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Synthesis of Daidzin Analogues as Potential Agents for Alcohol Abuse

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Abstract—Daidzin, the active principle of an herbal remedy for 'alcohol addiction', has been shown to reduce alcohol consumption in all laboratory animals tested to date. Correlation studies using structural analogues of daidzin suggests that it acts by raising the monoamine oxidase (MAO)/mitochondrial aldehyde dehydrogenase (ALDH-2) activity ratio (*J. Med. Chem.* **2000**, *43*, 4169). Structure–activity relationship (SAR) studies on the 7-*O*-substituted analogues of daidzin have revealed structural features important for ALDH-2 and MAO inhibition (*J. Med. Chem.* **2001**, *44*, 3320). We here evaluated effects of substitutions at 2, 5, 6, 8, 3′ and 4′ positions of daidzin on its potencies for ALDH-2 and MAO inhibition. Results show that analogues with 4′-substituents that are small, polar and with hydrogen bonding capacities are most potent ALDH-2 inhibitors, whereas those that are non-polar and with electron withdrawing capacities are potent MAO inhibitors. Analogues with a 5-OH group are less potent ALDH-2 inhibitors but are more potent MAO inhibitors. All the 2-, 6-, 8- and 3′-substituted analogues tested so far do not inhibit ALDH-2 and/or have decreased potencies for MAO inhibition. This, together with the results obtained from previous studies, suggests that a potent antidipsotropic analogue would be a 4′,7-disubstituted isoflavone. The 4′-substituent should be small, polar, and with hydrogen bonding capacities such as, –OH and $–NH_2$; whereas the 7-substituent should be a straight-chain alkyl with a terminal polar function such as $–(CH_2)_n–OH$ with $2 \leq n \leq 6$, $–(CH_2)_n–COOH$ with $5 \leq n \leq 10$, or $–(CH_2)_n–NH_2$ with $n \geq 4$.

Introduction

Alcohol dependence and abuse are among the most serious drug problems of Western societies. In the US, about 10% of the population abuse alcohol.[1] The economic cost to the nation is more than $185 billion per year.[2] Therefore, safe and effective treatments for this drug problem are sorely needed. Recent success in the development of pharmaotherapies for addictive disorders such as nicotine addiction and a more widespread recognition that alcohol dependence is a medical rather than moral problem have harnessed growing support in the search for and development of pharmaceutical agents for this medical condition. However, such efforts have been greatly hampered by (i) the lack of a thorough understanding of the molecular basis for alcohol craving, (ii) an adequate animal model for human 'alcohol addiction', and (iii) a truly predictive model for drug screening. We have taken an alternative approach to the problem, searching for effective agents from traditional Chinese medicines that have been used apparently safely and effectively for the treatment of 'alcohol addiction'.[3] This approach has led to the discovery of daidzin, the active principle of the medicinal plant *Pueraria lobata*, that selectively reduces ethanol intake in ethanol-preferring Syrian golden hamsters.[4,5] Since then, the antidipsotropic (ethanol intake suppressive) activity of daidzin has been confirmed in all ethanol drinking animal models tested to date.[6–9]

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 and rat liver mitochondria.[10] Further, daidzin does not affect the rates of mitochondria-catalyzed oxidative deamination of these monoamines. These findings suggest that its antidipsotropic activity may not be mediated by the monoamines themselves but rather by their respective metabolic intermediates, 5-hydroxyindole-3-acetaldehyde (5-HIAL) and/or 3, 4-dihydroxyphenylacetaldehyde

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(DOPAL) which accumulate in the presence of daidzin.[10] Later correlation studies using structural analogues of daidzin revealed a link between their antidipsotropic activities and their abilities to accumulate a biogenic aldehyde in isolated liver mitochondria preparations.[11] 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. This result, although not conclusive, lends support to the idea that one or more biogenic aldehyde derived from the action of MAO mediates the antidipsotropic action of daidzin (Fig. 1).[11,12] Further, it provided a molecular target based on which a simple in vitro screening assay was developed.[13] The potential role(s) of biogenic aldehyde(s) in the control of alcohol use and abuse has been proposed and thoroughly reviewed.[14]

The levels of biogenic aldehydes attained during mitochondria-catalyzed monoamine metabolism are regulated by the relative activities of MAO and ALDH-2. Therefore, in the design of more efficacious antidipsotropic analogues, structural features important for the inhibition of both ALDH-2 and MAO must be taken into consideration. Since the molecular details of daidzin binding to ALDH-2 and MAO are unknown at this time, studying the classical structure–activity relationship (SAR) remains a valuable approach for this purpose. In a recent study, we have studied the SAR of 7-*O*-substitued analogues of daidzin and documented a sufficient set of criteria for a potent antidipsotropic analogue.[15] As a continuing effort, we here evaluated effects of substitutions at the 2, 5, 6, 8, 3′, and 4′ positions of daidzin on the potencies for ALDH-2 and MAO inhibition.

Results and Discussion

Synthesis, purification and structural identification of analogues of daidzin

The 7-*O*-substituents of the derivatives of daidzein (**2a**–**5a**, **7a**–**9a**), puerarin (**8e**, **19**), 7-hydroxyisoflavone (**2b**–**5b**, **6**, **7b**, **8b**), 7-hydroxy-2-ethoxycarbonylisoflavone (**8c**, **9b**), and the 7-hydroxy-4′-fluoro (**11b**)-, -bromo (**12b**)-, -nitro (**13b**)-, and methyl (**14b**)-isoflavone were introduced by the general method of Williamson synthesis as described in previous reports (Table 1).[11,15] Synthesis of the 4′-substituted derivatives of daidzein (**11a**–**14a**, **15**, and **17**) were based on the formylation of substituted deoxybenzoins (Scheme 1, Step 1).[16,17] Deoxybenzoins undergo α-keto formylation, intramolecular acetal formation, and a facile dehydration to give the pyrone ring C and hence, the isoflavones (Steps 2 and 3). Substituted deoxybenzoins were prepared by reacting appropriate substituted phenyl acetic acids with properly substituted resorcinols in the presence of BF_3 (Step 1). The starting material (**1**) for the synthesis of the 7-*O*-substituted isoflavones (**2b**–**5b**, **6**, **7b**, **8b**,) was prepared by cyclization of 2, 4-dihydroxyphenyl benzyl ketone (Aldrich Chemical Co. Milwaukee, WI, USA) as described by Kállay et al. (Steps 2 and 3).[18] The 7-hydroxy-2-ethoxycarbonylisoflavone (**10a**) was prepared by cyclization of their corresponding deoxybenzoins in the presence of an appropriate formyl equivalent (Steps 2 and 3).[18,19] The 2-carboxy derivatives (**8d**, **10b**, **10c**) were obtained by hydrolyzing their respective ethyl esters. The 4′-amino derivatives (**16a, b**) were prepared by reduction of their corresponding 4′-nitro derivatives (**13a**, **b**) under the condition described by Li et al.[20] All compounds synthesized were purified by Sephadex-LH-20 and/or silica gel columns. Products were identified by ^{1}H NMR, ^{13}C NMR, MS, and elemental analyses. Purity of all compounds prepared in this study are greater than 97.8% as judged by HPLC with UV detection at 254 nm. The molecular formula, molecular weights, melting points, and data of elemental analyses are included in the Synthesis Section.

Structure–activity relationship (SAR)

In previous studies,[11,15,21,22] we have shown that all antidipsotropic analogues of daidzin are isoflavones and inhibit ALDH-2 preferentially. Analogues of daidzin that have a flavone backbone are not antidipsotropic and inhibit MAO preferentially. Hence, the 1,2-diphenylpropane

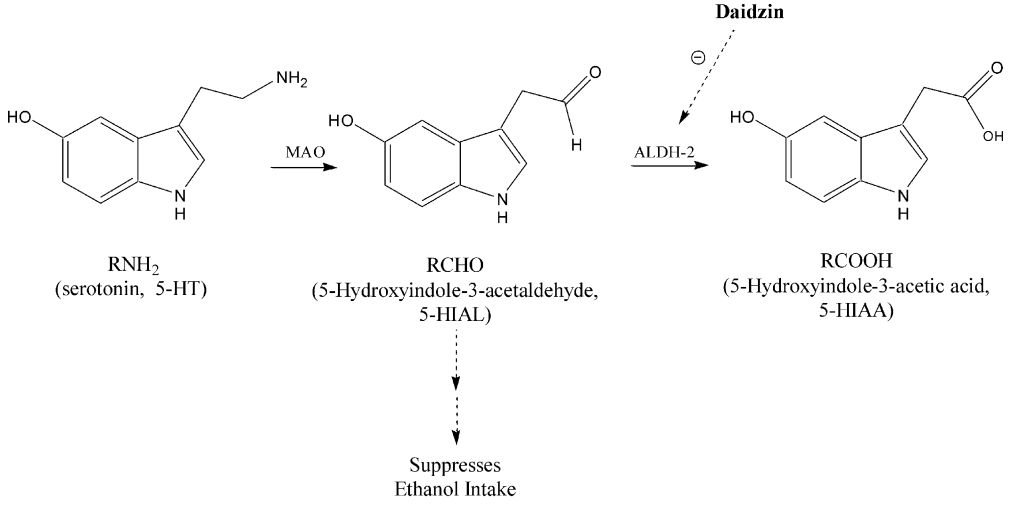
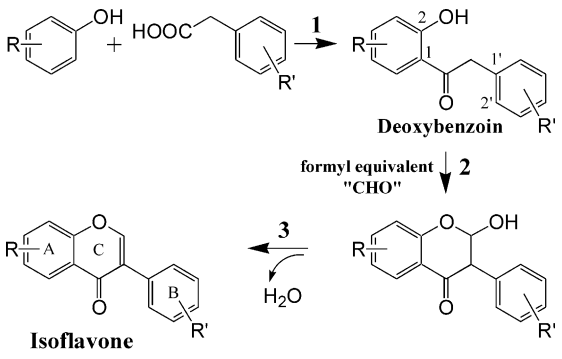


