Synthesis of Potential Antidipsotropic Isoflavones: Inhibitors of the Mitochondrial Monoamine Oxidase−Aldehyde Dehydrogenase Pathway

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Recently we have shown that daidzin, the major active principle of an ancient herbal treatment for "alcohol addiction", suppresses ethanol intake in alcohol-preferring laboratory animals. Further, we have identified the monoamine oxidase (MAO)−aldehyde dehydrogenase (ALDH-2) pathway of the mitochondria as the potential site of action of daidzin. Daidzin analogues that potently inhibit ALDH-2 but have no or little effect on MAO are most antidipsotropic, whereas those that also inhibit MAO exhibit little, if any, antidipsotropic activity. Therefore, in the design and synthesis of more potent antidipsotropic analogues, structural features important for the inhibition of both ALDH-2 and MAO must be taken into consideration. To gain further information on the structure−activity relationships at the inhibitor binding sites of ALDH-2 and MAO, we prepared 44 analogues of daidzin and determined their potencies for ALDH-2 and MAO inhibition. Results indicate that a sufficient set of criteria for a potent antidipsotropic analogue is an isoflavone with a free 4′-OH function and a straight-chain alkyl substituent at the 7 position that has a terminal polar function such as −OH, −COOH, or $-NH_2$. The preferable chain lengths for the 7-*O*-ω-hydroxy, 7-*O*-ω-carboxy, and 7-*O*-ω-amino subsitutents are $2 \leq n \leq 6$, $5 \leq n \leq 10$, and $n \geq 4$, respectively. Analogues that meet these criteria have increased potency for ALDH-2 inhibition and/or decreased potency for MAO inhibition and therefore are likely to be potent antidipsotropic agents.

Introduction

For more than a millennium, herbalists practicing traditional Chinese medicine have used extracts of the root and flower of kudzu (*Pueraria lobata*) to treat "alcohol addiction". The efficacy of the root extract was first demonstrated in alcohol-preferring golden hamsters under strictly controlled laboratory conditions. Further, the isoflavone daidzin was isolated from the root and identified as its major active principle.[1,2] These findings were subsequently confirmed by us and other investigators in additional animal models commonly used in alcohol research.[3−6] These provide for the first time a scientific basis for the traditional use of kudzu in the treatment of alcohol addiction.

In vitro, daidzin is a potent and selective inhibitor of mitochondrial aldehyde dehydrogenase (ALDH-2),[7] the major ALDH isozyme that catalyzes the detoxification of ethanol-derived acetaldehyde.[8] This finding has led to the suggestion that daidzin may act as an alcohol-sensitizing agent.[1,2] However, later in vivo studies showed that daidzin, at doses that significantly suppress hamster alcohol intake, does not inhibit overall ethanol and acetaldehyde metabolism in this animal species.[9,10] On the basis of these results, we suggested that daidzin does not act by alcohol sensitization but may act by modulating the activity of an as-yet-undefined physiological pathway catalyzed by ALDH-2.

Recently, we have shown that daidzin 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.[11] This, together with the finding that daidzin does not affect the rates of mitochondria-catalyzed oxidative deamination of these monoamines, suggests that the ethanol intake suppressive (antidipsotropic) activity 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.[11] Correlation studies using structural analogues of daidzin revealed a positive correlation between the antidipsotropic activities of these analogues and their abilities to increase 5-HIAL accumulation during 5-HT metabolism in isolated liver mitochondria.[12] These studies also showed that daidzin analogues that potently inhibit ALDH-2 but have little or no effect on monoamine oxidase (MAO) are most antidipsotropic, whereas those that also potently inhibit MAO exhibit little, if any, antidipsotropic activity. On the basis of these results, we proposed that the mitochondrial MAO−ALDH-2 pathway is the site of action of daidzin and that a biogenic aldehyde derived from the action of MAO may directly or indirectly mediate its antidipsotropic action (Figure 1). The level of biogenic aldehyde in isolated mitochondria is determined by the relative activities of MAO and ALDH-2. Therefore, in the design and synthesis of more potent antidipsotropic analogues, structural features important for the inhibition of both enzymes needed to be considered. To gain more information on the structure−activity relationships at the

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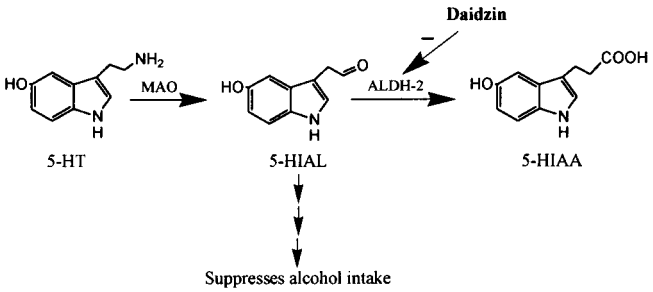


Figure 1. 5-HT metabolism in hamster liver mitochondria: a proposed site of action for daidzin.

inhibitor binding sites of ALDH-2 and MAO, we synthesized a series of analogues of daidzin and determined their potencies for ALDH-2 and MAO inhibition.

Results and Discussion

Synthesis, Purification, and Structural Identification of Analogues of Daidzin. The 7-*O*-mono- and 7,4′-*O*-disubstituted derivatives of daidzein were prepared via Williamson synthesis, by reacting the 7-*O*-mono- and 7,4′-*O*-diaroxide anions of daidzein with an appropriate alkyl halide RX (X = Br, Cl), respectively. The pK_a values of the 7- and 4′-hydroxyl groups of daidzein are 7.6 and 11.2, respectively, different enough to allow selective ionization of the 7-OH group under appropriate conditions ([base]/[daidzein] ≤ 1), thus permitting regioselective functionalization.[13] The 7,4′-disubstituted derivatives could be obtained by reacting daidzein with excessive amounts of base and RX. In this study, both the 7-*O*-mono- and 7,4′-*O*-disubstituted derivatives were obtained from one-pot reactions in which 1 < [base]/[daidzein] < 2. Reactions were carried out in acetone or DMF using KOH or K_2CO_3 as the bases, respectively.[14,15] The mono- and disubstituted species formed in the reaction mixtures were separated and purified by solvent extraction, column chromatography on Sephadex LH-20 and/or silica gel, and recrystallization from specified solvents and compositions (see the Experimental Section for details).

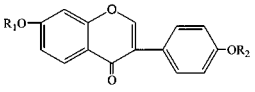
The structures and chemical compositions were determined by ^{1}H NMR, ^{13}C NMR, MS, and elementary analysis. NMR signals were assigned on the basis of standard spectra of daidzein.[12] Alkylation of the 7- and 4′-OH functions of daidzein resulted in additional H- and C-signals at high field (1.2−4.1 ppm for ^{1}H NMR, 24−33 ppm for ^{13}C NMR). The identities of various substituents were further verified on the basis of signals arising from their characteristic functional groups. For instance, (i) an α- or ω-carboxyl function on the 7- and 4′-substituents of **7**−**15**, **49,** and their ethyl esters **24**−**26**, **32**−**34**, and **43**−**45** was indicated by the presence of a carbonyl signal in the low field of ^{13}C NMR (168−174 ppm), (ii) terminal hydroxyl groups of **16**−**20** and **38** were identified by signals contributed by the hydroxymethylene function, which appeared at around 60 ppm, (iii) 7- and 4′-alkyl substituents in **4**−**6** were indicated by the signals of their terminal methyl groups, which appeared at 13.9 ppm for **6** and 21.6 ppm for **4**, and (iv) terminal double bonds on the side chains of **28** and **42** were verified by signals appearing at 5.01 (m, 2H) and 5.83 (m, 2H) ppm (^{1}H NMR), and at 138 ppm (^{13}C NMR) (see the Experimental Section for details). Molecular weights, melting points, and structural information data are listed in Table 1. The purities of all daidzin analogues prepared for this study are greater than 99.6% as judged by data from elementary analyses.

Structure−Activity Relationships (SARs): ALDH-2 Inhibition. 7-*O*-Substitution Is Crucial to ALDH-2 Inhibition. In previous studies,[9,12] we have shown that daidzein (7,4′-dihydroxyisoflavone) is a relatively poor ALDH-2 inhibitor (IC_{50} = 9 μM) whereas its 7-*O*-substituted derivatives, including daidzin (IC_{50} = 0.04 μM), inhibit the enzyme very potently (10−1000 times more potent). This result suggests that a variety of 7-*O*-substituted daidzein derivatives would be appropriate targets for designing novel daidzin analogues that could be more potent for ALDH-2 inhibition and therapeutically useful for the treatment of alcohol abuse and alcoholism. To gain further SAR information, we have synthesized and tested the potencies for ALDH-2 inhibition of nine series of 7-*O*-substituted daidzeins, namely, the 7-*O*-alkyl (**3**−**6**), 7-*O*-ω-carboxyalkyl (**7**−**15**), 7-*O*-ω-hydroxyalkyl (**16**−**20**), 7-*O*-ω-bromoalkyl (**21**−**23**), ethyl 7-*O*-ω-carboxyalkyl (**24**−**26**), 7-*O*-ω-alkenyl (**27, 28**), 7-*O*-α-carboxyalkyl (**29**−**31**), ethyl 7-*O*-α-carboxyalkyl (**32**−**34**), and 7-*O*-ω-aminoalkyl (**35, 36**) derivatives of daidzein. The results, expressed in IC_{50} values, are listed in Table 2. To facilitate dissolution, stock solutions of all analogues were prepared in DMSO/EtOH (9/1, v/v), and the final concentrations of these solvents in assay media were 0.09% and 0.01%, respectively. The IC_{50} values determined in the presence of these solvents are in general higher than those determined in their absence.[12]

Daidzein derivatives with a 7-*O*-triethyleneglycol (**20**) or a medium-chain-length (C-5 to C-10) 7-*O*-ω-carboxyalkyl (**9**−**13**) function are the most potent ALDH-2 inhibitors (IC_{50} values <0.06 μM), followed by the 7-*O*-ω-hydroxyalkyl (**16, 17**), 7-*O*-alkyl (**3, 4**), 7-*O*-glucosyl (**1**), and 7-*O*-ω-aminohexyl (**36**) derivatives (0.06 μM < IC_{50} ≤ 0.12 μM) and the 7-*O*-ω-bromoalkyl (**21**−**23**), 7-*O*-ω-alkenyl (**27, 28**), 7-*O*-ω-aminobutyl (**35**), and ethyl 7-*O*-α-carboxyethyl (**32**) derivatives (0.2 μM < IC_{50} ≤ 0.4 μM). Daidzein and the long-chain 7-*O*-alkyl (**5, 6**), 7-*O*-carboxyalkyl (**15**), 7-*O*-hydroxyalkyl (**18, 19**), and 7-*O*-α-carboxyalkyl (**29**−**31**, **33, 34**) derivatives of daidzein are the least potent with IC_{50} values ranging from 0.7 to ≫9 μM. Except for **6** and **15**, which have very long 7-*O*-substituents, alkylation of the 7-OH function of daidzein (**2**) improves potency for ALDH-2 inhibition.

