electrospray and spectra were recorded both in positive and negative modes (mass spectral data are reported in this order). Melting points were determined with a Hoover capillary melting point apparatus. Purity of all daidzin analogues synthesized in this laboratory was verified on a Waters HPLC system consisting of a 717 plus autosampler, 600s controller, 626 solvent delivering system, and 440 absorbance detector. Samples were analyzed on a Beckman ultrasphere ODS 5-μm, 4.6- × 250-mm column under two elution conditions: (a) a 30-min linear gradient: 12−80% acetonitrile in 0.1% TFA and (b) an isocratic condition in a mobile phase containing 0.1% TFA and a specified concentration of acetonitrile. Flow rates were set at 1 mL/min.

7-Hydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (Daidzein, 2). A solution of 5.000 g (45.41 mmol) of resorcinol and 6.910 g (45.41 mmol) of *p*-hydroxyphenylacetic acid in 111.7 mL (0.908 mol) of freshly distilled BF_3 etherate was stirred at 70 °C (oil bath) for 24 h in a 500 mL three-neck round-bottom flask fitted with a dropping funnel and a reflux condenser. The resulting yellow suspension was cooled to room

temperature, and 70 mL of dry DMF was added slowly via the dropping funnel. The reaction mixture was then heated to 50 °C, and a solution of 11 mL of mesyl chloride in 18 mL of dry DMF was added slowly. The resulting orange mixture was stirred at 65−70 °C and the reaction was monitored by HPLC. After about 4.5 h, the reaction mixture was quickly poured into 200 mL of ice water. The pH of the solution was adjusted slowly to 7 with 30% NaOH at 0 °C and the product was allowed to precipitate overnight at 5 °C. Precipitates were collected by filtration, washed 4 times each with 20 mL of H_2O, and dried over P_2O_5 to give 10.3 g (91% yield) of crude product. Recrystallization of this product from EtOH/$CHCl_3$/ether gave a total of 7.695 g of pure **2**, a 67% yield: mp 310−315 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.27 (s, 1 H, H-2), 7.95 (d, J = 8.5 Hz, 1 H, H-5), 7.37 (d, J = 9.0 Hz, 2 H, H-2′), 6.93 (d, J = 8.5 Hz, 1 H, H-6), 6.84 (s, 1 H, H-8), 6.79 (d, J = 9.0 Hz, 2 H, H-3′); ^{13}C NMR (DMSO-d_6) δ 174.72 (C-4), 162.51 (C-7), 157.45, 157.20, 152.79 (C-2), 130.10 (C-2′, 6′), 127.30 (C-5), 123.52 (C-3), 122.58 (C-1′), 116.66 (C-4a), 115.13 (C-3′, 5′), 114.98 (C-6), 102.12 (C-8); MS m/z 255 (M + H^+), 277 (M + Na^+), 293 (M + K^+), 253 (M − H^+), 289 (M + Cl^-), 367 (M + CF_3COO^-); HPLC t_R 14.9 min (gradient), 14.2 min (24% acetonitrile, isocratic).