Figure 1. Proposed site of action of daidzin.

skeleton of an isoflavone is critical for its antidipsotropic activity and selectivity toward ALDH-2 inhibition. Substitution of the 7-hydroxyl of daidzein increases potencies for both ALDH-2 and MAO inhibition. However, structural requirements of the 7-*O*-substituent for ALDH-2 and MAO inhibition are sufficiently different that analogues selectively inhibit ALDH-2 have been designed and synthesized. On the basis of these findings, we have concluded that a sufficient set of criteria for a potent antidipsotropic analogue is an isoflavone with a 7-*O*-straight-chain alkyl substituent that has a terminal polar function such as –OH, –COOH, or $–NH_2$; and the preferable chain lengths for the 7-*O*-ω-hydroxy, 7-*O*-ω-carboxy, and 7-*O*-ω-amino substituents to be $2 \geq n \geq 6$, $5 \geq n \geq 10$, and $n \geq 4$, respectively. As a continuing effort in the search of more efficacious analogues, we here evaluated effects of substitutions at the 2, 5, 6, 8, 3′, and 4′ positions of an isoflavone on the potencies for ALDH-2 and MAO inhibition.

Substitution of 4′-OH. In a preliminary study, we have synthesized nine 4′,7-*O*-disubstituted analogues of daidzin, tested and compared their ALDH-2 and MAO inhibitory activities with those of their respective 7-*O*-monosubstituted analogues. While all the monosubstituted analogues inhibit ALDH-2 ($IC_{50} = 0.08$–$9\,\mu M$), none of the disubstituted ones does.[15] Among the monosubstituted analogues studied, three inhibited MAO. None of the disubstituted analogues inhibits MAO. It appears that either a free 4′-hydroxyl and/or a smaller 4′-substituent are preferable for antidipsotropic activity. These results are consistent with our early finding that replacing the 4′-OH of daidzin with a methoxy function increased its IC_{50} for ALDH-2 inhibition by >100-fold.[22]

To further evaluate the SAR of 4′-substitution, we synthesized two series of 7-*O*-substituted analogues: the 7-*O*-substituted 4′-hydroxyisoflavones (**2a–5a**, **7a–8a**) and the 7-*O*-substituted isoflavones (**2b–5b**, **7b–8b**) (Fig. 2).



Scheme 1. Synthesis of daidzin analogues.

Table 1. Chemical and physical constants of new daidzin analogues

No.	Compd	Formula	M_r	Mp (°C)	Anal.
1	7-Hydroxyisoflavone	$C_{15}H_{10}O_3$	238.24	203–205	C,H
2a	7-Methoxydaidzein	$C_{16}H_{12}O_4$	268.27	210–211	C,H
2b	7-Methyoxyisoflavone	$C_{16}H_{12}O_3$	252.00	156–158	C,H
3a	7-*O*-Isopropyldaidzein	$C_{18}H_{16}O_4$	296.32	169–171	C,H
3b	7-*O*-Isopropylisoflavone	$C_{18}H_{16}O_3$	280.32	110–111	C,H
4a	7-*O*-ω-Ethoxycarbonylpentyldaidzein	$C_{23}H_{24}O_6$	396.44	130–131	C,H
4b	7-*O*-ω-Ethoxycarbonylpentylisoflavone	$C_{23}H_{24}O_5$	380.44	112–114	C,H
5a	7-*O*-ω-Hydroxyethyl-2-(2-oxyethyl)oxyethyldaidzein	$C_{21}H_{22}O_7$	386.40	149–150	C,H
5b	7-*O*-ω-Hydroxyethyl-2-(2-oxyethyl)oxyethylisoflavone	$C_{21}H_{22}O_6$	370.40	105–107	C,H
6	7-*O*-Acetylisoflavone	$C_{17}H_{12}O_5$	280.28	134–135	C,H
7a	7-*O*-Tetrahydro-2-(H)-pyran-2-*O*-propanyldaidzein	$C_{23}H_{24}O_6$	396.44	112–113	C,H
7b	7-*O*-Tetrahydro-2-(H)-pyran-2-*O*-propanylisoflavone	$C_{23}H_{24}O_5$	380.44	113–115	C,H
8a	7-*O*-Phthalimide-*N*-butyldaidzein	$C_{27}H_{21}O_6N$	455.47	195–197	C,H,N
8b	7-*O*-Phthalimide-*N*-butylisoflavone	$C_{27}H_{21}O_5N$	439.47	167–168	C,H,N
8c	2-Ethoxycarbonyl-7-*O*-phthalimide-*N*-butylisoflavone	$C_{30}H_{25}O_7N$	511.53	163–165	C,H,N
8d	2-Carboxy-7-*O*-phthalimide-*N*-butylisoflavone	$C_{28}H_{21}O_7N$	483.48	143–145	C,H,N
8e	7-*O*-Phthalimide-*N*-butylpuerarin	$C_{33}H_{31}O_{11}N$	617.61	68–69	C,H,N
9a	7-*O*-1H-Benzotriazole-1-methyldaidzein	$C_{22}H_{15}O_4N_3$	385.38	227–228	C,H,N
9b	2-Ethoxycarbonyl-7-*O*-1H-benzotriazole-1-methylisoflavone	$C_{25}H_{19}O_5N_3$	441.44	182–183	C,H,N
10a	2-Ethoxycarbonyl-7-hydroxyisoflavone	$C_{18}H_{14}O_5$	310.31	213–214	C,H
10b	2-Carboxy-7-hydroxyisoflavone	$C_{16}H_{10}O_5$	282.25	257–258	C,H
10c	2-Carboxy-7-*O*-ω-carboxypentylisoflavone	$C_{22}H_{20}O_6$	396.40	157–159	C,H
11a	7-Hydroxy-4′-fluoroisoflavone	$C_{15}H_9O_3F$	256.23	235–236	C,H
11b	7-*O*-ω-Ethoxycarbonylpentyl-4-fluoroisoflavone	$C_{23}H_{23}O_5F$	398.43	91–93	C,H
12a	7-Hydroxy-4′-bromoisoflavone	$C_{15}H_9O_3Br$	317.14	266–268	C,H
12b	7-*O*-ω-Ethoxycarbonylpentyl-4′-bromoisoflavone	$C_{23}H_{23}O_5Br$	459.34	122–124	C,H
13a	7-Hydroxy-4′-nitroisoflavone	$C_{15}H_9O_5N$	283.24		C,H,N
13b	7-*O*-ω-Ethoxycarbonylpentyl-4′-nitroisoflavone	$C_{23}H_{23}O_7N$	425.44	173–175	C,H,N
14a	7-Hydroxy-4′-methylisoflavone	$C_{16}H_{12}O_3$	252.27	241–243	C,H
14b	7-*O*-ω-Ethoxycarbonylpentyl-4′-methylisoflavone	$C_{24}H_{26}O_5$	394.47	141–142	C,H
15	4′,7-dimethoxyisoflavone	$C_{17}H_{14}O_4$	425.44	173–175	C,H
16a	7-Hydroxy-4′-aminoisoflavone	$C_{15}H_{10}O_3N$	253.26		C,H,N
16b	7-*O*-ω-Ethoxycarbonylpentyl-4′-aminoisoflavone	$C_{23}H_{25}O_5N$	395.46	147–148	C,H,N
17	7,8-Dimethoxyisoflavone	$C_{17}H_{14}O_4$	282.30	143–144	C,H
18	7-Ethylisoflavone	$C_{17}H_{14}O_2$	250.30	103–104	C,H
19	7-*O*-ω-Ethoxycarbonylpentylpuerarin	$C_{29}H_{34}O_{11}$	558.58	165–168	C,H

All members of the 7-*O*-substituted 4′-hydroxyisoflavone series are potent ALDH-2 inhibitors with IC_{50} values ranging from 0.04 to 0.28 μM. While the 7-*O*-substituted isoflavones also inhibit ALDH-2, they are less potent with IC_{50} values ranging from 0.1 to 1.5 μM. The effect of 4′-OH → 4′-H substitution on MAO inhibition, however, is more dramatic: while all members of the 4′-hydroxyl derivatives (**2a–5a**, **7a–8a**) inhibit MAO, those of the 4′-H series (**2b–5b**, **7b–8b**) do not. The fact that this effect is independent of the nature of the 7-*O*-substituents strongly suggests that the 4′-hydroxyl plays a more critical role in MAO inhibition. In this juncture, it is of interest to note that despite their decreased potencies for ALDH-2 inhibition, the 4′-H substituted derivatives may be more potent in vivo because unlike the 4′-OH-, the 4′-H- derivatives (i) do not inhibit MAO and (ii) is less likely to undergo phase II metabolism.