Analogue **3** has the smallest 7-*O*-substituent, and yet it is as potent as daidzin (**1**) or other daidzin analogues with long-chain 7-*O*-substituents. This suggests that an increase in the size and/or length of the 7-*O*-substituent per se does not improve analogue binding. The fact that daidzein is a poor ALDH-2 inhibitor may stem from the weak electron-donating property of its free 7-OH group. Therefore, replacing 7-OH with stronger electron-donating groups such as the alkoxyls should enhance enzyme binding. Indeed, all 7-*O*-substituted daidzeins synthesized in this study have increased electron density in the A ring as suggested by the deshielding effect observed in their NMR spectra (signals of H-5, H-6, and H-8 were shifted to lower field in 7-*O*-substituted derivatives), and all but **6** and **15** are more potent ALDH-2 inhibitors than daidzein. Alternatively, binding of daidzein to its binding site on ALDH-2 may bring its

Table 1. Physical Constants of the Compounds Synthesized



No.	R_1	R_2	Formula	Molecular weight	mp (°C)
1[a]	Glc-	H	$C_{21}H_{20}O_9$	416.386	230-238
2[a]	H-	H	$C_{15}H_{10}O_4$	254.243	335
3[b]	CH_3CH_2-	H	$C_{17}H_{14}O_4$	282.297	197-198.5
4	$(CH_3)_2CH$-	H	$C_{18}H_{16}O_4$	296.323	169-171
5	$CH_3(CH_2)_5$-	H	$C_{21}H_{22}O_4$	338.404	148-149.5
6	$CH_3(CH_2)_{11}$-	H	$C_{27}H_{34}O_4$	422.565	131-133
7	HOOCCH$_2$-	H	$C_{17}H_{12}O_6$	312.280	244-245
8	HOOC$(CH_2)_4$-	H	$C_{20}H_{18}O_6$	354.339	232-235
9[b]	HOOC$(CH_2)_5$-	H	$C_{21}H_{20}O_6$	368.367	223-225
10[b]	HOOC$(CH_2)_6$-	H	$C_{22}H_{22}O_6$	382.393	193-198
11	HOOC$(CH_2)_7$-	H	$C_{23}H_{24}O_6$	396.441	192-193
12[b]	HOOC$(CH_2)_9$-	H	$C_{23}H_{28}O_6$	424.474	248-251
13[b]	HOOC$(CH_2)_{10}$-	H	$C_{24}H_{30}O_6$	438.501	68-71
14	HOOC$(CH_2)_{11}$-	H	$C_{27}H_{32}O_6$	452.549	238-240
15	HOOC$(CH_2)_{15}$-	H	$C_{31}H_{40}O_6$	508.656	151-152
16	HO-$(CH_2)_2$-	H	$C_{17}H_{14}O_5$	298.296	210-212
17	HO-$(CH_2)_6$-	H	$C_{21}H_{22}O_5$	354.404	177-178.5
18	HO-$(CH_2)_9$-	H	$C_{24}H_{28}O_5$	396.440	165-167
19	HO-$(CH_2)_{12}$-	H	$C_{27}H_{34}O_5$	438.547	142-144
20	H-$(OCH_2CH_2)_3$-	H	$C_{21}H_{22}O_7$	386.403	149-150
21[b]	Br-$(CH_2)_3$-	H	$C_{18}H_{15}O_4Br$	375.219	178-179
22[b]	Br-$(CH_2)_4$-	H	$C_{19}H_{17}O_4Br$	389.246	170-172
23[b]	Br-$(CH_2)_6$-	H	$C_{21}H_{21}O_4Br$	417.30	140-142
24	$C_2H_5OOCCH_2$-	H	$C_{19}H_{16}O_6$	340.334	169-171
25	$C_2H_5OOC(CH_2)_4$-	H	$C_{22}H_{22}O_6$	382.414	146-148
26	$C_2H_5OOC(CH_2)_5$-	H	$C_{23}H_{24}O_6$	396.441	130-131
27[b]	CH_2=CHCH$_2$-	H	$C_{18}H_{14}O_4$	294.308	176-178
28	CH_2=CH$(CH_2)_4$-	H	$C_{21}H_{20}O_4$	336.388	156-157
29	HOOC CH$_3$CH-	H	$C_{18}H_{14}O_6$	326.129	185-187
30	HOOC $CH_3(CH_2)_3CH$-	H	$C_{21}H_{20}O_6$	368.387	162-163
31	HOOC $CH_3(CH_2)_5CH$-	H	$C_{23}H_{24}O_6$	396.441	123-125
32	C_2H_5OOC CH$_3$CH-	H	$C_{20}H_{18}O_6$	354.339	167-169
33	C_2H_5OOC $CH_3(CH_2)_3CH$-	H	$C_{23}H_{24}O_6$	396.441	129-131
34	C_2H_5OOC $CH_3(CH_2)_5CH$-	H	$C_{25}H_{28}O_6$	424.495	118-120
35[c]	$H_2N(CH_2)_4$-	H	$C_{19}H_{19}O_4N$	325.365	242-245
36[c]	$H_2N(CH_2)_6$-	H	$C_{21}H_{23}O_4N$	353.419	235-238
37	HOOCCH$_2$-	R_1	$C_{19}H_{14}O_8$	370.317	268-270
38	HO-$(CH_2)_9$-	R_1	$C_{33}H_{46}O_6$	538.704	141-142
39	$(CH_3)_2CH_2$-	R_1	$C_{21}H_{22}O_4$	338.404	134-136
40	$CH_3(CH_2)_5$-	R_1	$C_{27}H_{34}O_4$	422.565	119-121
41	$CH_3(CH_2)_{11}$-	R_1	$C_{39}H_{58}O_4$	590.888	140-141
42	CH_2=CH$(CH_2)_4$-	R_1	$C_{27}H_{30}O_4$	418.534	112-115
43	$C_2H_5OOC(CH_2)_4$-	R_1	$C_{29}H_{34}O_8$	510.557	96-98
44	$C_2H_5OOC(CH_2)_5$-	R_1	$C_{31}H_{38}O_8$	538.640	99-101
45	C_2H_5OOC CH$_3$CH-	R_1	$C_{25}H_{26}O_8$	454.478	97-99

[a] Commercial products, over 99.5% pure as judged by HPLC analysis. [b] Compounds synthesized in a previous study (see ref 12). [c] Compounds synthesized and provided by Dr. X. Shen (current address: American Cyanamid Co., P.O. Box 400, Princeton, NJ 08543-0400).

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

	IC_{50}, μM			IC_{50}, μM			IC_{50}, μM	
compd	ALDH-2	MAO	compd	ALDH-2	MAO	compd	ALDH-2	MAO
1	0.08	ni	**20**	0.04	ni	**39**	ni	ni
2	9	14	**21**	0.26	0.15	**40**	ni	ni
3	0.08	0.3	**22**	0.27	4	**41**	ni	ni
4	0.08	0.2	**23**	0.3	2	**42**	ni	ni
5	1.5	ni	**24**	0.13	ni	**43**	ni	ni
6	≫9	ni	**25**	0.24	>9	**44**	ni	ni
7	1.2	≫9	**26**	0.28	5.3	**45**	ni	ni
8	0.1	5	**27**	0.18	0.45	**46**	>6	ni
9	0.06	4	**28**	0.15	5	**47**	ni	0.03
10	0.05	2.1	**29**	2.5	ni	**48**	ni	0.12
11	0.04	6.5	**30**	1	ni	**49**	3.5	7
12	0.05	10	**31**	2	ni	**50**	7	4.5
13	0.04	13	**32**	0.32	≫9	**51**	ni	13
14	0.13	ni	**33**	4.5	≫9	**52**	1.5	0.5
15	>9	ni	**34**	7.2	≫9	**53**	1.5	ni
16	0.07	1.5	**35**	0.25	>9	**54**	0.15	ni
17	0.12	>9	**36**	0.1	≫9	**55**	ni	ni
18	0.7	>9	**37**	≫9	ni	**56**	ni	ni
19	1.7	ni	**38**	ni	ni			

[a] MAO and ALDH-2 activity were assayed as described in the Experimental Section, using 10 μM 5-HT and 0.3 mM formaldehyde as the stubstrates, respectively. IC_{50} values were estimated graphically with inhibition data determined at more than six inhibitor concentrations. ni = no inhibition up to 10 μM.

free 7-OH group in close contact with a nonpolar region in the inhibitor binding pocket of the enzyme. Therefore, replacing the 7-OH of daidzein with a less polar alkoxyl function should lead to more potent inhibition. Further, this nonpolar region in the enzyme binding pocket must be relatively short and narrow because shifting the OH function two carbon atoms away from the 7 position increases potency of inhibition dramatically (from IC_{50} = 9 μM for daidzein to IC_{50} = 0.07 μM for **16**), whereas replacing the terminal hydroxymethylene function in **16** with a carboxyl group in **7** dramatically decreases its ability to inhibit ALDH-2 (from 0.07 to ≫9 μM).