7-*O*-(5-Carboxypentyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Carboxypentyldaidzein, 3). To a suspension of 5.000 g (19.68 mmol) of **2** and 75 mL of acetone was added 19 mL of 2 *N* aq KOH (39.37 mmol). The resulting mixture was stirred vigorously at room temperature until **2** was totally dissolved. To this solution, 6-bromohexanoic acid (3.837 g, 19.68 mmol) was added and the reaction mixture was stirred under gentle reflux for 3 days. After 12 h at 4 °C, the carboxylic acid potassium salt precipitate was collected on a fritted funnel and washed with small portions of cold acetone. The product was resuspended in 75 mL of H_2O, and the pH of the solution was adjusted to 2 with 1 N HCl. The white fluffy precipitates were collected on a fritted funnel, rinsed with cold H_2O until the pH of the filtrate was close to neutral, and dried over P_2O_5 to give 1.410 g of the desired product **3**. Unreacted **2** was recovered by concentrating the acetone/aqueous KOH filtrate on rotary evaporator. The remaining aqueous solution was acidified to pH 3 with 1 N HCl, kept at 5 °C for 12 h, and the precipitates were collected and washed with H_2O to recover 3.963 g of **2**. Compound **3**: mp 223−225 °C; ^{1}H NMR (DMSO-d_6) δ 9.58 (broad s, 1 H, 4′-OH), 8.35 (s, 1 H, H-2), 8.00 (d, J = 8.9 Hz, 1 H, H-5), 7.39 (d, J = 8.6 Hz, 2 H, H-2′), 7.12 (d, J = 2.3 Hz, 1 H, H-8), 7.06 (dd, J = 8.9, 2.3 Hz, 1 H, H-6), 6.80 (d, J = 8.6 Hz, 2 H, H-3′), 4.11 (t, J = 7.0 Hz, 2 H, CH_2O), 2.24 (t, J = 7.3 Hz, 2 H, CH_2C═O), 1.76 (quintet, J = 6.9 Hz, 2 H), 1.57 (quintet, J = 7.4 Hz, 2 H), 1.43 (quintet, J = 7.0 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 174.70 (C-4), 174.42 (CO_2H), 163.03 (C-7), 157.40 (C-8a), 157.23 (C-4′), 153.09 (C-2), 130.06 (C-2′, 6′), 126.90 (C-5), 123.67 (C-3), 122.38 (C-1′), 117.50 (C-4a), 114.96 (C-3′, 5′, 6 overlap), 100.95 (C-8), 68.35 (C−O), 33.59, 28.15, 25.05, 24.22; MS m/z 369 (M + H^+), 391 (M + Na^+), 407 (M + K^+), 253 (M-[$(CH_2)_5CO_2H]^+$), 367 (M − H^+), 403 (M + Cl^-), 481 (M + CF_3COO^-); HPLC t_R 20.3 min (gradient), 19.5 min (32% acetonitrile, isocratic).

7-*O*-(6-Carboxyhexyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Carboxyhexyldaidzein, 4). To a suspension of 2.540 g (10.00 mmol) of **2** and 40 mL of acetone was added 10 mL of 2 N aq KOH (20.00 mmol). The resulting mixture was stirred vigorously at room temperature until **2** was completely dissolved. To this solution, 2.09 g of 7-bromoheptanoic acid (10.00 mmol) was added and the reaction mixture was stirred under gentle reflux for 3 days. After 12 h at 4 °C, the carboxylic acid potassium salt formed was collected on a fritted funnel and washed with small portions of cold acetone. The precipitate was resuspended in 100 mL of H_2O and the pH of the suspension was adjusted to 2 with 15% HCl. The white fluffy precipitate collected on a fritted funnel was dissolved in 100 mL of MeOH with heating. The resulting solution was slowly cooled to room temperature and kept at 4 °C overnight. The white precipitate was collected on a fritted funnel to give 775 mg of **4**: mp 193−198 °C; ^{1}H NMR (DMSO-d_6) δ 8.24 (s, 1 H, H-2), 7.93 (d, J = 8.9 Hz, 1 H, H-5), 7.34 (d, J = 8.4 Hz, 2 H, H-2′), 7.02 (s, 1 H, H-8), 7.00 (d, J = 8.9 Hz, 1 H, H-6), 6.82 (d, J = 8.4 Hz, 2 H, H-3′), 4.02 (t, J = 7.0 Hz, 2 H, CH_2O), 1.88 (t, J = 7.1 Hz, 2 H, CH_2C═O), 1.70 (quintet, J = 7.3 Hz, 2 H), 1.46 (quintet, J = 7.0 Hz, 2 H), 1.37 (quintet, J = 7.0 Hz, 2 H), 1.29 (quintet, J = 7.0 Hz, 2 H); ^{13}C NMR (DMSO-d_6) δ 174.72 (C-4, COOH), 163.03 (C-7), 157.33 (C-8a, 4′), 152.84 (C-2), 129.85 (C-2′, 6′), 126.84 (C-5), 123.75 (C-3), 121.59 (C-1′), 117.42 (C-4a), 115.19 (C-3′, 5′), 114.87 (C-6), 100.83 (C-8), 68.51 (C−O), 38.29, 29.08, 28.53, 26.27, 25.38; MS m/z 383 (M + H^+), 421 (M + K^+), 253 (M − $(CH_2)_6CO_2H^+$), 381 (M − H^+), 417 (M + Cl^-), 495 (M + CF_3COO^-); HPLC t_R 22.1 min, (gradient), 41.4 min (32% acetonitrile, isocratic).