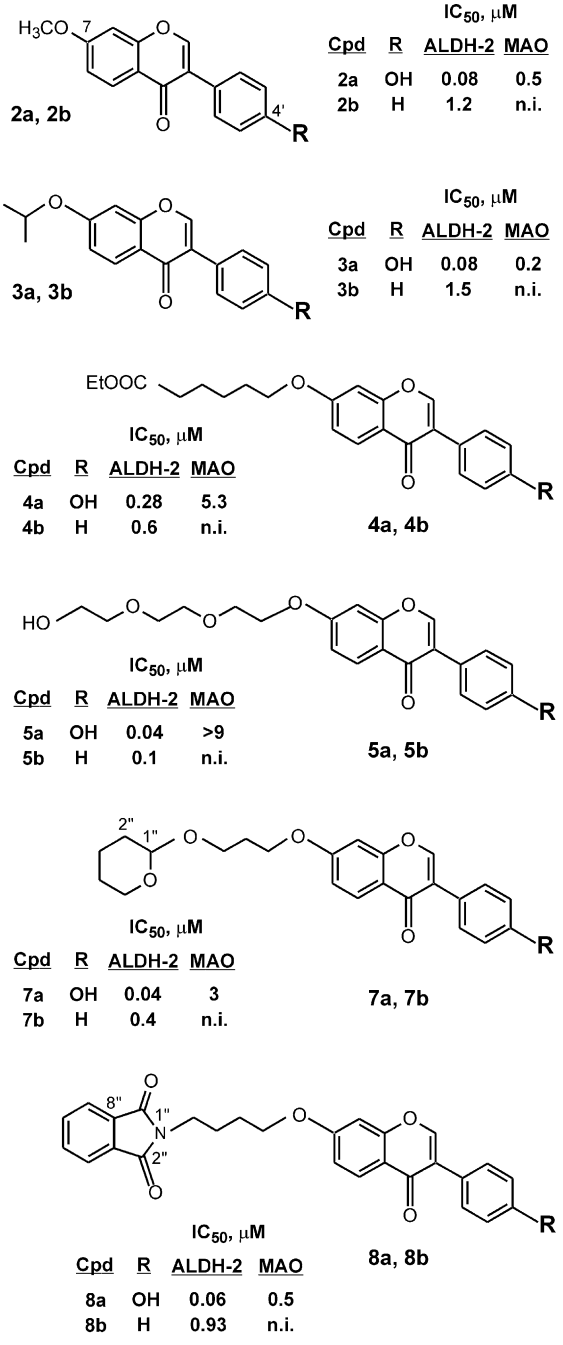
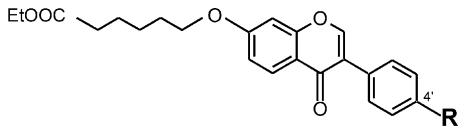


Figure 2. Effect of 4′-OH → 4′-H substitution on the potency of various 7-*O*-substituted isoflavones for MAO and ALDH-2 inhibition.

To explore the potential effects of polarity, size and hydrogen bonding capacity of the 4′-substituents on the potencies for MAO and ALDH-2 inhibition, we synthesized and studied two series of 4′-substituted analogues: the 7-*O*-ω-ethoxycarbonylpentyl (Fig. 3) and the 7-hydroxy-isoflavones (Fig. 4). Among the 7-*O*-ω-ethoxycarbonylpentylisoflavones, the 4′-NH_2 (**16b**), 4′-OH (**4a**), and 4′-H (**4b**) are more potent ALDH-2 inhibitors than the 4′-F (**11b**), 4′-Br (**12b**), and 4′-CH_3 (**14b**) derivatives. The 4′-NO_2 derivative (**13b**) is not inhibitory at all (Fig. 3). It appears that in addition to size, polarity and hydrogen bonding capacity of the 4′-substituent also affects potency for ALDH-2 inhibition. The most potent inhibitors are those with small, polar, and hydrogen bond forming 4′-substituents, such as analogues **16b** (4′-NH_2) and **4a** (4′-OH). The 4′-CH_3 (**14b**), although small, is lipophilic and hence is much less potent. The 4′-NO_2 derivative (**13b**) is not inhibitory. The relative large NO_2 group might have prohibited its binding to the daidzin-binding site.

Only three of the seven 4′-substituted 7-*O*-ω-ethoxycarbonylpentyl derivatives inhibit MAO (Fig. 3). Among them, only the 4′-NO_2 derivative (**13b**) inhibits MAO to any significant extent (IC_{50} = 0.8 μM). Therefore, little SAR information can be deduced from this series of analogues. On the contrary, all members of the 4′-substituted 7-hydroxyl derivatives (Fig. 4) inhibit MAO, with the 4′-NO_2 (**13a**) being most potent (IC_{50} = 0.1 μM), followed by 4′-Br (**12a**) (1.0 μM), 4′-F (**11a**) (1.5 μM), 4′-H (**1**) (8.6 μM), 4′-CH_3 (**14a**) (9 μM), 4′-OH (**23**) (12 μM), and 4′-NH_2-7-hydroxyisoflavone (**16a**) (12 μM). Potencies for MAO inhibition appear to be linked more to the electron withdrawing properties of the 4′-substituents rather than to their sizes: analogue that has the strongest electron withdrawing 4′-substituent (NO_2) also inhibits MAO most potently (IC_{50} = 0.1 μM), whereas that with the strongest electron donating 4′-substituent (NH_2) is least inhibitory (IC_{50} > 9 μM). Polarities of the 4′-substituents do not seem to

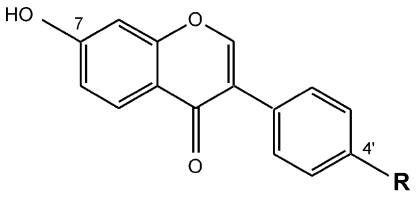


Cpd	R	ALDH-2	MAO
		IC_{50}, μM	
16b	NH_2	0.2	n.i.
4a	OH	0.28	5.3
4b	H	0.6	n.i.
11b	F	6.6	n.i.
12b	Br	>9	>9
14b	CH_3	>9	n.i.
13b	NO_2	n.i.	0.8

Figure 3. Effect of substitutions at the 4′ position on the potency of 7-*O*-ω-ethoxycarbonyl-pentylisoflavones for MAO and ALDH-2 inhibition.

affect MAO inhibition, for example, the 4′-H, 4′-CH_3, and 4′-OH derivatives have similar potency for MAO inhibition.

Substitution of 8-H. Early studies have shown that unlike daidzin, puerarin (**31**) does not inhibit ALDH-2 in vitro[22] and is not antidipsotropic in vivo (Fig. 5).[4,5] This, together with the fact that daidzein (**23**) is about 100-fold less potent than daidzin in ALDH-2 inhibition, suggest that substituents on both the C-7 and C-8 of daidzein are important for binding to ALDH-2. Since most, if not all, 7-*O*-substitutions of daidzein enhance ALDH-2 inhibition (Fig. 2), a free 7-OH appears to contribute negatively to ALDH-2 inhibitory activity. Therefore, the inactivity of puerarin could be attributed to (i) the bulky glucosyl group on the C-8 and/or (ii) the presence of a free hydroxyl group on C-7.



		IC_{50}, μM	
Cpd	R	ALDH-2	MAO
23	OH	4	12
16a	NH_2	4.5	>9
13a	NO_2	>9	0.1
14a	CH_3	>9	9
11a	F	n.i.	1.5
12a	Br	n.i.	1
1	H	n.i.	8.6

Figure 4. Effect of substitutions at the 4′ position on the potency of 7-hydroxyisoflavones for MAO and ALDH-2 inhibition.

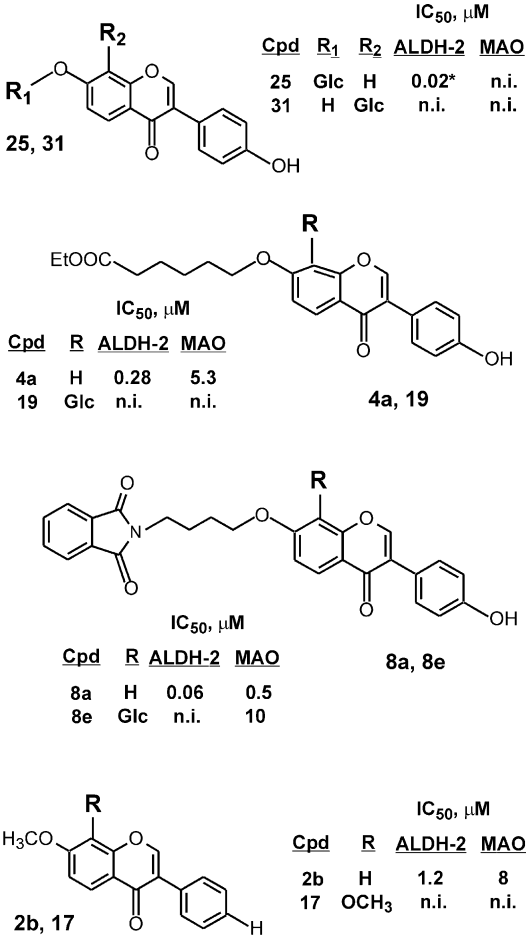


Figure 5. Effect of substitutions at the 8 position on the potency for MAO and ALDH-2 inhibition. *Data taken from ref 22.