Potencies for ALDH-2 inhibition of the 7-*O*-alkyl series are inversely proportional to the alkyl chain lengths. The IC_{50} value for ALDH-2 inhibition increases from 0.08 to 1.5 and ≫9 μM as the chain length increases from C-2 (**3**, **4**) to C-6 (**5**) and C-12 (**6**), respectively (Table 2). This trend also pertains to the series of 7-*O*-ω-hydroxyalkyl, ethyl 7-*O*-α-carboxyalkyl, and 7-*O*-ω-carboxyalkyl derivatives. However, potencies for ALDH-2 inhibition of the 7-*O*-alkyl derivatives with terminal hydroxyl and carboxyl functions do not drop off significantly until chain lengths are greater than 6 (**18, 19**) and 11 (**15**) carbon atoms, respectively. Further, addition of either a bromo (**23**) or an amino (**36**) end group also improves ALDH-2 inhibition. These, together with the fact that derivatives with 7-*O*-glucosyl (**1**), triethylene glycol (**20**), or *O*-ω-carboxyalkyl (**9**−**13**) functions are all potent inhibitors of ALDH-2, suggest that the subsite of the binding pocket of ALDH-2 that accommodates the 7-*O*-substituent of a daidzin analogue begins with a nonpolar bottleneck region, which is about 2−3 carbon atoms long measuring from the 7 position of the chromone structure, followed by a long, spacious and relatively hydrophilic pocket. It accommodates poorly nonpolar alkyls having chain lengths greater than three carbon atoms but receives very well long 7-*O*-substituents with hydrophilic end groups.

The Isoflavone Skeleton Is Important for ALDH-2 Inhibition. Flavonoid is one of the most abun-

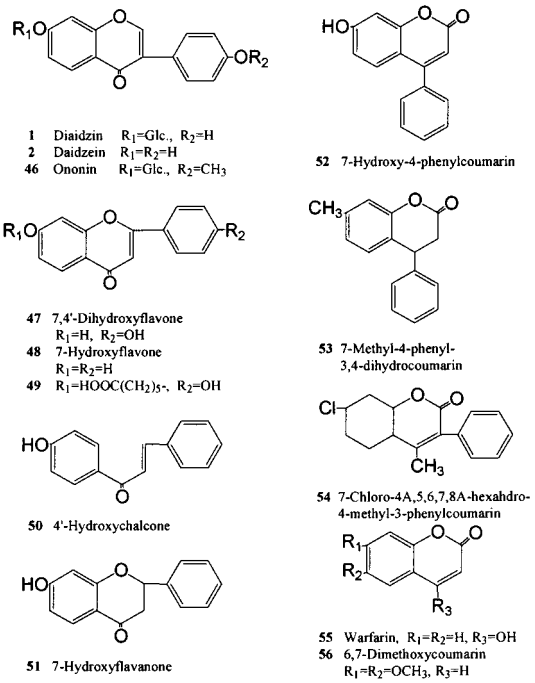


Figure 2. Structures of some isoflavones and chemically or biologically related compounds.

dant classes of phenolic compounds found in the plant kingdom that share a 1,3-diphenylpropane (2-phenylated) skeleton. They are structurally and biogenetically related to the isoflavonoids that have a 1,2-diphenylpropane (3-phenylated) skeleton. Despite their structural similarities, none of the flavones tested so far suppress alcohol intake in alcohol-preferring Syrian golden hamsters.[1] In an early study, we surveyed 29 commercially available flavones and isoflavones and showed that all flavones studied exhibit low, if any, ALDH-2 inhibitory activity.[7] To further evaluate the importance of the 1,2-diphenylpropane skeleton for ALDH-2 inhibition, we synthesized 7-*O*-*ω*-carboxypentyl-4′-hydroxyflavone (**49**) (Figure 2), a flavone analogue of 7-*O*-*ω*-carboxypentyl-4′-hydroxyisoflavone (**9**), and tested and compared their potencies for ALDH-2 inhibition. Results show that the isoflavone (**9**) is at least 50 times more potent than its flavone analogue (**49**) (IC_{50} = 0.06 and 3.5 μM, respectively). This, together with the fact that none of the flavones tested (**47, 48**) inhibit ALDH-2, suggests that the isoflavone skeleton is important for ALDH-2 inhibition (Table 2).

Potencies for ALDH-2 inhibition of five coumarins (**52**−**56**), one chalcone (**50**), and one flavanone (**51**) (see Figure 2 for the structures) have been studied before[7] and were reexamined under the same assay conditions described in this study. Results are consistent with the previous finding: only 4-phenyl (**52**, **53)** and 3-phenyl (**54**) substituted coumarins inhibit ALDH-2 (Table 2). In this context, it is of interest to note that, among the coumarin compounds tested, **54** is the most potent and has a skeletal ring structure similar to that of an isoflavone. Nevertheless, a direct comparison between this particular coumarin (**54**) and the isoflavones could be misleading because its saturated cyclohexyl ring is both spatially and electronically different from that of the aromatic A ring of an isoflavone so that its mode of binding may differ significantly from the SAR derived from the isoflavones.

Free 4′-OH Is Important for ALDH-2 Inhibition. To study the effect of 4′-*O*-substitution on the potency for ALDH-2 inhibition, we have synthesized nine 7,4′-*O*-disubstituted derivatives of daidzein, and tested and compared their ALDH-2 inhibitory activities with those of their 7-*O*-monosubstituted analogues. As shown in Table 2, none of the disubstituted derivatives inhibit ALDH-2. These results are consistent with our earlier finding that replacement of the 4′-OH of daidzin with a methoxy group (as in **46**) increases the inhibition constant by more than 100-fold.[7] On the basis of these results, we conclude that blocking the free 4′-OH of an isoflavone abolishes its ability to inhibit ALDH-2.

Structure−Activity Relationships: MAO Inhibition. 7-*O*-Substitution and MAO Inhibition. The structural requirements of the 7-*O*-substituent for MAO inhibition appear to be similar to those for ALDH-2 except that, in MAO, the 7 position of a bound inhibitor is located in a more spatially restricted area. MAO, like ALDH-2, is relatively resistant to daidzein inhibition (Table 2). The free 7-OH group again is the culprit for poor inhibition because its replacement with a short-chain hydrophobic alkyl (**3, 4**), bromoalkyl (**21**), or allyl (**27**) moiety greatly enhances the potency for MAO inhibition. Long-chain 7-*O*-alkyl derivatives of daidzein (**5**, **6**), however, are not inhibitory at all. This trend also pertains to the 7-*O*-*ω*-hydroxyalkyl (**16**−**19**), ethyl 7-*O*-α-carboxyalkyl (**29**−**31**), and 7-*O*-*ω*-carboxyalkyl (**10**−**15**) series. Replacement of the alkyl side chains of the noninhibitory analogues (**5**, **6**) with *ω*-carboxyalkyls of equal chain lengths (**9, 10**) improves the potency for MAO inhibition. Nevertheless, none of the 7-*O*-*ω*-carboxyalkyl derivatives (**7**−**15**), nor for that matter the 7-*O*-*ω*-hydroxyalkyl, 7-*O*-*ω*-bromoalkyl, and 7-*O*-*ω*-aminoalkylderivatives of daidzein with chain lengths greater than three carbon atoms, potently inhibit MAO. It appears that, in MAO, the 7-*O*-substituent of a bound inhibitor is located in a spatially restricted area. This area is likely to be hydrophobic because short-chain carboxyalkyl (**7**) and hydroxyalkyl (**16**) derivatives are poor MAO inhibitors.

4′-*O*-Substitution and MAO Inhibition. The effects of 4′-*O*-substitution on the potency for MAO inhibition were evaluated by testing and comparing the MAO inhibitory activities of a series of 7-*O*-monosubstituted derivatives (**4**−**7, 18, 25, 26, 28,** and **32**) and their 7,4′-*O*-disubstituted counterparts (**39**−**41, 37, 38, 43, 44, 42,** and **45**). It appears that the free 4′-OH groups of these analogues are important for MAO inhibition because replacement of the 4′-OH of an MAO inhibitory analogue (**4, 26,** or **28)** with their respective 7-*O*-substituents (**39, 44,** and **42**) invariably destroys their abilities to inhibit MAO (Table 2).

Flavones Rather Than Isoflavones Are Better MAO Inhibitors. Unlike ALDH-2, MAO appears to be more sensitive to flavone than isoflavone inhibition. Daidzein, a 7,4′-*O*-dihydroxyisoflavone, is a poor MAO inhibitor (IC_{50} = 14 μM), whereas **47**, its flavonoid counterpart, inhibits MAO very potently (IC_{50} = 0.03 μM). Like its isoflavone counterpart, substituting 7-OH with a six-carbon carboxyalkyl function (**49**) is detrimental to its potency for MAO inhibition (IC_{50} = 7 μM). 7-Hydroxyflavone (**48)** is also a potent inhibitor of MAO (IC_{50} = 0.12 μM). Therefore, unlike the isoflavones, the

4′-OH function of a flavonoid compound might not be critical for MAO inhibition. Coumarin compounds with proper substituents could be potent MAO inhibitors. For instance, 7-hydroxy-4-phenylcoumarin (**52**) is a fairly potent inhibitor of MAO (IC_{50} = 0.5 μM), whereas 7-methyl-4-phenylcoumarin (**53**) does not inhibit MAO at all. This suggests that the mode of binding of the flavones to the enzyme is significantly different from that of the coumarins.