7-*O*-(9-Carboxynonyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Carboxynonyldaidzein, 5). To a suspension of 1.362 g (5.36 mmol) of **2** and 22 mL of acetone was added 5.35 mL of 2 N aq KOH (10.72 mmol). The resulting mixture was stirred vigorously at room temperature until **2** was completely dissolved. To this solution, 1.345 g of 10-bromodecanoic acid (5.36 mmol) was added and the reaction mixture was stirred under gentle reflux for 3 days. The carboxylic acid potassium salt formed was precipitated at 5−6 °C for 12 h, collected on a fritted funnel, and washed with small portions of cold acetone to give 675 mg of pure **5**: mp 248−251 °C; ^{1}H NMR (DMSO-d_6, 58 °C) δ 8.28 (s, 1 H, H-2), 8.01 (d, J = 8.9 Hz, 1 H, H-5), 7.38 (d, J = 8.3 Hz, 2 H, H-2′), 7.07 (s, 1 H, H-8), 7.05 (d, J = 8.9 Hz, 1 H, H-6), 6.82 (d, J = 8.3 Hz, 2 H, H-3′), 4.12 (t, J = 7.0 Hz, 2 H, CH_2O), 2.24 (t, J = 7.1 Hz, 2 H, CH_2C═O), 1.76 (quintet, J = 7.3 Hz, 2 H), 1.53 (broad quintet, 2 H), 1.43 (broad quintet, 2 H), 1.30−1.2 (m, 8 H); HPLC t_R 27.7 min (gradient), 35.7 min (44% acetonitrile, isocratic).

7-*O*-(10-Carboxydecyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Carboxydecyldaidzein, 6). To a suspension of 1.500 g of **2** (5.90 mmol) and 35 mL of acetone was added 8.8 mL of 2 N aq KOH (17.71 mmol). The resulting mixture was stirred vigorously at room temperature until **2** was completely dissolved. To this solution, 6-bromoundecanoic acid (3.132 g, 11.81 mmol) was added and the reaction mixture was stirred under gentle reflux for 3 days. Acetone was removed by flash evaporation. The remaining aqueous solution was neutralized by the addition of 17 mL of 1 N HCl and the resulting suspension was kept at 5−6 °C for 24 h. The precipitates were collected on a fritted funnel, washed with small portions of H_2O and 2 × 25 mL of petroleum ether, and dried over P_2O_5 to yield 3.45 g of crude product. The dry product was then washed with 3 × 75 mL of hot $CHCl_3$ on a fritted funnel and filtrates were combined and evaporated to dryness (2.174 g). The resulting solid was washed with several portions of diethyl ether until bromoundecanoic acid was no longer detectable (by NMR) in the wash. This procedure yielded 1 g of **6**: mp 68−71 °C; ^{1}H NMR (DMSO-d_6) δ 9.53 (broad s, 1 H, 4′-OH), 8.36 (s, 1 H, H-2), 8.01 (d, J = 8.7 Hz, 1 H, H-5), 7.39 (d, J = 8.1 Hz, 2 H, H-2′), 7.13 (s, 1 H, H-8), 7.06 (d, J = 8.7 Hz, 1 H, H-6), 6.81 (d, J = 8.1 Hz, 2 H, H-3′), 4.11 (t, J = 7.0 Hz, 2 H, CH_2O), 2.15 (t, J = 7.3 Hz, 2 H, CH_2C═O), 1.75 (quintet, J = 7.3 Hz, 2 H), 1.48 (quintet, J = 7.3 Hz, 2 H), 1.42 (quintet, J = 7.3 Hz, 2 H), 1.32−1.23 (m, 10 H); ^{13}C NMR (DMSO-d_6) δ 174.70 (C-4, CO_2H), 163.06 (C-7), 157.42 (C-8a), 157.23 (C-4′), 153.11 (C-2), 130.06 (C-2′, 6′), 126.91 (C-5), 123.67 (C-3), 122.38 (C-1′), 117.48 (C-4a), 114.96 (C-3′, 5′, 6 overlap), 100.95 (C-8), 68.46 (C−O), 33.65, 28.91, 28.83, 28.71 (2 C), 28.54, 28.39, 25.41, 24.48; HPLC t_R 29.7 min (gradient), 43.7 min (46% acetonitrile, isocratic).