To evaluate the effect of C-8 substituents on ALDH-2 and MAO inhibition, we synthesized two 8-C-C-glucosylated 7-*O*-substituted analogues, 7-ethoxycarbonyl-pentoxy-puerarin (**19**), 7-*O*-phthalimide-*N*-butylpuerarin (**8e**) and one 8-methoxylated 7-*O*-substituted analogues 7,8-dimethoxyisoflavone (**17**); determined and compared their potencies for ALDH-2 and MAO inhibition with their 8-H substituted counterparts, 7-ethoxycarbonyl-pentoxydaidzein (**4a**), 2-carboxy-7-*O*-phthalimide-*N*-butyldaidzein (**8a**), and 7-methoxy-isoflavone (**2b**), respectively. Results (Fig. 5) indicate that replacing the 8-H with either a glucosyl (**19** and **8e**) or a methoxy function (**17**) completely abolishes ALDH-2 inhibitory activities and substantially decreases potencies for MAO inhibition. Since ALDH-2 inhibition is essential for antidipsotropic activity, it is unlikely that better leads can be obtained by modifying the substituent on the 8 position.

Substitutions at the 5, 2, 6, and 3′ positions. In an early study, we showed that hydroxyl substitution at the 5 position of daidzin and daidzein decreases their potencies for ALDH-2 inhibition by at least an order of magnitude: **25** versus **27** and **23** versus **26**.[22] In this study, we showed that the 5-hydroxyl substituted 7-methoxydaidzein (**2a** vs **28**) also has decreased potency

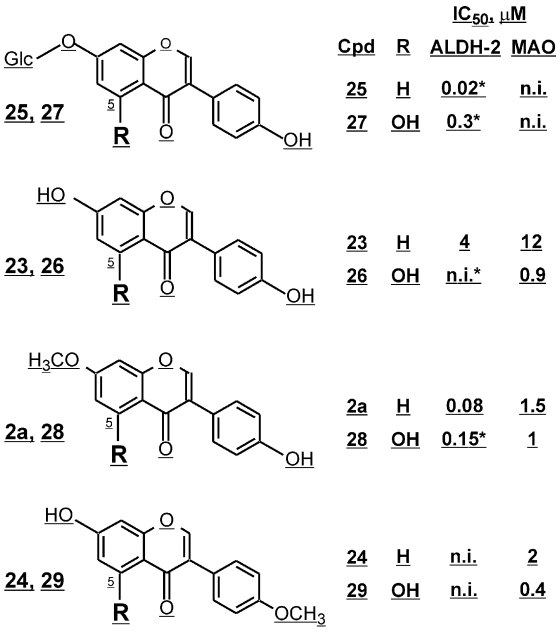


Figure 6. Effect of substitutions at the 5 position on the potency for MAO and ALDH-2 inhibition. *Data taken from ref 22.

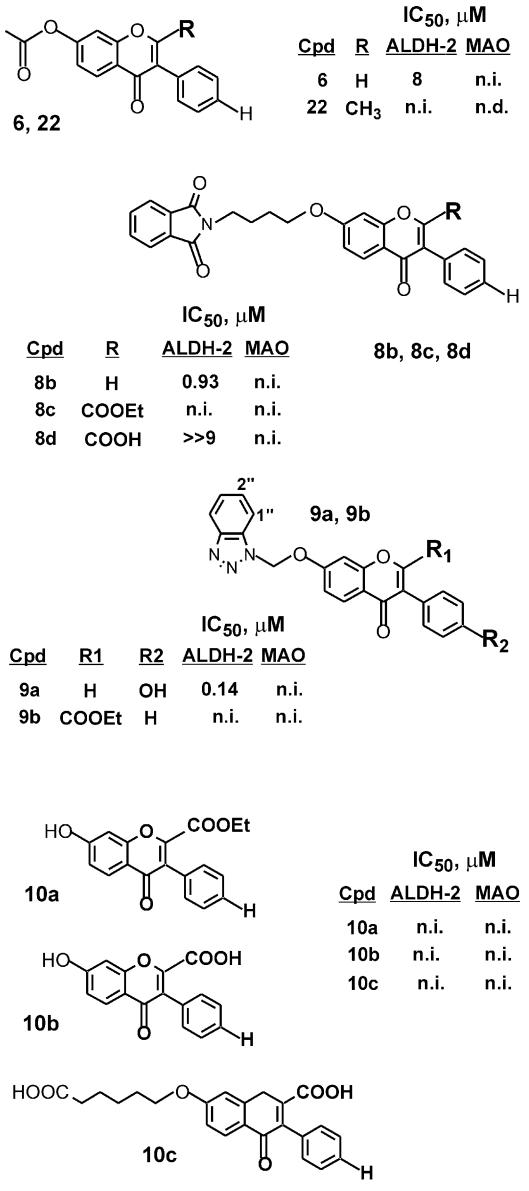


Figure 7. Effect of substitutions on the 2 position on the potency for MAO and ALDH-2 inhibition.

for ALDH-2 inhibition (Fig. 6). This, together with the finding that hydroxyl substitution at the 5 position increases potency for MAO inhibition (**23** vs **26**, **2a** vs **28**, **24** vs **29**), suggest that a 5-OH function contribute negatively to antidipsotropic activity and should be avoided in the design and synthesis of new antidipsotropic analogues.

Preliminary attempts were also made to reveal the effect of substitution at the 2 position on the potency for ALDH-2 inhibition (Fig. 7). Toward this end, we have prepared one 2-methyl (**22**), one 2-carboxy (**8d**), and two 2-ethoxycarbonyl (**8c**, **9b**) derivatives, determined and compared their potencies for ALDH-2 inhibition with their respective non-substituted counterparts, analogues **6**, **8b**, and **9a**. While analogues **6**, **8b**, and **9a** inhibit ALDH-2 fairly potently with IC_{50} values ranging from 8 to 0.14 μM, none of their 2-substituted derivatives exhibits any inhibitory activity toward ALDH-2.

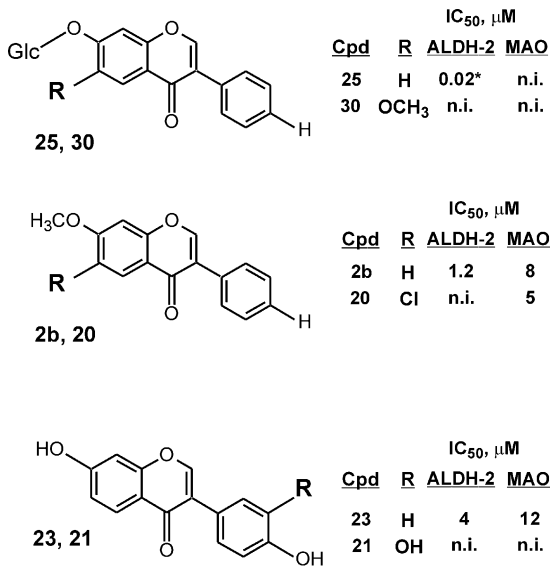


Figure 8. Effect of substitutions on the 6, and 3′ positions on the potency for MAO and ALDH-2 inhibition. *Data taken from ref 22.

Three additional 2-substituted analogues, **10a**, **10b**, and **10c** were also tested and shown to have no effect on ALDH-2 activity. These results suggest that the 2 position of bound daidzin occupies a critical area in the enzyme binding site and replacing its –H abolishes ALDH-2 inhibitory activity. This tentative conclusion may also prove to be true for the 6 and 3′ positions as substituting either 6- or 3′-H with OCH_3 (**25** vs **30**), Cl (**2b** vs **20**) or OH (**23** vs **21**) abolished ALDH-2 inhibitory activity (Fig. 8).