Summary

We have prepared a series of daidzein (**2**) derivatives in which the 7-OH group of the parent molecule was replaced with 7-*O*-alkyl (**3**−**6**), 7-*O*-*ω*-carboxyalkyl (**7**−**15**), 7-*O*-*ω*-hydroxyalkyl (**16**−**19**), 7-*O*-*ω*-bromoalkyl (**21**−**23**), ethyl 7-*O*-*ω*-carboxyalkyl (**24**−**26**), 7-*O*-*ω*-allyl (**27, 28**), 7-*O*-α-carboxyalkyl (**29**−**31**), ethyl 7-*O*-α-carboxyalkyl (**32**−**34**), and 7-*O*-*ω*-aminoalkyl (**35, 36**) substituents. Their potencies for ALDH-2 and MAO inhibition were determined and compared among each other and with those of the 7-*O*-glucosyl derivative daidzin (**1**). Results reveal that alkylation of the 7-OH of **2** is essential for potent inhibition of both ALDH-2 and MAO. However, long-chain 7-*O*-substituents have no or decreased inhibitory activities toward both enzymes. The optimal chain length varies for both ALDH-2 and MAO inhibition, depending on the chemical nature of the end group and the enzyme. It appears that a straight-chain alkyl substituent with a polar end group at the 7 position of an isoflavone is the key to a potent and yet selective inhibitor of ALDH-2 and hence a potent antidipsotropic agent.

We have also begun to explore the effect of 4′-substitution and the isoflavone skeleton on ALDH-2 and MAO inhibition. Preliminary results suggest that a free −OH at the 4′ position may be crucial for both ALDH-2 and MAO inhibition and that isoflavones, as opposed to flavones, inhibit ALDH-2 more potently than MAO and hence are more likely to be antidipsotropic.

Three-dimensional structures of ALDH-2− and MAO−isoflavonoid inhibitor complexes are still unknown at this time, which hence precludes the discovery of potential lead compounds based solely on rational design. However, interactive docking using selected analogues and the X-ray structure of ALDH-2[17,18] should provide useful information on the mode of enzyme−inhibitor binding, and this approach is currently being pursued.

Experimental Section

General chemicals were purchased from either Aldrich Chemical Co. (Milwaukee, WI) or Lancaster Synthesis Inc. (Windham, NH). All organic solvents used were of HPLC grade and were supplied by J.P. Baker (Phillipsburg, NJ) or Fisher Scientific Co. (Pittsburgh, PA). Daidzin (**1**) was purchased from LC Laboratories (Woburn, MA). Daidzein (**2**) was synthesized by Tyger Scientific Inc. (Princeton, NJ). Ononin (**46**), 7,4′-dihydroxyflavone (**47**), 7-hydroxyflavone (**48**), 4′-hydroxychalcone (**50**), and 7-hydroxyflavanone (**51**) were purchased from Indofine Chemical Co. (Somerville, NJ). 7-Hydroxy-4-phenylcoumarin (**52**), 7-methyl-4-phenyl-3,4-dihydrocoumarin (**53**), 7-chloro-4A,5,6,7,8A-hexahydro-4-methyl-3-phenylcoumarin (**54**), warfarin (**55**), and 6,7-dimethoxycoumarin (**56**) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 7-*O*-Ethyldaidzein (**3**), 7-*O*-*ω*-carboxypentyldaidzein (**9**), 7-*O*-*ω*-carboxyhexyldaidzein (**10**), 7-*O*-*ω*-carboxynonyldaidzein (**12**), 7-*O*-*ω*-carboxydecyldaidzein (**13**), 7-*O*-*ω*-bromopropyldaidzein (**21**), 7-*O*-*ω*-bromobutyldaidzein (**22**), 7-*O*-*ω*-bromohexyldaidzein (**23**), and 7-O-allyldaidzein (**27**) were synthesized as described in a previous study.[12] The remaining compounds (**4**−**8, 11, 14**−**20, 24**−**26, 28**−**34,** and **37**−**45**) were synthesized as described below. Serotonin (5-HT) was purchased from Research Biochemical International (Natick, MA), and its 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.[16] All other reagents used were of the best grade available.

MAO and ALDH-2 Assays. The membrane and supernatant fractions of the lysate of a gradient-purified hamster liver mitochondria preparation were used as sources of MAO and ALDH-2, respectively. ALDH-2 and MAO activity assays were performed according to the procedures reported before.[12] To facilitate dissolution, stock solutions of all test compounds were prepared in DMSO/ethanol (90/10, v/v), and their final concentrations in all assay media, including controls, were 0.09% and 0.01%, respectively.

Synthesis. General Methods. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 BQ spectrometer at 500 MHz and a Bruker AM-500 spectrometer at 126 MHz (NuMega Resonace Laboratories Inc., San Diego, CA), respectively, using DMSO as solvent and as internal standard (2.50 and 39.51 ppm for ^{1}H and ^{13}C, respectively) unless otherwise indicated. Mass spectra were measured on a Perkin-Elmer PE-SCIEX API 100 mass spectrometer by infusion. Samples were ionized by electrospray, and spectra were recorded in positive mode. Melting points were determined with a Hoover capillary melting point apparatus. Elementary analyses were performed by NuMega Resonance Laboratories Inc. Crude synthetic products were purified by one or a combination of the following methods: chromatography on a Sephadex LH-20 (Fluka, 25−100 μm) or a silica gel 60 (70−230 mesh, EM Science) column and recrystallization from acetone or chloroform/methanol of various proportions. Analytical thin-layer chromatography (TLC) was performed on Kiselgel 60F_{254} plates (Merck KgaA, Darmstadt, Germany).

7-*O*-Isopropyldaidzein (4) and 7,4′-Di-*O*-isopropyldaidzein (39). To a suspension of 5.1 g of **2** (20.08 mmol) and 60 mL of acetone was added 15 mL of 2 N KOH (30 mmol) with vigorous stirring until **2** was completely dissolved. To this solution was added 6 mL of 2-bromopropane (63.9 mmol), and the reaction mixture was refluxed with gentle stirring for 72 h. Solvent was removed on a rotary evaporator, and the residue was suspended in water and extracted with ethyl acetate. Ethyl acetate was removed, and the residue was fractionated on a Sephadex LH-20 column equilibrated in chloroform/methanol (7/3, v/v). Fractions that contained **4** or **39** were pooled separately (monitored by TLC), dried, and recrystallized from chloroform/methanol (7/3) to give 1.8 g of **4** and 1.31 g of **39**. Analyses (**4**): colorless crystalline plates; yield 30.3% (**4**/**2**, mol/mol); mp169−171 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (m, 3H, $-CH_3$), 1.31 (m, 3H, $-CH_3$), 4.85 (m, 1H, −OCH), 6.81 (dd, 2H, J = 8.15, 1.7 Hz, H-3′, H-5′), 7.02 (dd, 1H, J = 8.86, 2.1 Hz, H-6), 7.11 (d, 1H, J = 2.5 Hz, H-8), 7.39 (dd, 2H, J = 8.15, 1.68 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.55 (s, 1H, OH-4′); ^{13}C NMR δ 21.5 ($-CH_3$), 21.8 ($-CH_3$), 70.4 (−OCH<), 101.7 (C-8), 114.9 (C-6), 115.1 (C-3′, C-5′), 117.3 (C-10), 122.4 (C-1′), 123.6 (C-3), 127.0 (C-5), 130.0 (C-2′, C-6′), 153.0 (C-2), 157.1 (C-9), 157.2 (C-4′), 161.9 (C-7), 174.6 (C-4); MS m/z 297.5 $(M + H)^+$. Anal. Calcd ($C_{18}H_{16}O_4$): C, 72.96; H, 5.44. Found: C, 72.96; H, 5.25. Analyses (**39**): colorless crystalline needles; yield 19.3%; mp134−136 °C; ^{1}H NMR and ^{13}C NMR similar to those of **4** and consistent with the interpretation that **39** is a 7,4′-disubstituted analogue of **4**; MS m/z 339.5 $(M + H)^+$. Anal. Calcd ($C_{21}H_{22}O_4$): C, 74.54; H, 6.55. Found: C, 74.70; H, 6.68.