Preparation of ω-Bromoalkyldaidzein 7-*O*-Ethers from 2. To a suspension of **2** in acetone was added 2 N aqueous KOH (1 equiv) and a dibromoalkane (10 equiv). The mixtures were refluxed for 18−24 h. After the reaction had reached completion, as indicated by TLC, the mixture was cooled to room temperature and acetone was evaporated on a rotary evaporator. The residue was diluted with H_2O and the pH of the solution was adjusted to 4 with 1 N HCl. An aqual volume of petroleum ether was then added to precipitate the crude products. The suspension was stirred with a spatula for 10 min. The precipitates were collected on a fritted funnel, washed

successively with H_2O and petroleum ether (3 times each), and dried over P_2O_5. The crude solid was washed with hot $CHCl_3$ (as many times as TLC proved necessary), leaving the insoluble unreacted **2**. The combined chloroform filtrates were concentrated to an oil on a rotary evaporator. The desired product was precipitated with petroleum ether, collected on a fritted funnel, washed with small portions of $CHCl_3$/petroleum ether 2/1, and dried under vacuum over P_2O_5. The powders were recrystallized from an appropriate solvent to give pure desired products as off-white solids.

7-*O*-(3-Bromopropyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Bromopropyldaidzein, 7). This compound was prepared as described in the general procedure from 2 g of **2** (7.87 mmol) and 15.898 g of 1,3-dibromopropane (78.74 mmol) in 40 mL of acetone. The product was recrystallized from 95% ethanol to yield 1.353 g of **7**: mp 178−179 °C; ^{1}H NMR (DMSO-d_6) δ 9.58 (s, 1 H, OH), 8.37 (s, 1 H, H-2), 8.02 (d, J = 8.8 Hz, 1 H, H-5), 7.39 (d, J = 8.5 Hz, 2 H, H-2′), 7.18 (d, J = 2.3 Hz, 1 H, H-8), 7.09 (dd, J = 8.8, 2.3 Hz, 1 H, H-6), 6.80 (d, J = 8.5 Hz, 2 H, H-3′), 4.24 (t, J = 6.1 Hz, 2 H, CH_2O), 3.68 (t, J = 6.1 Hz, 2 H, CH_2Br), 2.30 (quintet, J = 6.1 Hz, 2 H, CH_2); ^{13}C NMR (DMSO-d_6) δ 174.68 (C-4), 162.66 (C-7), 157.35 (C-8a), 157.23 (C-4′), 153.15 (C-2), 130.05 (C-2′, 6′), 127.00 (C-5), 123.69 (C-3), 122.33 (C-1′), 117.71 (C-4a), 114.95 (C-3′, 5′), 114.89 (C-6), 101.13 (C-8), 66.26 (C−O), 31.55 (C−Br), 30.97; MS m/z 375 (M + H$^+$), 377 (M + 2 + H$^+$), 397 (M + Na$^+$), 399 (M + 2 + Na$^+$); HPLC t_R 25.6 min (gradient), 30.3 min (40% acetonitrile, isocratic).

7-*O*-(4-Bromobutyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Bromobutyldaidzein, 8). This compound was synthesized as described in the general procedure from 2 g of **2** (7.87 mmol) and 8.501 g of 1,4-dibromobutane (39.35 mmol) in 40 mL of acetone. The product was recrystallized from $CHCl_3$/diethyl ether (3/1) to yield 1.310 g of **8**: mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 9.53 (s, 1 H, OH), 8.37 (s, 1 H, H-2), 8.02 (d, J = 8.9 Hz, 1 H, H-5), 7.40 (d, J = 8.5 Hz, 2 H, H-2′), 7.15 (d, J = 2.2 Hz, 1 H, H-8), 7.08 (dd, J = 8.9, 2.2 Hz, 1 H, H-6), 6.81 (d, J = 8.5 Hz, 2 H, H-3′), 4.17 (t, J = 6.2 Hz, 2 H, CH_2O), 3.63 (t, J = 6.6 Hz, 2 H, CH_2Br), 1.99 (quintet, J = 6.8 Hz, 2 H), 1.89 (quintet, J = 6.8 H, 2 H); ^{13}C NMR (DMSO-d_6) δ 174.69 (C-4), 162.87 (C-7), 157.37 (C-8a), 157.23 (C-4′), 153.09 (C-2), 130.05 (C-2′, 6′), 126.92 (C-5), 123.67 (C-3), 122.36 (C-1′), 117.57 (C-4a), 114.96 (C-3′, 5′, 6 overlap), 101.00 (C-8), 67.59 (C−O), 34.72, 28.95, 27.10; MS m/z 389 (M + H$^+$), 391 (M + 2 + H$^+$), 411 (M + Na$^+$), 413 (M + 2 + Na$^+$), 427 (M + K$^+$), 429 (M + 2 + K$^+$), 387 (M − H$^+$), 389 (M + 2 − H$^+$), 423 (M + Cl$^-$), 425 (M + 2 + Cl$^-$); HPLC t_R 27.4 min (gradient), 14.3 min (48% acetonitrile, isocratic).