Conclusion

We have prepared a series of daidzin analogues in which their 4′-OH groups were replaced with H (**2b–5b**, **6**, **7b–9b**), F (**11a**,**b**), Br (**12a**,**b**), CH_3 (**14a**,**b**), NH_2 (**16a**,**b**), NO_2 (**13a**,**b**), or OCH_3 (**24**) substituents. Their potencies for ALDH-2 and MAO inhibition were determined and compared among each other and with those of their respective 7-*O*-substituted derivatives of daidzein. Results reveal that the potency for ALDH-2 inhibition of a daidzin analogue is associated with a small, polar 4′-substituent with hydrogen bonding capacity such as $–NH_2$ and –OH, whereas that for MAO inhibition is positively linked to the electron withdrawing property of the 4′-substituent. In ALDH-2, the 2, 5, 6, 8, and 3′ positions of a bound isoflavone are apparently located in relatively restricted areas as all attempts to replace the –H atom had resulted in complete loss of ALDH-2 inhibitory activity. On the basis of these results and those from previous studies,[11,15] we conclude that a potent antidipsotropic analogue of daidzin should be a 4′, 7-disubstituted isoflavone. The 4′-substituent should be small, polar, and with hydrogen bonding capacities, such as –OH and $–NH_2$, whereas the 7-substituent should be a straight-chain alkyl with a terminal polar function such as $–(CH_2)_n–OH$ with $2 \leq n \leq 6$, $–(CH_2)_n–COOH$ with $5 \leq n \leq 10$, or $–(CH_2)_n–NH_2$ with $n \geq 4$.

Experimental

General chemicals were purchased from either Aldrich Chemical Co. (Milwaukee, WI, USA) or Lancaster Synthesis Inc. (Windham, NH, USA). All organic solvents used were of HPLC grade and were supplied by J.P. Baker (Phillipsburg, NJ, USA) or Fisher Scientific Company (Pittsburgh, PA, USA). Daidzin (**25**) and Glycitin (**30**) were purchased from LC Laboratories (Woburn, MA, USA). Daidzein (**23**) was first synthesized by Tyger Scientific Inc. (Princeton, NJ, USA) and later obtained from LC Laboratories. 6-Chloro-7-methylisoflavone (**20**), 7,3′, 4′-trihydroxyisoflavone (**21**), formononetin (**24**), genistein (**26**), genistin (**27**), prunetin (**28**), biochanin A (**29**), and puerarin (**31**) were products of Indofine Chemical Company (Somerville, NJ, USA). 7-Acetoxy-2-methylisoflavone (**22**) was purchased from Aldrich (Milwaukee, WI, USA). The 7-*O*-substituted daidzein (**3a**–**5a**) were prepared as described in previous studies.[11,15] Serotonin (5-HT) was purchased from Research Biochemical International (Natick, MA, USA) and its metabolic intermediate 5-HIAL was produced in this laboratory by monoamine oxidase (MAO)-catalysed oxidative deamination of 5-HT using rat liver mitochondrial membrane as a source of MAO.[23] All other reagents used were best grade available.

MAO and ALDH-2 assays

The membrane and supernatant fraction 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.[11] 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

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 500 BQ spectrometer at 500 MHz and Bruker AM-500 spectrometer at 126 MHz (NuMega Resonace Labs. Inc., San Diego, CA, USA), 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 and negative mode. Melting points were determined with a Hoover capillary melting point apparatus. Elemental analyses were performed by NuMega Resonance Labs. Inc. Crude synthetic products were purified by one or a combination of the following methods: chromatography on Sephadex LH-20 (Fluka, 25–100 μm) or 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-Hydroxyisoflavone (1). To a solution of 6.3 g of 2, 4-dihydroxyphenyl-benzyl ketone (27.4 mmol) in 10.5 mL of 2-propanol was added 0.5 mL of morpholine and 6 mL of ethyl orthoformate (36.0 mmol). The mixture was stirred at 80 °C for 7 h and concentrated by flash evaporation. Residue was dissolved in 30 mL of methanol, stirred at 50–60 °C for 20 min and allowed to precipitate at 4 °C for 24 h. The yellowish precipitates formed were collected by filtration, washed with small portions of methanol, and dried to give 5.16 g of amorphous powder (1), a yield of 81.6% (w/w): mp 203–205 °C; ^{1}H NMR (DMSO-d_6) δ 6.88 (d, 1H, J = 2.0 Hz, 8-H), 6.95 (dd, 1H, J = 8.80, 1.90 Hz, 6-H), 7.38 (dd, 1H, J = 2.01, 7.24 Hz, 4′-H), 7.42 (dd, 1H, J = 1.70, 7.30 Hz, 3′, 5′-H), 7.57 (dd, 1H, J = 1.55, 7.84 Hz, 2′, 6′-H), 7.97 (d, 1H, J = 8.85 Hz, 5-H), 8.38 (s, 1H, 2-H), 10.80 (br. 1H, 7-OH). ^{13}C NMR (DMSO-d_6) δ 102.2 (C-8), 115.3 (C-6), 116.6 (C-10), 123.5 (C-3), 127.3 (C-1′), 127.7 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 132.1 (C-4′), 153.8 (C-2), 157.5 (C-9), 162.7 (C-7), 174.4 (C-4). MS (m/z) 239.2 $(M+H)^+$, 237.2 $(M-H)^-$. Anal. ($C_{15}H_{10}O_3$) for C, H. calcd 75.62, 4.23; found: 75.62, 4.21.

7-Methoxy-4′-hydroxyisoflavone (2a). To a solution of 5.1 g of daidzein (20.06 mmol) in 40 mL of DMSO was added 3.5 g of anhydrous K_2CO_3 (25.4 mmol) and 6 mL of iodomethane (96.4 mmol). The mixture was stirred at RT for 2 h and then poured into ice water to precipitate product. Precipitates were extracted with ethyl acetate, dried by flash evaporation and purified on a Sephadex LH-20 column (chloroform/methanol, 7:3). Final product was recrystallized from acetone to give 2.3 g of crystalline **2a**, a yield of 45.1% (w/w): mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (–OCH_3), 6.81 (d, 2H, J = 8.43 Hz, 3′, 5′-H), 7.05 (dd, 1H, J = 8.89, 2.39 Hz, 6-H), 7.11 (d, 1H, J = 2.07 Hz, 8-H), 7.39 (d, 2H, J = 8.62 Hz, 2′, 6′-H), 8.01 (d, 1H, J = 8.87 Hz, 5-H), 8.35 (s, 1H, 2-H), 9.54 (s, 1H, 4′-OH). Anal. ($C_{16}H_{12}O_4$) for C, H. calcd 71.64, 4.51; found: 71.24, 4.47.

7-Methoxyisoflavone (2b). This compound was prepared by the same method as **2a** using 500 mg of **1** instead of daidzein as the starting material. The total product obtained was 515 mg, a yield of 97.1% (mol/mol): mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (–OCH_3), 7.08 (dd, 1H, J = 8.94, 2.41 Hz, 6-H), 7.15 (d, 1H, J = 2.07 Hz, 8-H), 7.38 (m, 1H, 4′-H), 7.43 (t, 2H, J = 7.22, 7.76 Hz, 3′, 5′-H), 7.58 (d, 2H, J = 7.26 Hz, 2′, 6′-H), 8.03 (d, 1H, J = 8.86 Hz, 5-H), 8.45 (s, 1H, 2-H). Anal. ($C_{16}H_{12}O_3$) for C, H. calcd 76.26, 4.80; found: 76.07, 4.76.

7-*O*-Isopropylisoflavone (3b). This compound was prepared based on the same method described in a previous study.[5] To a solution of 2.50 g of **1** (10.5 mmol) in 30 mL of DMF was added 1.80 g of anhydrous K_2CO_3 (13.0 mmol) and 3.0 mL of 2-bromopropane (32.0 mmol). The mixture was stirred at 80 °C for 4 h and then poured into ice water. Precipitates were collected by filtration, washed with small portions of water, and dried to give a residue of crude product. The residue was loaded onto a Sephadex LH-20 column (chloroform/methanol, 7:3) and fractions that contained pure product were pooled,

concentrated, and recrystallized from acetone to give 1.05 g of crystalline **3b**, a yield of 42% (w/w): mp 110–111 °C. ^{1}H NMR (DMSO-d_6) δ 1.33 (–CH_3), 1.34 (–CH_3), 4.86 (m, 1H, >CH_2–), 7.05 (dd, 1H, J = 8.86, 2.20 Hz, 6-H), 7.16 (d, 1H, J = 2.50 Hz, 8-H), 7.38 (t, 1H, J = 6.78 Hz, 4′-H), 7.44 (t, 2H, J = 7.76, 1.6 Hz, 3′, 5′-H), 7.59 (d, 2H, J = 8.09, 1.6 Hz, 2′, 6′-H), 8.02 (d, 1H, J = 8.86 Hz, 5-H), 8.46 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 21.5 (–CH_3), 21.5 (–CH_3), 70.4 (–CH_2–O–), 101.8 (C-8), 115.7 (C-6), 117.3 (C-10), 123.7 (C-3), 127.0 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 132.0 (C-4′), 154.0 (C-2), 157.5 (C-9), 162.0 (C-7), 174.1 (C-4). MS (m/z) 281.4 $(M+H)^+$, 303.3 $(M+Na)^+$, 319.4 $(M+K)^+$, 279.5 $(M-H)^-$. Anal. ($C_{23}H_{24}O_5$) for C, H. calcd 77.12, 5.75; found: 76.50, 5.69.