7-*O*-Hexyldaidzein (5) and 7,4′-Di-*O*-hexyldaidzein (40). The monohexyl (**5**) and dihexyl (**40**) daidzeins were synthesized and purified by the same methods described for **4** and **39** using 6-bromohexane (3.5 mL) as the alkylating agent. Analyses (**5**): colorless crystalline needles; yield 26.9%; mp145−148 °C;

^{1}H NMR (DMSO-d_6) δ 0.874 (t, 3H, $-CH_3$), 1.30 (m, 4H, $-CH_2-CH_2-$), 1.45 (m, 2H, $-CH_2-$), 1.75 (m, 2H, $-CH_2-$), 4.09 (m, $-OCH_2-$), 6.81 (dd, 2H, J = 8.7, 1.77 Hz, H-3′, H-5′), 7.03 (dd, 1H, J = 8.86, 1.9 Hz, H-6), 7.10 (d, 1H, J = 1.9 Hz, H-8), 7.40 (dd, 2H, J = 8.7, 1.77 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.53 (s, 1H, OH-4′); ^{13}C NMR δ 13.9 ($-CH_3$), 22.0 ($-CH_2-$), 25.1 ($-CH_2-$), 28.4 ($-CH_2-$), 30.9 ($-CH_2-$), 68.4 ($-OCH_2-$), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 153.0 (C-2), 157.2 (C-9), 157.3 (C-4′), 163.0 (C-7), 174.7 (C-4); MS m/z 339.3 $(M + H)^+$. Anal. Calcd ($C_{21}H_{22}O_4$): C, 74.54; H, 6.55. Found: C, 74.60; H, 6.82. Analyses (**40**): colorless crystalline plates; yield 4.2%; mp 119−121 °C; MS m/z 425.5 $(M + H)^+$; ^{1}H NMR and ^{13}C NMR spectra similar to those of **5** and consistent with the interpretation that **40** is a 7,4′-disubstituted analogue of **5**. Anal. Calcd ($C_{27}H_{34}O_4$): C, 76.74; H, 8.11. Found: C, 76.30; H, 7.92.

7-*O*-Dodecyldaidzein (6) and 7,4′-Di-*O*-dodecyldaidzein (41). The monododecyl (**6**) and didodecyl (**41**) derivatives of daidzein were also synthesized and purified according to the methods described for **4** and **39** using 1-bromododecane as the alkylating agent. Analyses (**6**): colorless crystalline plates; yield 42%; mp 131−133 °C; ^{1}H NMR and ^{13}C NMR spectra virtually identical to those of **5** except signals contributed by the alkyl group on the seventh position; MS m/z 423.5 $(M + H)^+$. Anal. Calcd ($C_{27}H_{34}O_4$): C, 76.74; H, 8.11. Found: C, 77.11; H, 8.10. Analyses (**41**): colorless crystalline plates; yield 30%; mp 140−141 °C; ^{1}H NMR and ^{13}C NMR spectra similar to those of **6** and consistent with the interpretation that it is the 7,4′-disubstituted analogue of **6**; MS m/z 591.8 $(M + H)^+$. Anal. Calcd ($C_{39}H_{58}O_4$): C, 79.28; H, 9.89. Found: C, 79.18; H, 9.91.

7-*O*-Carboxymethyldaidzein (7), Its Ethyl Ester (24), and 7,4′-Di-*O*-carboxymethyldaidzein (37). To a solution of 5.1 g of **2** (20.08 mmol), 60 mL of acetone, and 10 mL of 2 N KOH (20 mmol) was added 2.5 mL of ethyl bromoacetate (21.54 mmol). The reaction mixture was refluxed under gentle stirring for 72 h and refrigerated for 12 h. The precipitates were collected on a fritted funnel and recrystallized from acetone to yield 1.42 g of pure **24**. Analyses (**24**): colorless crystalline needles; yield 20.9%; mp169−171 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, 3H, $-CH_3$), 4.19 (dd, 2H, $-CH_2O-$), 4.98 (s, 2H, $-CH_2O-$), 6.81 (dd, 2H, J = 8.47, 1.6 Hz, H-3′, H-5′), 7.10 (dd, 1H, J = 8.88, 2.6 Hz, H-6), 7.16 (d, 1H, J = 2.6 Hz, H-8), 7.40 (dd, 2H, J = 8.47, 1.6 Hz, H-2′, H-6′), 8.03(d, 1H, J = 8.88 Hz, H-5), 8.36 (s, 1H, H-2), 9.55 (s, 1H, OH-4′); ^{13}C NMR δ 14.02($-CH_3$), 60.87 ($-CH_2O-$), 65.08 ($-CH_2O-$), 101.6 (C-8), 114.8 (C-6), 114.9 (C-3′, C-5′), 118.1 (C-10), 122.3 (C-1′), 123.7 (C-3), 127.1 (C-5), 130.1 (C-2′, C-6′), 153.2 (C-2), 157.1 (C-9), 157.3 (C-4′), 161.9 (C-7), 168.1 ($-OCO-$), 174.7 (C-4); MS m/z 341.2 $(M + H)^+$. Anal. Calcd ($C_{19}H_{16}O_6$): C, 67.05; H, 4.73. Found: C, 67.20; H, 4.67. The filtrate was concentrated and fractionated on a Sephadex LH-20 column in chloroform/methanol (7/3). Fractions that contained the diethyl ester of **37** were pooled and recrystallized in acetone to give 0.5 g of white crystalline 7,4′-diethylcarbonylmethoxyisoflavone. The mono- and dicarboxymethyldaidzeins **7** and **37** were obtained by hydrolyzing 0.5 g of their respective ethyl esters in 10 mL of methanol, 1 mL of 2 N KOH, and 5 mL of water. The reaction mixtures were refluxed with gentle stirring for 2 h. Methanol was evaporated, and the residue was partitioned in water and ethyl acetate. The ethyl acetate layer was collected, dried, and recrystallized from acetone to yield pure **7** or **37**. Analyses (**7**): colorless crystalline plates; yield 22.7%; mp 244−245 °C; ^{1}H NMR (DMSO-d_6) δ 4.98 (s, 2H, $-CH_2O-$), 6.81 (d, 2H, J = 8.47 Hz, H-3′, H-5′), 7.10 (dd, 1H, J = 8.88, 2.6 Hz, H-6), 7.16 (d, 1H, J = 1.94 Hz, H-8), 7.40 (d, 2H, J = 8.33 Hz, H-2′, H-6′), 8.03 (d, 1H, J = 8.86 Hz, H-5), 8.36 (s, 1H, H-2), 9.55 (s, 1H, OH-4′), 12.0 (br, 1H, −COOH); MS m/z 313.1 $(M + H)^+$, 311.3 $(M - H)^+$. Anal. Calcd ($C_{17}H_{12}O_6$): C, 65.39; H, 3.87. Found: C, 64.16; H, 3.87. Analyses (**37**): colorless crystalline plates; yield 3.2%; mp 268−270 °C;^{1}H NMR spectrum similar to that of **7** and consistent with the interpretation that it is the 7,4′-disubstituted analogue of **7**; MS m/z 371.2 $(M + H)^+$, 369.3 $(M - H)^+$, 393.2 $(M + Na)^+$. Anal. Calcd ($C_{19}H_{14}O_8$): C, 61.63; H, 3.81. Found: C, 61.46; H, 3.71.

7-*O*-ω-Carboxybutyldaidzein (8), Its Ethyl Ester (25), and 7,4′-Di-*O*-ω-carboxybutyldaidzein (43). Compounds **25**, **43,** and **8** were synthesized and purified according to the procedures described for **7**, **24**, and **37**, respectively, using ethyl 5-bromovalerate as the alkylating agent. Analyses (**25**): colorless crystalline plates; yield 8.8%; mp 146−148 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H, $-CH_3$), 1.69 (m, 2H, $-CH_2-$), 1.77 (m, 2H, $-CH_2-$), 2.38 (t, 2H, $-CH_2-$), 4.05 (m, 2H, $-CH_2O-$), 4.12 (m, 2H, $-CH_2O-$), 6.81 (dd, 2H, J = 8.67, 1.5 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.89, 2.1 Hz, H-6), 7.11 (d, 1H, J = 2.1 Hz, H-8), 7.39 (dd, 2H, J = 8.7, 1.5 Hz, H-2′, H-6′), 8.0(d, 1H, J = 8.89 Hz, H-5), 8.35 (s, 1H, H-2), 9.55 (s,1H, HO-4′); ^{13}C NMR δ 14.1 ($-CH_3$), 21.1 ($-CH_2-$), 27.7 ($-CH_2-$), 59.7 ($-CH_2O-$), 68.1 ($-CH_2O-$), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.1 (C-2′, C-6′), 153.1 (C-2), 157.3 (C-9), 157.4 (C-4′), 162.9 (C-7), 172.7 (O═CO−), 174.7 (C-4); MS m/z 383.3 $(M + 1)^+$. Anal. Calcd ($C_{22}H_{22}O_6$): C, 69.09; H, 5.80. Found: C, 68.60; H, 5.84. Analyses (**43**): colorless crystalline plates; yield 30%; mp 96−98 °C; ^{1}H NMR and ^{13}C NMR spectra similar to those of **25** and consistent with the interpretation that **43** is a 7,4′-disubstituted analogue of **25**; MS m/z 511 $(M + H)^+$. Anal. Calcd ($C_{29}H_{34}O_8$): C, 68.22; H, 6.71. Found: C, 68.11; H, 6.35. Analyses (**8**): white amorphous powder; yield 20.6%; mp 232−235 °C; ^{1}H NMR and ^{13}C NMR spectra with the characteristic features of those of **25** and consistent with the interpretation that **8** is the free acid of **25**; MS m/z 355.2 $(M + H)^+$. Anal. Calcd ($C_{20}H_{18}O_6$): C, 67.79; H, 5.12. Found: C, 66.09; H, 4.67.