7-*O*-(6-Bromohexyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-ω-Bromohexyldaidzein, 9). This compound was prepared as described in the general procedure from 2.520 g of **2** (9.92 mmol) and 24.583 g of 1,6-dibromohexane (0.10075 mol) in 50 mL of acetone. The crude product was recrystallized from chloroform/diethyl ether (2/1) to give 2.229 g of pure **9**: mp 140−142 °C; ^{1}H NMR (DMSO-d_6) δ 9.58 (broad s, 1 H, OH), 8.36 (s, 1 H, H-2), 8.01 (d, J = 8.8 Hz, 1 H, H-5), 7.39 (d, J = 7.0 Hz, 2 H, H-2′), 7.13 (s, 1 H, H-8), 7.06 (d, J = 8.8 Hz, 1 H, H-6), 6.81 (d, J = 7.0 Hz, 2 H, H-3′), 4.12 (t, J = 6.5 Hz, 2 H, CH_2O), 3.54 (t, J = 6.5 Hz, 2 H, CH_2Br), 1.83 (m, 2 H, CH_2), 1.76 (m, 2 H, CH_2), 1.46 (m, 4 H, CH_2); ^{13}C NMR (DMSO-d_6) δ 174.67 (C-4), 163.01 (C-7), 157.38 (C-8a), 157.22 (C-4′), 153.08 (C-2), 130.04 (C-2′, 6′), 126.90 (C-5), 123.65 (C-3), 122.36 (C-1′), 117.48 (C-4a), 114.94 (C-3′, 5′, 6 overlap), 100.93 (C-8), 68.33 (C−O), 35.07, 32.14, 28.22, 27.24, 24.57; MS m/z 417 (M + H$^+$), 419 (M + H$^+$ + 2); HPLC t_R 29.1 min (gradient), 31.7 min (46% acetonitrile, isocratic).

7-*O*-[2-(1,3-Dioxanyl)ethyl]-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-[2-(1,3-Dioxanyl)ethyl]daidzein, 10). To a solution containing 3.000 g of **2** (11.81 mmol), 6.5 mL of aqueous 2 N KOH (12.99 mmol) and 60 mL of acetone was added 2.1 mL of 2-(2-bromoethyl)-1,3-dioxane (2.995 g, 15.35 mmol). The reaction mixture was stirred under gentle reflux for 40 h and acetone was removed by flash evaporation. The remaining aqueous solution was diluted with 30 mL of H_2O (30 mL) and pH was adjusted to 7 with 1 N HCl. The product was precipitated by stirring this aqueous solution with 50 mL of petroleum ether for a few minutes. The precipitate, containing the product and unreacted **2**, was collected on a fritted funnel, washed successively with H_2O (3 × 10 mL) and petroleum ether (3 × 10 mL), and dried under vacuum over P_2O_5. The desired product was collected by washing the precipitate with hot chloroform (4 × 100 mL) and the combined chloroform extracts were evaporated to a solid residue which was recrystallized from 2-propanol to give 854 mg of pure **10** as a pale yellow solid: mp 178−179.5 °C; ^{1}H NMR (DMSO-d_6) δ 9.52 (s, 1 H, OH), 8.35 (s, 1 H, H-2), 8.00 (d, J = 8.8 Hz, 1 H, H-5), 7.39 (d, J = 8.5 Hz, 2 H, H-2′), 7.13 (d, J = 2.2 Hz, 1 H, H-8), 7.06 (dd, J = 8.8, 2.2 Hz, 1 H, H-6), 6.80 (d, J = 8.5 Hz, 2 H, H-3′), 4.75 (t, J = 5.2 Hz, 1 H, CH), 4.17 (t, J = 6.4 Hz, 2 H, CH_2OAr), 4.01 (dd, J = 11.2, 4.8 Hz, 2 H), 3.72 (dd, J = 11.2, 9.0 Hz, 2 H), 1.98 (td, J = 6.4, 5.2 Hz, 2 H), 1.88 (m, 2 H); ^{13}C NMR (DMSO-d_6) δ 174.69 (C-4), 162.76 (C-7), 157.37 (C-8a), 157.23 (C-4′), 153.11 (C-2), 130.06 (C-2′, 6′), 126.96 (C-5), 123.68 (C-3), 122.36 (C-1′), 117.62 (C-4a), 114.96 (C-3′, 5′), 114.86 (C-6), 101.03 (C-8), 98.59, 66.09, 64.17, 34.35, 25.35; MS m/z 369 (M + H$^+$), 367 (M − H$^+$), 403 (M + Cl$^-$); HPLC t_R 21.5 min (gradient), 26.3 min (32% acetonitrile, isocratic).