7-*O*-Ethoxycarbonylpentylisoflavone (4b). This compound was prepared by the same method described for **3b** except that ethyl 6-bromohexanoate was used as the alkylating agent. From 2.52 g of **1**, 3.42 g of **4b** was obtained, a yield of 85% (mol/mol): mp 112–114 °C; ^{1}H NMR (DMSO-d_6) δ1.17 (t, 3H, –CH_3), 1.45 (m, 2H, –CH_2–), 1.60 (m, 2H, –CH_2–), 1.77 (m, 2H, –CH_2–), 2.32 (t, 2H, –CH_2–), 4.05 (q, 2H, –CH_2–O–), 4.12 (t, 2H, –CH_2–O–), 7.07 (dd, 1H, J = 8.86, 1.94 Hz, 6-H), 7.16 (d, 1H, J = 1.97 Hz, 8-H), 7.38 (dd, 1H, J = 7.75, 1.85 Hz, 4′-H), 7.44 (d, 2H, J = 7.67 Hz, 3′, 5′-H), 7.58 (d, 2H, J = 1.94, 7.19 Hz, 2′, 6′-H), 8.02 (d, 1H, J = 8.94 Hz, 5-H), 8.47 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (–CH_3), 24.2 (–CH_2–), 25.0 (–CH_2–), 28.1 (–CH_2–), 33.4 (–CH_2–), 59.7 (–CH_2–O–), 68.4 (–CH_2–O–), 101.1 (C-8), 115.1 (C-6), 117.5 (C-10), 123.7 (C-3), 126.9 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 132.0 (C-4′), 154.1 (C-2), 157.5 (C-9), 163.1 (C-7), 172.8 (>C=O), 174.4 (C-4). MS (m/z) 381.5 $(M+H)^+$, 403.6 $(M+Na)^+$, 419.3 $(M+K)^+$. Anal. ($C_{23}H_{24}O_5$) for C, H. calcd 72.61, 6.36; found: 72.57, 6.33.

7-(Hydroxyethylethoxy)ethoxyisoflavone (5b). This compound was prepared by the same method described for **3b** except that 2-[2-(2-chloroethoxy)ethoxy]ethanol was used as the alkylating agent. From 2 g of **1**, 280 mg of **5b** was obtained, a yield of 14% (w/w): mp 105–107 °C. ^{1}H NMR (DMSO-d_6) δ 3.43 (m, 2H, –CH_2–), 3.50 (m, 2H, –CH_2–), 3.56 (m, 2H, –CH_2–), 3.61 (m, 2H, –CH_2–), 3.80 (m, 2H, –CH_2–), 4.26 (m, 2H, –CH_2–), 4.58 (t, 2H, –CH_2–), 7.10 (dd, 1H, J = 8.97, 2.57 Hz, 6-H), 7.18 (d, 1H, J = 2.68 Hz, 8-H), 7.37 (t, 1H, J = 7.26 Hz, 4′-H), 7.44 (t, 2H, J = 7.75 Hz, 3′, 5′-H), 7.57 (dd, 1H, J = 1.60, 7.32 Hz, 2′, 6′-H), 8.03 (d, 1H, J = 8.88 Hz, 5-H), 8.46 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 60.2 (–CH_2–), 68.1 (–CH_2–), 68.6 (–CH_2–), 69.8 (–CH_2–), 70.0 (–CH_2–), 72.4 (–CH_2–), 101.2 (C-8), 115.1 (C-6), 117.8 (C-10), 123.7 (C-3), 126.9 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 132.1 (C-4′), 154.1 (C-2), 157.4 (C-9), 162.9 (C-7), 174.4 (C-4). MS (m/z) 371.5 $(M+H)^+$, 393.3 $(M+Na)^+$, 409.2 $(M+K)^+$. Anal. ($C_{21}H_{22}O_6$) for C, H. calcd 68.10, 5.99; found: 67.92, 6.05.

7-*O*-Acetylisoflavone (6). To a solution of 500 mg of **1** in 15 mL of anhydrous pyridine 2.0 mL of acetic anhydride was added. The mixture was gently stirred for 6 min and left at room temperature for 72 h. Reaction product was precipitated in 100 mL of ice water, collected by filtration, washed with small portions of cold water, and dried under vacuum to give 490 mg of **6** (crystalline needle), a yield of 98% (mol/mol): mp 134–135 °C. ^{1}H NMR (DMSO-d_6) δ 2.34 (–CH_3), 7.33 (dd, 1H, J = 8.47, 2.47 Hz, 6-H), 7.39 (m, 1H, 4′-H), 7.45 (t, 2H, J = 7.75 Hz, 3′, 5′-H), 7.58 (d, 1H, J = 2.3 Hz, 8-H), 7.60 (d, 2H, J = 7.75, 1.79 Hz, 2′, 6′-H), 8.18 (d, 1H, J = 8.86 Hz, 5-H), 8.55 (s, 1H, 2-H). Anal. ($C_{17}H_{12}O_4$) for C, H. calcd 72.85, 4.35; found: 72.36, 4.34.

7-*O*-[Tetrahydro-2-(H)-pyran-2-*O*-propanyl]-daidzein (7a). This compound was prepared by the same method described for **3b** except that daidzein and 2-(3-bromopropoxy)tetrahydro-2H-pyran were used as starting materials. From 5.1 g of daidzein, 4.20 g of **7a** (white amorphous powder) was obtained, a yield of 82.4% (w/w): mp 112–113 °C. ^{1}H NMR (DMSO-d_6) δ 1.39 (m, 2H, –CH_2), 1.49 (m, 2H, 3″-H), 1.59–1.71 (m, 2H, 2″-H), 2.01 (m, 2H, –O–CH_2–), 3.34–3.53 (m, 2H, 4″-H), 3.70–3.82 (m, 2H, 5″-H), 4.19 (t, 2H, –CH_2–O–), 4.58 (d, 1H, J = 3.62 Hz, 1″-H), 6.82 (d, 2H, J = 8.53, 2.59, Hz, 3′,5′-H), 7.06 (dd, 1H, J = 8.86, 2.42 Hz, 6-H), 7.14 (d, 1H, J = 2.13 Hz, 8-H), 7.39 (d, 2H, J = 8.64 Hz, 2′,6′-H), 8.01 (d, 1H, J = 8.88 Hz, 5-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 19.1 (–CH_2–), 25.0 (C-3″), 28.9 (C-4″), 30.2 (C-2″), 61.3 (C-5″), 63.1 (–CH_2–O–), 65.6 (–CH_2–O–), 98.0 (C-1″), 101.0 (C-8), 114.9 (C-6), 114.9 (C-3′, 5′), 117.6 (C-10), 122.4 (C-1′), 123.7 (C-3), 126.9 (C-5), 130.0 (C-2′, 6′), 153.1 (C-2), 157.2 (C-9), 157.4 (C-4′), 162.9 (C-7), 174.7 (C-4). MS (m/z) 397.2 $(M+H)^+$, 419.4 $(M+Na)^+$, 435.4 $(M+K)^+$, 395.4 $(M-H)^-$. Anal. ($C_{23}H_{24}O_6$) for C, H. calcd 69.68, 6.10; found: 69.43, 6.08.

7-*O*-[Tetrahydro-(2H)-pyran-2-*O*-]propanylisoflavone (7b). This compound was prepared by the same method described for **3b** except that 2-(3-bromopropoxy) tetrahydro-2H-pyran was used as the alkylating agent. From 2.38 g of **1**, 2.67 g of **7b** (crystalline needle) was obtained, a yield of 70.2% (mol/mol): mp 113–115 °C. ^{1}H NMR (DMSO-d_6) δ 1.41 (m, 2H, –CH_2–), 1.47 (m, 2H, 3″-H), 1.59–1.73 (m, 2H, 2″-H), 2.0 (m, 2H, –O–CH_2–), 3.40–3.54 (m, 2H, 4″-H), 3.70–3.83 (m, 2H, 5″-H), 4.21 (t, 2H, –CH_2–O–), 4.58 (d, 1H, J = 3.5 Hz, 1″-H), 7.08 (dd, 1H, J = 8.9, 1.97 Hz, 6-H), 7.17 (d, 1H, J = 1.97 Hz, 8-H), 7.38 (t, 1H, J = 6.98 Hz, 4′-H), 7.43 (t, 2H, J = 7.38 Hz, 3′, 5′-H), 7.58 (d, 2H, J = 7.56 Hz, 2′, 6′-H), 8.03 (d, 1H, J = 8.88 Hz, 5-H), 8.46 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 19.1 (–CH_2–), 25.0 (C-3″), 28.9 (C-4″), 30.2 (C-2″), 61.3 (C-5″), 63.1 (–CH_2–*O*–), 65.7 (–CH_2–O–), 98.0 (C-1″), 101.1 (C-8), 115.1 (C-6), 117.6 (C-10), 123.7 (C-3), 126.96 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 132.0 (C-4′), 154.1 (C-2), 157.4 (C-9), 163.1 (C-7), 174.1 (C-4). MS (m/z) 381.4 $(M+H)^+$, 403.4 $(M+Na)^+$, 419.2 $(M+K)^+$, 379.6 $(M-H)^-$. Anal. ($C_{23}H_{24}O_5$) for C, H. calcd 72.61, 6.36; found: 72.02, 6.34.