7-*O*-ω-Carboxyheptyldaidzein (11) was synthesized according to the procedure described before.[1] In this preparation, 5.1 g of **2** (20.02 mmol), 20 mL of 2 N KOH (40 mmol), and 4.4 g of 8-bromo-1-octanoic acid (19.72 mmol) were refluxed in 60 mL of acetone for 72 h. The reaction mixture was refrigerated for 12 h, and the precipitates were collected on a fritted funnel and recrystallized from acetone to give 100 mg of **11**. Analyses (**11**): white amorphous powder; yield 1.6%; mp192−193 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 [m, 4H, $-(CH_2)_2-$], 1.41 (m, 2H, $-CH_2-$), 1.51 (m, 2H, $-CH_2-$), 1.74 (m, 2H, $-CH_2-$), 2.20 (t, 2H, $-CH_2-$), 4.10 (t, $-CH_2O-$), 6.80 (dd, 2H, J = 8.2, 1.7 Hz, H-3′, H-5′), 7.05 (dd, 1H, J = 8.85, 1.7 Hz, H-6), 7.12 (d, 1H, J = 1.7 Hz, H-8), 7.39 (dd, 2H, J = 8.2, 1.7 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.85 Hz, H-5), 8.35 (s, 1H, 2-H), 9.55 (s, 1H, OH-4′), 12.0 (s, 1H, −COOH); ^{13}C NMR δ 24.4 ($-CH_2-$), 25.3 ($-CH_2-$), 28.3 ($-CH_2-$), 28.4 ($-CH_2-$), 28.4 ($-CH_2-$), 33.6 ($-CH_2-$), 68.4 ($-OCH_2-$), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 153.1 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 174.5 (C-4, −COOH); MS m/z 397 $(M + H)^+$. Anal. Calcd ($C_{23}H_{24}O_6$): C, 69.68; H, 6.10. Found: C, 69.52; H, 5.99.

7-*O*-ω-Carboxyundecyldaidzein (14) was synthesized according to the procedure described for **11** using 12-bromo-1-dodecanoic acid (5.83 g, 20.87 mmol) as the alkylating agent. The precipitates collected after refrigeration and purified on a Sephadex LH-20 column in chloroform/methanol (7/3). The pooled fractions were dried and recrystallized in chloroform/methanol (6/4) to give 500 mg of **14**. Analyses (**14**): white amorphous powder; yield 5.3%; mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 1.22−1.24 [m, 10H, $(-CH_2-)_5$], 1.40 (m, 2H, $-CH_2-$), 1.50 (m, 2H, $-CH_2-$), 1.75 (m, 2H, $-CH_2-$), 2.17 (t, 2H, $-CH_2-$), 3.40 (m, 2H, $-CH_2-$), 4.09 (t, $-CH_2O-$), 6.80 (dd, 2H, J = 8.4, 1.7 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.9, 1.9 Hz, H-6), 7.10 (d, 1H, J = 1.9 Hz, H-8), 7.40 (dd, 2H, J = 8.4, 1.7 Hz, H-2′, H-6′), 8.0(d, 1H, J = 8.9 Hz, H-5), 8.34 (s, 1H, H-2), 9.5 (s, 1H, OH-4′), 12.0 (br, 1H, −COOH); ^{13}C NMR δ 24.5 ($-CH_2-$), 25.4 ($-CH_2-$), 28.4 ($-CH_2-$), 28.5 ($-CH_2-$), 28.7 ($-CH_2-$), 28.7 ($-CH_2-$), 28.9 ($-CH_2-$), 28.9 ($-CH_2-$), 29.0 ($-CH_2-$), 33.7 ($-CH_2-$), 68.4 ($-OCH_2-$), 100.9 (C-8), 115.0 (C-6), 115.0 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 153.0 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 174.5 (C-4, −COOH); MS m/z 453.4 $(M + H)^+$. Anal. Calcd ($C_{27}H_{32}O_6$): C, 71.66; H, 7.13. Found: C, 71.32; H, 7.13.

7-*O*-*ω*-Carboxypentadecyldaidzein (15) was synthesized and purified according to the procedures described for **14** using 6-bromo-1-hexadecanoic acid (5.15 g, 15.36 mmol) as the alkylating agent. Analyses (**15**): white amorphous powder; yield 36%; mp151−152 °C; ^{1}H NMR (DMSO-d_6) δ 1.22−1.24 [m, 20H, $(-CH_{2-})_{10}$], 1.40 (m, 2H, $-CH_{2-}$), 1.46 (m, 2H, $-CH_{2-}$), 1.70 (m, 2H, $-CH_{2-}$), 2.16 (t, 2H, $-CH_{2-}$), 3.97 (t, $-CH_2O-$), 6.82 (dd, 2H, J = 8.7, 1.7 Hz, H-3′, H-5′), 7.03 (dd, 1H, J = 8.86, 1.9 Hz, H-6), 7.10 (d, 1H, J = 1.9 Hz, H-8), 7.48 (dd, 2H, J = 8.7, 1.7 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.5 (s, 1H, OH-4′); ^{13}C NMR δ 24.5 ($-CH_{2-}$), 25.4 ($-CH_{2-}$), 25.5 ($-CH_{2-}$), 28.9−29.0 [$(-CH_{2-})_{10}$], 33.8 ($-CH_{2-}$), 67.4 ($-OCH_{2-}$), 100.9 (C-8), 114.1 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 124.1 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 152.9 (C-2), 157.2 (C-9), 157.4 (C-4′), 162.9 (C-7), 174.6 (C-4, −COOH); MS m/z 509.7 $(M + H)^+$. Anal. Calcd ($C_{31}H_{40}O_6$): C, 73.2; H, 7.93. Found: C, 73.41; H, 7.83.

7-*O*-*ω*-Hydroxyethyldaidzein (16). To a suspension of 5.3 g of **2** (20.87 mmol) and 60 mL of acetone was added with vigorous stirring 13 mL of 2 N KOH (26 mmol). After **2** was completely dissolved, 2 mL of 2-bromoethanol (28.21 mmol) was added, and the reaction mixture was refluxed with gentle stirring for 72 h. Solvent was evaporated, and the residue was partitioned in water and ethyl acetate. The ethyl acetate layer was further purified on a silica gel column in petroleum ether/ethyl acetate/methanol (6/3/1) followed by a Sephadex LH-20 column in methanol. Fractions that contained the major product were pooled, dried, and recrystallized from acetone to give 1.7 g of **16**. Analyses (**16**): white amorphous powder; yield 28.5%; mp 210−212 °C; MS m/z 299.1 $(M + H)^+$. Anal. Calcd ($C_{17}H_{14}O_5$): C, 68.45; H, 4.73. Found: C, 67.86; H, 4.23.

7-*O*-*ω*-Hydroxyhexyldaidzein (17) was synthesized according to the method described for **16** using 6-bromo-1-hexanol (3 mL, 22.93 mmol) as the alkylating agent. After reflux, the reaction mixture was refrigerated for 12 h, and the precipitates were collected and fractionated on a Sephadex LH-20 column in methanol. Fractions that contained pure product were pooled, dried, and recrystallized from acetone to give 1.16 g of **17**. Analyses (**17**): white amorphous powder; yield 16.4%; mp177−178.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.35 (m, 2H, $-CH_{2-}$), 1.43 (m, 4H, $-CH_2CH_{2-}$), 1.75 (m, 2H, $-CH_{2-}$), 3.40 (m, 2H, $-CH_{2-}$), 4.1 (t, $-CH_2O-$), 6.81 (dd, 2H, J = 8.7, 1.6 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.86, 1.98 Hz, H-6), 7.10 (d, 1H, J = 1.99 Hz, H-8), 7.40 (dd, 2H, J = 8.7, 1.58 Hz, H-2′, H-6′), 8.0-(d, 1H, J = 8.86 Hz, 5-H), 8.34 (s, 1H, H-2), 9.53 (s, 1H, OH-4′); ^{13}C NMR δ 25.2 ($-CH_{2-}$), 25.3 ($-CH_{2-}$), 28.5 ($-CH_{2-}$), 32.5 ($-CH_{2-}$), 60.6 ($-CH_2O-$), 68.4 ($-OCH_{2-}$), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.1 (C-2′, C-6′), 153.1 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 174.7 (C-4); MS m/z 355.3 $(M + H)^+$. Anal. Calcd ($C_{21}H_{22}O_5$): C, 71.17; H, 6.26. Found: C, 70.82, H, 6.44.

7-O-*ω*-Hydroxynonyldaidzein (18) and 7,4′-di-*O*-*ω*-hydroxynonyldaidzein (38) were synthesized according to the procedure described for **16** using 9-bromo-1-nonanol (5 g, 22.41 mmol) as the alkylating agent. After reflux, the reaction mixture was refrigerated, and the precipitates formed were collected on a fritted funnel and further purified on a Sephadex LH-20 column in chloroform/methanol (7/3). Fractions that contained **18** or **38** were pooled separately, concentrated, and recrystallized from acetone to yield 305 mg of **18** and 1.4 g of **38**, respectively. Analyses (**18**): colorless crystalline plates; yield 3.8%; mp 165−167 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 [m, 8H, $(-CH_{2-})_4$], 1.40 (m, 4H, $-CH_2CH_{2-}$), 1.73 (m, 2H, $-CH_{2-}$), 3.37 (m, 2H, $-CH_{2-}$), 4.09 (t, $-CH_2O-$), 6.81 (d, 2H, J = 8.75, 1.58 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.87, 2.1 Hz, H-6), 7.10 (d, 1H, J = 2.1 Hz, H-8), 7.39 (d, 2H, J = 8.75, 1.58 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.53 (s, 1H, OH-4′); ^{13}C NMR δ 25.4 ($-CH_{2-}$), 25.5 ($-CH_{2-}$), 28.4 ($-CH_{2-}$), 28.7 ($-CH_{2-}$), 28.9 ($-CH_{2-}$), 29.0 ($-CH_{2-}$), 32.5 ($-CH_{2-}$), 60.7($-CH_2O-$), 68.4 ($-OCH_{2-}$), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 153.0 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 174.7 (C-4); MS m/z 397.5 $(M + H)^+$. Anal. Calcd ($C_{24}H_{28}O_5$): C, 72.71, H, 7.12. Found: C, 73.11; H, 7.13. Analyses (**38**): white amorphous powder; yield 13%; mp141−142 °C; ^{1}H NMR and ^{13}C NMR spectra with the characteristic features of those of **18** and consistent with the interpretation that **38** is a 7,4′-disubstituted analogue of **18**; MS m/z 539.8 $(M + H)^+$. Anal, Calcd ($C_{33}H_{46}O_6$): C, 73.58; H, 8.61. Found: C, 73.68; H, 8.66.