7-*O*-Allyl-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-Allyldaidzein, 11). To a suspension of 1.000 g of **2** (3.93 mmol) and 22 mL of acetone was added 2 mL of 11.2% KOH (4.00 mmol) and 5.000 g of allyl bromide (3.93 mmol). The resulting orange solution was refluxed for 18 h. The reaction mixture was cooled to room temperature and solvent was removed by rotary evaporation. The product was precipitated by adding 25 mL of petroleum ether, 4 mL (8 mmol) of 1 N HCl and 25 mL of H_2O. The precipitate formed upon stirring was collected on a fritted funnel, washed with 2 × 10 mL of petroleum ether and 2 × 10 mL of H_2O, and dried under vacuum over P_2O_5. The solid, containing the desired product and unreacted **2**, was washed with hot chloroform and (2 × 100 mL) and the combined chloroform filtrates were concentrated on a rotary evaporator and the desired product was precipitated from the concentrate by adding three pipets full of petroleum ether. The product was further purified by recrystallization (95% ethanol) to give 441 mg of pure **11** as a beige powder: mp 176−178 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (broad s, 1 H, OH), 8.35 (s, 1 H, H-2), 8.02 (d, J = 8.8 Hz, 1 H, H-5), 7.38 (d, J = 8.5 Hz, 2 H, H-2′), 7.15 (s, 1 H, H-8), 7.09 (d, J = 8.8 Hz, 1 H, H-6), 6.80 (d, J = 8.5 Hz, 2 H, H-3′), 6.06 (ddt, J = 17.3, 10.5, 4.4 Hz, 1 H, CH=), 5.44 (d, J = 17.3 Hz, 1 H, CH_2=), 5.30 (d, J = 10.5 Hz, 1 H, CH=), 4.72 (d, J = 4.4 Hz, 2 H, CH_2); ^{13}C NMR (DMSO-d_6) δ 174.69 (C-4), 162.47 (C-7), 157.31 (C-8a), 157.25 (C-4′), 153.14 (C-2), 132.81, 130.07 (C-2′, 6′), 126.97 (C-5), 123.70 (C-3), 122.35 (C-1′), 118.24, 117.68 (C-4a), 115.03 (C-3′, 5′), 114.97 (C-6), 101.36 (C-8), 68.98 (C−O); MS m/z 295 (M + H$^+$), 317 (M + Na$^+$), 293 (M − H$^+$); HPLC t_R 23.6 min (gradient), 43.6 min (32% acetonitrile, isocratic).