7-*O*-(Phthalimide-*N*-)butyldaidzein (8a). This compound was prepared according to the method described before.[15] To a suspension of 5.3 g of daidzein (20.85 mmol) and 50 mL of acetone, 11 mL of 2 N KOH

(22 mmol) and 5.6 g of *N*-(4-bromobutyl)-phthalimide (19.9 mmol) were added. The mixture was stirred under gentle reflux for 72 h and concentrated. The residue was dissolved in chloroform/methanol (7:3) and loaded onto a Sephadex LH-20 column (chloroform/methanol, 7:3). Fractions that contained pure product were pooled, concentrated, and recrystallized from acetone to give 1.76 g of **8a**, a yield of 33.2% (w/w): mp 195–197 °C. ^{1}H NMR (DMSO-d_6) δ 1.78 (–CH_2–CH_2–), 3.65 (–N–CH_2–), 4.13 (–O–CH_2–), 6.81 (d, 2H, J = 8.5 Hz, 3′, 5′-H), 7.01 (dd, 1H, J = 8.89, 2.5 Hz, 6-H), 7.07 (d, 1H, J = 2.2 Hz, 8-H), 7.39 (d, 2H, J = 8.6 Hz, 2′, 6′-H), 7.80 (m, 2H, 5″, 6″-H), 7.83 (m, 2H, 4″, 7″-H), 7.96 (d, 1H, J = 8.89 Hz, 5-H), 8.32 (s, 1H, 2-H), 9.54 (s, 1H, 4′-OH). ^{13}C NMR (DMSO-d_6) δ 24.6 (–CH_2–), 25.8 (–CH_2–), 37.1 (–N–CH_2–), 67.9 (–CH_2–O–), 101.0 (C-8), 114.8 (C-6), 114.9 (C-3′, 5′), 117.5 (C-10), 122.4 (C-1′), 122.9 (C-3″, 8″), 123.6 (C-3), 126.9 (C-5), 130.0 (C-2′, 6′), 131.6 (C-5″, 6″), 134.3 (C-4″, 7″), 153.0 (C-2), 157.2 (C-9), 157.3 (C-4′), 162.8 (C-7), 168.0 (C-2″, 9″), 174.6 (C-4). MS (*m*/*z*) 456.5 $(M+H)^+$. Anal. ($C_{27}H_{21}O_6N$) for C, H, N. calcd 71.20, 4.65, 3.08; found: 70.89, 4.61, 3.09.

7-*O*-Phthalimide-*N*-butylisoflavone (8b). This compound was prepared by the same method described for **8a** except that **1** was used instead of daidzein. From 1.6 g of **1**, 1.26 g of **8b** (white crystals) was obtained, a yield 78.5% (w/w): mp 167–168 °C. ^{1}H NMR (DMSO-d_6) δ 1.78 (–CH_2–CH_2–), 3.65 (–N–CH_2–), 4.14 (–O–CH_2–), 7.03 (dd, 1H, J = 8.9, 2.0 Hz, 6-H), 7.10 (d, 1H, J = 1.8 Hz, 8-H), 7.37 (dd, 1H, J = 6.92 Hz, 4′-H), 7.42 (dd, 2H, J = 7.7 Hz, 3′, 5′-H), 7.57 (dd, 2H, J = 7.6, 2′, 6′-H), 7.80 (m, 2H, 5″, 6″-H), 7.83 (m, 2H, 4″, 7″-H), 7.96 (d, 1H, J = 8.89 Hz, 5-H), 8.32 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 24.6 (–CH_2–), 25.8 (–CH_2–), 37.1 (–N–CH_2–), 67.9 (–CH_2–O–), 101.0 (C-8), 115.0 (C-6), 117.5 (C-10), 122.9 (C-3″, 8″), 123.7 (C-3), 126.9 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 131.6 (C-5″, 6″), 132.0 (C-4′), 134.3 (C-4″, 7″), 154.1 (C-2), 157.4 (C-9), 163.0 (C-7), 168.0 (C-2″, 9″), 174.6 (C-4). MS (*m*/*z*) 440.1 $(M+H)^+$. Anal. ($C_{27}H_{21}O_5N$) for C, H, N. calcd 73.79, 4.82, 3.19; found: 73.40, 4.81, 3.20.

2-Ethoxycarbonyl-7-*O*-phthalimide-*N*-butylisoflavone (8c). To a solution of 2.53 g (8.16 mmol) of **10a** (see below for method of synthesis) in 50 mL of DMF, 1.59 g of K_2CO_3 (11.49 mmol) and 3.0 g of *N*-4-bromobutyl-phthalimide (10.63 mmol) were added, respectively. The mixture was stirred at 80 °C for 1 h and then poured into ice water. Precipitates were collected by filtration, washed with small portions of water, dried, and recrystallized in acetone to give 3.85 g of **8c**, a yield of 92.2% (mol/mol): mp 163–165 °C. ^{1}H NMR (DMSO-d_6) δ 0.90 (–CH_3), 1.78 (–CH_2–CH_2–), 3.65 (–N–CH_2–), 4.08 (–O–CH_2–), 4.16 (–O–CH_2–), 7.05 (dd, 1H, J = 8.97, 2.42 Hz, 6-H), 7.16 (d, 1H, J = 2.44 Hz, 8-H), 7.24 (d, 2H, J = 8.53 Hz, 3′, 5′-H), 7.39 (m, 3H, 2′, 4′, 6′-H), 7.79–7.81 (m, 2H, 5″, 6″-H), 7.82–7.85 (m, 2H, 4″, 7″-H), 7.92 (d, 1H, J = 8.89 Hz, 5-H). ^{13}C NMR (DMSO-d_6) δ 13.2 (–CH_3), 24.5 (–CH_2–), 25.8 (–CH_2–), 37.1(–N–CH_2–), 62.1 (–CH_2–O–), 68.1 (–CH_2–O–), 101.0 (C-8), 115.9 (C-6), 116.7 (C-10), 122.9 (C-3″, 8″), 125.2 (C-1′), 126.9 (C-3), 127.8 (C-3′, 5′), 127.9 (C-5), 129.7 (C-2′, 6′), 131.5 (C-5″, 6″), 134.3 (C-4″, 7″), 150.0 (C-2), 156.7 (C-9), 160.9 (>C=O), 163.7 (C-7), 168.0 (C-2″, 9″), 175.3 (C-4). MS (*m*/*z*) 533.7 $[(M-1)+Na]^+$, 549.6 $[(M-1)+K]^+$, 439.1 $[M-COOC_2H_5-1]^-$. Anal. ($C_{30}H_{25}O_7N$) for C, H, N. calcd 70.44, 4.93, 2.74; found: 70.35, 4.92, 2.76.

2-Carboxy-7-*O*-phthalimide-*N*-butylisoflavone (8d). This compound was prepared by hydrolyzing **8c**. To a solution of 1.0 g of **8c** in 20 mL of methanol was added 80 mL of 0.02 N aqueous KOH. The mixture was stirred under gentle reflux for 3 h until all samples were hydrolyzed (TLC). Methanol was removed by flash evaporation. The remaining solution was acidified with 1 N HCl to pH 2–3 to precipitate produce. Precipitates were collected by filtration and washed with small portions of water until pH of filtrate became neutral. The solution was then dried and residue was recrystallized from acetone to give 630 mg of **8d** (white crystals): mp 143–145 °C. MS (*m*/*z*) 484.5 $(M+H)^+$, 506.4 $(M+Na)^+$, 482.5 $(M-H)^-$. Anal. ($C_{28}H_{21}O_7N$) for C, H, N. calcd 69.56, 4.38, 2.90; found: 69.21, 4.42, 2.89.

7-*O*-Phthalimide-*N*-butylpuerarin (8e). This compound was prepared by the same method described for **8a** except that puerarin was used instead of daidzein. From 500 mg of puerarin, 512 mg of **8e** (white powder) was obtained, a yield of 69.0%: mp 68–69 °C. ^{1}H NMR (DMSO-d_6) δ 1.78 (–CH_2–CH_2–), 3.65 (–N–CH_2–), 4.14 (–O–CH_2–), 4.82 (d, 1H, J = 9.7 Hz, glucosyl 1‴-H), 7.03 (dd, 1H, J = 8.9, 2.0 Hz, 6-H), 7.10 (d, 1H, J = 1.8 Hz, 8-H), 7.37 (dd, 1H, J = 6.92 Hz, 4′-H), 6.81 (dd, 2H, J = 7.7 Hz, 3′, 5′-H), 7.41 (dd, 2H, J = 7.6, 2′, 6′-H), 7.80 (m, 2H, 5″, 6″-H), 7.83 (m, 2H, 4″, 7″-H), 7.96 (d, 1H, J = 8.89 Hz, 5-H), 8.40 (s, 1H, 2-H), 9.54 (s, 1H, 4′-OH). ^{13}C NMR (DMSO-d_6) δ 24.6 (–CH_2–), 25.8 (–CH_2–), 37.1(–N–CH_2–), 61.5 (glucosyl C-6‴), 67.9 (–CH_2–O–), 70.7 (glucosyl C-4‴), 70.75 (glucosyl C-2‴), 73.39 (glucosyl C-1‴), 78.71 (glucosyl C-3‴), 81.79 (glucosyl C-5‴), 112.6 (C-8), 115.0 (C-6), 117.5 (C-10), 122.9 (C-3″, 8″), 123.7 (C-3), 126.9 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 131.6 (C-5″, 6″), 132.0 (C-4′), 134.3 (C-4″, 7″), 154.1 (C-2), 157.4 (C-9), 163.0 (C-7), 168.0 (C-2″, 9″), 174.6 (C-4). MS (*m*/*z*) 618.4 $(M+H)^+$, 640.5 $(M+Na)^+$, 616.7 $(M-H)^-$. Anal. ($C_{33}H_{31}O_{11}N$) for C, H, N. calcd 64.18, 5.06, 2.27; found: 63.89, 5.05, 2.24.