7-*O*-*ω*-Hydroxydodecyldaidzein (19) was synthesized and purified according to the procedures described for **18** using 12-bromo-1-dodecanol (5 g, 18.85 mmol) as the alkylating agent. Analyses (**19**): white amorphous powder; yield 20.5%; mp 142−144 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 [m, 14H, $(-CH_{2-})_7$], 1.37 (m, 4H, $-CH_2CH_{2-}$), 1.73 (m, 2H, $-CH_{2-}$), 3.36 (m, 2H, $-CH_2O-$), 4.08 (t, $-CH_2O-$), 6.81 (dd, 2H, J = 8.69, 1.58 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.86, 1.9 Hz, H-6), 7.09 (d, 1H, J = 1.9 Hz, H-8), 7.40 (dd, 2H, J = 8.69, 1.58 Hz, H-2′, H-6′), 7.98 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.55 (s,1H, OH-4′); ^{13}C NMR δ 25.4 [$(-CH_{2-})_2$], 25.5 ($-CH_{2-}$), 28.4 ($-CH_{2-}$), 28.7 ($-CH_{2-}$), 28.9 ($-CH_{2-}$), 29.1 ($-CH_{2-}$), 32.5 ($-CH_{2-}$), 60.7 ($-CH_2O-$), 68.4 ($-OCH_{2-}$), 100.9 (C-8), 114.8 (C-6), 114.8 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 153.0 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 174.7 (C-4); MS m/z 439.6 $(M + H)^+$. Anal. Calcd ($C_{27}H_{34}O_5$): C, 73.95; H, 7.81. Found: C, 74.20; H, 7.61.

7-*O*-*ω*-Hydroxyethyl-2-(2-oxyethyl)oxyethyldaidzein (20). To a solution of 5.1 g of **2** (20.08 mmol) in 60 mL of DMF were added 4.15 g of K_2CO_3 (30.03 mmol) and 6.74 g of 2-(2-(2-chloroethoxy)ethoxy)ethanol (40.0 mmol), and the mixture was refluxed (80 °C) with gentle stirring for 12 h. After reflux, the reaction mixture was poured into 100 mL of ice/water followed by ethyl acetate extraction. Ethyl acetate was evaporated, and the residue was fractionated on a Sephadex LH-20 column in chloroform/ methanol (7/3). Fractions that contained **20** were pooled, dried, and recrystallized from acetone to yield 3.08 g of pure product. Analyses (**20**): colorless crystalline needles; yield 39.9%; mp 149−150 °C; ^{1}H NMR (DMSO-d_6) δ 3.43 (m, 2H, $-CH_{2-}$), 3.48 (m, 2H, $-CH_{2-}$), 3.55 (m, 2H, $-CH_{2-}$), 3.60 (m, 2H, $-CH_{2-}$), 3.79 (m, 2H, $-CH_{2-}$), 4.25 (m, 2H, $-CH_{2-}$), 6.81 (d, 2H, J = 8.66 Hz, H-3′, H-5′), 7.08 (d, 1H, J = 8.86 Hz, H-6), 7.15 (d, 1H, J = 1.77 Hz, H-8), 7.40 (d, 2H, J = 8.66 Hz, H-2′, H-6′), 8.01 (d, 1H, J = 8.18 Hz, H-5), 8.35 (s, 1H, H-2), 9.54 (s, 1H, OH-4′); ^{13}C NMR 60.2 ($-CH_2O-$), 68.1 ($-CH_2O-$), 68.6 ($-CH_2O-$), 69.8 ($-CH_2O-$), 69.9 ($-CH_2-O-$), 72.4 ($-CH_2O-$), 101.1 (C-8), 114.9 (C-6), 115.0 (C-3′, C-5′), 117.6 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.1 (C-2′, C-6′), 153.1 (C-2), 157.2 (C-9), 157.3 (C-4′), 162.8 (C-7), 174.7 (C-4); MS m/z 387 $(M + H)^+$. Anal. Calcd ($C_{21}H_{22}O_7$): C, 65.28; H, 5.74. Found: C, 65.15; H, 5.59.

7-*O*-*ω*-Ethoxycarbonylpentyldaidzein (26) and 7,4′-Di-*O*-*ω*-ethoxycarbonylpentyldaidzein (44). To a solution of 7.7 g of **2** (30.31 mmol), 30 mL of 2 N KOH (60 mmol), and 120 mL of acetone was added 12.5 mL of ethyl-6-bromo-1-hexanoate (70.60 mmol). The mixture was refluxed with gentle stirring for 72 h, and the products were allowed to precipitate under refrigeration. The precipitates were collected on a fritted funnel and fractionated on a Sephadex LH-20 column in chloroform/methanol (7/3). Fractions that contained **26** or **44** were pooled separately, dried, and recrystallized from acetone to give 670 mg of **26** and 5.39 g of **44**. Analyses (**26**): white crystalline needles; yield 5.6%; mp 130−131 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H, $-CH_3$), 1.45 (m, 2H, $-CH_{2-}$), 1.58 (m, 2H, $-CH_{2-}$), 1.74 (m, 2H, $-CH_{2-}$), 2.31 (t, 2H, $-CH_{2-}$), 4.04 (m, 2H, $-CH_2O-$), 4.09 (m, 2H, $-CH_2O-$), 6.82 (dd, 2H, J = 9.76, 2.7 Hz, H-3′, H-5′), 7.04 (dd, 1H, J = 8.87, 2.79 Hz, H-6), 7.09 (d, 1H, J = 2.1 Hz, H-8), 7.40 (dd, 2H, J = 9.76, 2.7 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.86 Hz, H-5), 8.34 (s, 1H, H-2), 9.55 (s, 1H, OH-4′); ^{13}C NMR δ 14.1($-CH_3$), 24.2 ($-CH_{2-}$), 24.9 ($-CH_{2-}$), 28.1 ($-CH_{2-}$), 33.4 ($-CH_{2-}$), 59.7 ($-CH_2O-$), 68.3 ($-CH_2O-$), 100.9 (C-8), 114.9 (C-6), 115 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.1 (C-2′, C-6′), 153.1 (C-2), 157.2 (C-9), 157.4 (C-4′), 163.0 (C-7), 172.8 (−OCO-), 174.7 (C-4); MS m/z 397.3 $(M + H)^+$. Anal. Calcd ($C_{23}H_{24}O_6$): C, 69.68; H, 6.10. Found: C, 69.84; H, 6.15. Analyses (**44**): yield 50%; mp 99−101 °C; MS m/z 539.6 (M +

H)$^+$. Anal. Calcd ($C_{31}H_{38}O_8$): C, 69.13; H, 7.11. Found: C, 69.34; H, 7.11.

7-*O*-ω-Hexenyldaidzein (28) and 7,4′-Di-*O*-ω-hexenyldiadzein (42). The ω-hexenyl derivatives of daidzein were synthesized and purified according to the same procedure described for **26** and **44** using 6-bromo-1-hexene (5 g, 30.66 mmol) as the alkylating agent. Analyses (**28**): yield 39%; mp 156−157 °C; ^{1}H NMR (DMSO-d_6) δ 1.51 (m, 2H, −CH_2−), 1.74 (m, 2H, −CH_2−), 2.08 (m, 2H, −CH_2−), 4.09 (t, −OCH_2−), 5.01 (m, 2H, −CH_2═), 5.83 (m, 1H, −CH═), 6.81 (dd, 2H, J = 8.66, 1.53 Hz, H-3′, H-5′), 7.03 (dd, 1H, J = 8.88, 2.6 Hz, H-6), 7.09 (d, 1H, J = 1.8 Hz, H-8), 7.40 (dd, 2H, J = 8.66, 1.53 Hz, H-2′, H-6′), 8.0 (d, 1H, J = 8.88 Hz, H-5), 8.33 (s, 1H, H-2), 9.54 (s, 1H, OH-4′); ^{13}C NMR δ 24.6 (−CH_2−), 27.8 (−CH_2−), 32.8 (−CH_2−), 68.2 (−OCH_2−), 100.9 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 117.5 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, C-6′), 138.4 (CH_2═CH−), 153.0 (C-2), 157.2 (C-9), 157.4 (C-4′), 162.9 (C-7), 174.7 (C-4); MS m/z 337.5 (M + H)$^+$. Anal. Calcd ($C_{21}H_{20}O_4$): C, 74.98; H, 5.99. Found: C, 74.52; H, 6.08. Analyses (**42**): yield 20%; mp 112−115 °C; ^{1}H NMR and ^{13}C NMR spectra with the characteristic features of those of **28**; MS m/z 419.4 (M + H)$^+$. Anal. Calcd ($C_{27}H_{30}O_4$): C, 77.48; H, 7.22. Found: C, 77.50; H, 7.27.