7-*O*-(2,3-Dihydroxypropyl)-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-(2,3-Dihydroxypropyl)daidzein, 12). To a solution of 647 mg of **11** (2.20 mmol) and 309 mg methylmorpholine *N*-oxide (2.64 mmol) in 60 mL of acetone/H_2O (3/2) was added 1.4 mL OsO_4 solution (2.5% in t-BuOH). The resulting solution was stirred at room temperature for 48 h. Then 35 mL of a 3.6% $Na_2S_2O_4$ solution was added to the solution and the resulting black mixture was stirred at room temperature for another 4 h. The mixture was filtered through Celite and the filtrate was evaporated to dryness. The residue was further dried over P_2O_5 and then washed with small portions (5−10 mL) of H_2O to give 486 mg of **12**: mp 188−191 °C; ^{1}H NMR (DMSO-d_6) δ 9.52 (s, 1 H, 4′-OH), 8.35 (s, 1 H, H-2), 8.01 (d, J = 8.9 Hz, 1 H, H-5), 7.39 (d, J = 8.3 Hz, 2 H, H-2′), 7.13 (s, 1 H, H-8), 7.07 (d, J = 8.9 Hz, 1 H, H-6), 6.80 (d, J = 8.3 Hz, 2 H, H-3′), 5.04 (d, J = 5.0 Hz, 1 H, CH-*OH*), 4.72 (t, J = 5.5 Hz, 1 H, CH_2-*OH*), 4.15 (m, 1 H, *CH*$_2$-OAr), 4.02 (m, 1 H, *CH*$_2$OAr), 3.83 (m, 1 H, *CH*-OH), 3.46 (t, J = 5.5 Hz, 2 H, *CH*$_2$−OH); ^{13}C NMR (DMSO-d_6) δ 174.72 (C-4), 163.20 (C-7), 157.39 (C-8a), 157.24 (C-4′), 153.12 (C-2),

130.08 (C-2′, 6′), 126.93 (C-5), 123.70 (C-3), 122.40 (C-1′), 117.54 (C-4a), 115.04 (C-3′, 5′), 114.98 (C-6), 101.02 (C-8), 70.51, 69.76, 62.50; MS *m*/*z* 329 ($M + H^+$), 351 ($M + Na^+$), 367 ($M + K^+$), 327 ($M - H^+$), 363 ($M + Cl^-$); HPLC t_R 12.3 min (gradient), 13.4 min (16% acetonitrile, isocratic).

7-Ethoxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one (7-*O*-Ethyldaidzein, 13). Finely ground KOH (442 mg, 7.87 mmol) was supended in 10 mL of DMSO (dried over 4 Å molecular sieves) for 10 min. Then 2 g of **2** (7.87 mmol) and 0.8 mL of ethyl iodide (1.596 g, 10.24 mmol) were added. Reaction was allowed to proceed with stirring at room temperature until completion as indicated by TLC (about 3.5 h). The reaction mixture was poured into 40 mL of ice water. The off-white precipitate was collected on a fritted funnel, washed with 2 × 10 mL of H_2O, and dried over P_2O_5. The dried product was recrystallized from 95% EtOH to give 2.043 g of **13**: mp 197−198.5 °C; ^{1}H NMR (DMSO-d_6) δ 9.58 (s, 1 H, OH), 8.34 (s, 1 H, H-2), 8.00 (d, J = 8.9 Hz, 1 H, H-5), 7.38 (d, J = 8.4 Hz, 2 H, H-2′), 7.10 (s, 1 H, H-8), 7.04 (d, J = 8.9 Hz, 1 H, H-6), 6.80 (d, J = 8.4 Hz, 2 H, H-3′), 4.16 (quartet, J = 6.4 Hz, 2 H, CH_2O), 1.36 (t, J = 6.4 Hz, 3 H, CH_3); ^{13}C NMR (DMSO-d_6) δ 174.69 (C-4), 162.89 (C-7), 157.40 (C-8a), 157.23 (C-4′), 153.07 (C-2), 130.06 (C-2′, 6′), 126.90 (C-5), 123.67 (C-3), 122.38 (C-1′), 117.48 (C-4a), 114.96 (C-3′, 5′), 114.90 (C-6), 100.89 (C-8), 64.18 (C−O), 14.35; MS *m*/*z* 283 ($M + H^+$), 305 ($M + Na^+$), 281 ($M - H^+$), 317 ($M + Cl^-$); HPLC: t_R 22.7 min (gradient), 33.7 min (32% acetonitrile, isocratic).

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AR, aldehyde reductase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; DOPAL, 3,4-dihydroxyphenylacetaldehyde; 5-HIAL, 5-hydroxyindole-3-acetaldehyde; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HTOL, 5-hydroxytryptophol; 5-HT, 5-hydroxytryptamine (serotonin); MAO, monoamine oxidase; AUC, area under the curve.

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