7-*O*-α-Carboxyethyldaidzein (29), Its Ethyl Ester (32), and the Diethyl Ester of 7,4′-Di-*O*-α-carboxyethyldaidzein (45). The ethyl esters **32** and **45** were synthesized according to the method described for **20** using ethyl 2-bromopropionate (3 mL, 23.1 mmol) as the alkylating agent. The reaction mixture was refluxed at 80 °C for 2 h and poured into 100 mL of ice/water. The products were extracted with ethyl acetate and further purified on a Sephadex LH-20 column in chloroform/methanol (3/7). Fractions that contained **32** or **45** were pooled separately, concentrated, and recrystallized from acetone to give 5.615 g of **32** and 866 mg of **45**. Analyses (**32**): colorless crystalline needles; yield 79%; mp 167−169 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7.04 Hz, −CH_3), 1.57 (d, 3H, J = 6.84 Hz, −CH_3), 4.17 (dd, 2H, J = 6.95, 14.07 Hz, −CH_2−), 5.23 (d, 1H, J = 6.80 Hz, −CH−), 6.82 (dd, 2H, J = 8.76, 1.6 Hz, H-3′, H-5′), 7.06 (dd, 1H, J = 8.2, 1.83 Hz, H-6), 7.08 (d, 1H, J = 1.83 Hz, H-8), 7.40 (dd, 2H, J = 8.65, 1.6 Hz, H-2′, H-6′), 8.03 (d, 1H, J = 8.2 Hz, H-5), 8.35 (s, 1H, H-2), 9.55 (s, 1H, HO-4′); ^{13}C NMR (DMSO-d_6) δ 13.9 (−CH_3), 17.9 (−CH_3), 61.1 (−OCH_2−), 72.1 (−CH−), 101.8 (C-8), 114.9 (C-6), 114.9 (C-3′, C-5′), 118.1 (C-10), 122.3 (C-1′), 123.7 (C-3), 127.2 (C-5), 130.1 (C-2′, C-6′), 153.2 (C-2), 157.1 (C-9), 157.3 (C-4′), 161.5 (C-7), 170.7 (C═O), 174.6 (C-4); MS m/z 355 (M + H)$^+$. Anal. Calcd ($C_{20}H_{18}O_6$): C, 67.79; H, 5.12. Found: C, 67.65, H, 5.17. Analyses (**45**): colorless crystalline plates; yield 9.5%; mp 97−99 °C. Anal. Calcd ($C_{25}H_{26}O_8$): C, 66.07; H, 5.77. Found: C, 66.07; H, 5.77. The free acid **29** was prepared by hydrolyzing 2.5 g of **32** (7.06 mmol) in 20 mL of methanol, 5.0 mL of 2 N KOH, and 10 mL of water at 60−80 °C for 4 h. After removal of methanol, the white residue was recrystallized from acetone to give 2.0 g of **29**. Analyses (**29**): yield 86.9%; MS m/z 327 (M + 1)$^+$. Anal. Calcd ($C_{18}H_{14}O_6$): C, 66.24; H, 4.33. Found: C, 66.19%, H, 4.23.

7-*O*-α-Carboxypentyldaidzein (30) and Its Ethyl Ester (33). The ethyl ester **33** was synthesized and purified by the same methods described for **32** using ethyl 2-bromo-1-hexanoate (8 mL, 0.044 mol) as the alkylating agent. Analyses (**33**): colorless crystalline needles; yield 85%; mp 129−131 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.09 Hz, −CH_3), 1.18 (t, 3H, J = 7.07 Hz, −CH_3), 1.35 (m, 2H, −CH_2−), 1.43 (m, 2H, −CH_2−), 1.91 (m, 2H, −CH_2−), 4.17 (m, 2H, −CH_2−), 5.12 (t, 1H, J = 6.52 Hz, −CHO), 6.81 (dd, 2H, J = 8.76, 1.55 Hz, H-3′, H-5′), 7.07 (dd, 1H, J = 8.63, 2.3 Hz, H-6), 7.08 (d, 1H, J = 2.57 Hz, H-8), 7.39 (dd, 2H, J = 8.63, 1.52 Hz, H-2′, H-6′), 8.03 (d, 1H, J = 8.63 Hz, H-5), 8.36 (s, 1H, H-2), 9.55(s, 1H, HO-4′); ^{13}C NMR (DMSO-d_6) δ 13.8 (−CH_3), 14.0 (−CH_3), 21.7 (−CH_2−), 26.6 (−CH_2−), 31.5 (−CH_2−), 61.0 (−OCH_2−), 75.8 (−CHO−), 101.8 (C-8),114.9 (C-6), 114.9 (C-3′, C-5′), 118.1 (C-10), 122.3 (C-1′), 123.7 (C-3), 127.2 (C-5), 130.1 (C-2′, C-6′), 153.2 (C-2), 157.1 (C-9), 157.3 (C-4′), 161.8 (C-7), 170.2 (C═O), 174.6 (C-4); MS m/z 397 (M + H)$^+$. Anal. Calcd ($C_{23}H_{24}O_6$): C, 69.68, H, 6.10. Found: C, 69.98, H, 5.83. The free acid **30** was prepared by hydrolyzing 1 g of **33** under the conditions described for the preparation of **29** to give 855 mg of product. Analyses (**30**): white amorphous powder; yield 86%; mp162−163 °C; MS m/z 369 (M + 1)$^+$. Anal. Calcd ($C_{21}H_{20}O_6$): C, 68.47; H, 5.47. Found: C, 68.08; H, 5.45.

7-*O*-α-Carboxyheptyldaidzein (31) and Its Ethyl Ester (34). Compounds **31** and **34** were synthesized and purified according to the methods described for **30** and **33** using ethyl 2-bromo-1-octanoate as the alkylating agent. Analyses (**34**): yield 80%; mp118−120 °C; ^{1}H NMR (DMSO-d_6) δ 0.85(t, 3H, J = 6.66 Hz, −CH_3), 1.18(t, 3H, J = 7.35 Hz, −CH_3), 1.26−1.35 [(m, 6H, (−CH_2−)$_3$], 1.45 (m, 2H, −CH_2−), 1.90 (m, 2H, −CH_2−), 4.16 (m, 2H, −CH_2−), 5.12 (t, 1H, J = 6.08 Hz, −CHO), 6.81 (dd, 2H, J = 8.49, 1.6 Hz, H-3′, H-5′), 7.06 (dd, 1H, J = 8.8, 2.8 Hz, H-6), 7.08 (d, 1H, J = 2.55 Hz, H-8), 7.39 (dd, 2H, J = 8.72, 1.6 Hz, H-2′, H-6′), 8.03 (d, 1H, J = 8.79 Hz, H-5), 8.36 (s, 1H, H-2), 9.55 (s, 1H, HO-4′); ^{13}C NMR (DMSO-d_6) δ 13.9 (−CH_3), 14.0 (−CH_3), 21.9 (−CH_2−), 24.4 (−CH_2−), 28.2 (−CH_2−), 31.0 (−CH_2−), 31.8 (−CH_2−), 61.0 (−OCH_2−), 75.8 (−CHO−), 101.8 (C-8),114.9 (C-6), 115.0 (C-3′, C-5′), 118.1 (C-10), 122.3 (C-1′), 123.7 (C-3), 127.2 (C-5), 130.1 (C-2′, C-6′), 153.2 (C-2), 157.1 (C-9), 157.3 (C-4′), 161.8 (C-7), 170.2 (C═O), 174.6 (C-4); MS m/z 425 (M + H)$^+$. Anal. Calcd ($C_{25}H_{28}O_6$): C, 70.74, H, 6.65. Found: C, 70.82, H, 6.51. Analyses (**31**): yield 96%; mp 123−125 °C; MS m/z 397 (M + H)$^+$. Anal. Calcd ($C_{23}H_{24}O_6$): C, 69.68; H, 6.10. Found: C, 68.05; H, 6.11.

7-*O*-ω-Carboxypentylflavone (49). A mixture of 600 mg of 7,4′-hydroxyflavone (3.15 mmol), 515 mg of anhydrous K_2CO_3 (3.7 mmol), and 611 mg of 6-bromo-1-hexanoic acid (3.13 mmol) were refluxed in 40 mL of anhydrous DMF with vigorous stirring for 4 h. The mixture was poured into 100 mL of ice/water and extracted with ethyl acetate. The organic phase was dried and further purified on a Sephadex LH-20 column in chloroform/methanol (7/3). Fractions that contained **49** were pooled and solvents removed on a rotary evaporator. The residue was recrystallized from acetone to yield 50 mg of **49**. Analyses (**49**): yellow crystalline needles; yield 60%; mp 209−211 °C; ^{1}H NMR (DMSO-d_6) δ 1.44 (m, 2H, −CH_2−), 1.55 (m, 2H, −CH_2−), 1.74 (m, 2H, −CH_2−), 2.23 (t, 2H, −CH_2−), 4.05 (t, 2H, −CH_2O−), 6.78 (s, 1H, H-3), 6.90 (dd, 1H, J = 8.16, 2.0 Hz, H-6), 6.98 (d, 1H, J = 2.0 Hz, H-8), 7.07 (dd, 2H, J = 8.82, 1.6 Hz, H-3′, H-5′), 7.86 (d, 1H, J = 8.16 Hz, H-5), 7.98 (dd, 2H, J = 8.82, 1.6 Hz, H-2′, H-6′); ^{13}C NMR δ 24.3 (−CH_2−), 25.1 (−CH_2−), 28.3 (−CH_2−), 33.6 (−CH_2−), 67.7 (−OCH_2−), 102.5 (C-8), 105.0 (C-3), 114.8 (C-6), 114.9 (C-3′, C-5′), 116.1 (C-10), 123.3 (C-1′), 126.5 (C-5), 128.0 (C-2′, C-6′), 157.4 (C-9), 161.3 (C-4′), 162.0 (C-2), 162.6(C-7), 174.5 (−COOH), 176.3 (C-4); MS m/z 369 (M + H)$^+$. Anal. Calcd ($C_{21}H_{20}O_6$): C, 68.47; H, 5.47. Found: C, 68.05; H, 5.85.

Abbreviations

The abbreviations used are ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; 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); and MAO, monoamine oxidase.

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