7-*O*-1H-Benzotriazole-1-methyldaidzein (9a). This compound was prepared by the same method described for **3b** except that daidzein and 1-chloromethyl-1H-benzotriazole were used as the starting material. From 5.1 g of daidzein, 4.35 g of **9a** was obtained, a yield of 85.3% (w/w): mp 227–228 °C. ^{1}H NMR (DMSO-d_6) δ 6.83 (dd, 2H, J = 8.63, 2.62 Hz, 3′, 5′-H), 6.98 (s, 2H, –CH_2–), 7.21(dd, 1H, J = 8.89, 2.31 Hz, 6-H), 7.41 (dd, 2H, 2′, 6′-H), 7.48 (t, 1H, J = 7.84 Hz, 1″-H), 7.54 (d, 1H, J = 2.02 Hz, 8-H), 7.66 (t, 1H, J = 7.69, 2.02 Hz, 4″-H), 8.03 (t, 1H, J = 8.85, 8.04 Hz, 2″, 3″-H), 8.11 (d, 1H, J = 8.52 Hz, 5-H), 8.40 (s, 1H, 2-H), 9.56 (s, 1H, 4′-OH). ^{13}C NMR (DMSO-d_6) δ 73.7 (–N–CH_2–O–), 103.2 (C-8), 110.7 (C-1″), 115.0 (C-3′, 5′), 115.4 (C-6), 118.9 (C-4″), 119.5 (C-10), 122.2 (C-1′), 123.8 (C-3), 124.8 (C-2″),

127.3 (C-5), 128.5 (C-3″), 130.1 (C-2′, 6′), 132.7 (C-6″), 145.3 (C-5″), 153.4 (C-2), 156.9 (C-9), 157.3 (C-4′), 159.9 (C-7), 174.7 (C-4). MS (*m*/*z*), 386.4 $(M+H)^+$. Anal. ($C_{29}H_{20}O_4N_3$) for C, H, N. calcd 68.57, 3.92, 10.90, found: 68.28, 3.92, 10.94.

2-Ethoxycarbonyl-7-*O*-1H-benzotriazole-1-methylisoflavone (9b). This compound was prepared by the same method described for **9a** except that **10a** (see below for method of synthesis) was used instead of daidzein. From 3.1 g of **10a**, 1.43 g of **9b** was obtained, a yield of 46% (w/w): mp 182–183 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (–CH_3), 4.09 (–CH_2–O–), 7.03 (s, 2H, –N–CH_2–O–), 7.23 (dd, 1H, J = 8.78, 2.79 Hz, 6-H), 7.25 (dd, 2H, J = 8.78, 2.77 Hz, 3′, 5′-H), 7.40 (dd, 2H, J = 8.53, 1.50 Hz 2′, 6′-H), 7.40 (d, 1H, J = 1.5 Hz, 8-H), 7.49 (t, 1H, J = 7.99, 7.46 Hz, 4′-H), 7.65–7.68 (m, 2H, 1″, 4″-H), 7.99–8.04 (m, 2H, 2″, 3″-H), 8.12 (d, 1H, J = 8.38 Hz, 5-H). ^{13}C NMR (DMSO-d_6) δ 13.2 (–CH_3), 62.1 (–CH_2–O–), 73.6 (–N–CH_2–O–), 103.2 (C-8), 110.7 (C-1″), 116.3 (C-6), 118.1 (C-10), 119.5 (C-4″), 124.8 (C-3), 125.5 (C-1′), 127.3 (C-5), 127.8 (C-3′, 5′), 128.0 (C-2″), 128.6 (C-3″), 129.7 (C-2′, 6′), 131.4 (C-6″), 132.7 (4′-H), 145.3 (C-5″), 150.1 (C-2), 156.3 (C-9), 160.8 (>C=O, C-7), 175.5 (C-4). MS (*m*/*z*) 442.2 $(M+H)^+$, 463.9 $(M+Na)^+$, 479.8 $(M+K)^+$. Anal. ($C_{25}H_{19}O_5N_2$) for C, H, N. calcd 68.02, 4.34, 9.52; found: 68.66, 4.36, 9.58.

2-Ethoxycarbonyl-7-hydroxyisoflavone (10a). This compound was prepared according to the method described in the reference.[6,7] To a solution of 4.56 g of 2,4-dihydroxyphenylbenzylketone (20.24 mmol) in 20 mL of pyridine was added 3.5 mL of ethyl chlorooxoacetate (31.28 mmol). The mixture was stirred gently at room temperature for 1 h and yellowish precipitates were formed. The precipitates were collected by filtration and dried, redissolved in chloroform/methanol (7:3), and loaded onto a Sephadex LH-20 (chloroform/methanol, 7:3) column. Fractions that contained pure product were pooled, concentrated and recrystallized from acetone to give 4 g of **10a**, a yield of 87.61%: mp 213–214 °C. ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, –CH_3), 4.05 (–CH_2–), 6.90 (d, 1H, J = 1.91 Hz, 8-H), 6.98 (dd, 1H, J = 8.82, 1.91 Hz, 6-H), 7.23 (d, 1H, J = 7.15 Hz, 3′, 5′- H), 7.39–7.42 (m, 3H, 2′, 4′, 6′-H), 7.92 (d, 1H, J = 8.84 Hz, 5-H), 11.02 (s, 1H, 7-OH). ^{13}C NMR (DMSO-d_6) δ 13.2 (–CH_3), 62.0 (–CH_2–O–), 102.2 (C-8), 115.9 (C-6), 115.9 (C-10), 125.1 (C-1′), 127.4 (C-3), 127.8 (C-3′, 5′), 127.9 (C-5), 129.8 (C-2′, 6′), 131.7 (C-4′), 149.8 (C-2), 156.8 (C-9), 161.0 (>C=O), 163.5 (C-7), 175.3 (C-4). MS (*m*/*z*) 311.3 $(M+H)^+$, 333.1 $(M+Na)^+$, 309.2 $(M-H)^-$. Anal. ($C_{18}H_{14}O_5$) for C, H. calcd 69.67, 4.55; found: 69.27, 4.59.

2-Carboxy-7-hydroxyisoflavone (10b). This compound was obtained by hydrolyzing **10a**. To a solution of 1 g of **10a** in 10 mL of methanol was added 20 mL of 0.2 N aqueous KOH. The mixture was stirred under gentle reflux for 2 h until **10a** was completely hydrolyzed (monitored by TLC). Methanol was removed on a rotary evaporator. Hydrolyzed product in remaining solution was precipitated by acidification (HCl, pH 2–3). Precipitates were collected by filtration, washed with small portions of water until pH of filtrate approached neutral, and recrystallized from acetone to give 630 mg of pure **10b**, a yield of 63% (w/w): mp 257–258 °C. MS (*m*/*z*) 283.2 $(M+H)^+$, 305.3 $(M+Na)^+$, 321.3 $(M+K)^+$, 237.4 $(M-COOH)^+$, 281.5 $(M-H)^-$. Anal. ($C_{16}H_{10}O_5$) for C, H. calcd 68.09, 3.57; found: 68.17, 3.54.

2-Carboxy-7-ω-carboxylpentyl isoflavone (10c). To a solution of 5.0 g (16.1 mmol) of **10a** in 60 mL of DMF was added 2.6 g of K_2CO_3 (18.8 mmol) and 3 mL of ethyl 6-bromo hexanoate (16.86 mmol). The mixture was refluxed for 1 h and poured into 100 mL of ice/water. Products were extracted with ethyl acetate and evaporated to dryness. Dry residue was dissolved in 20 mL of methanol followed by addition of 20 mL of 1 N KOH (20 mmol). The mixture was stirred under gentle reflux for 2 h until the ethyl ester was completely hydrolyzed (monitored by TLC). Solvent was removed under reduced pressure. Residue was dissolved in chloroform/methanol (7:3) and purified on a Sephadex-LH-20 column in chloroform/methanol (7:3). Fractions that contained pure product were pooled, concentrated and recrystallized from acetone to give 5.34 g of **10c**, a yield of 83.7%: mp 157–159 °C. ^{1}H NMR (DMSO-d_6) δ 1.44 (m, 2H, –CH_2), 1.60 (m, 2H, –CH_2), 1.76 (m, 2H, –CH_2), 2.33 (m, 2H, –CH_2), 4.14 (–CH_2–), 7.09 (d, 1H, J = 1.8 Hz, 6-H), 7.21 (d, 1H, J = 2.5 Hz, 8-H), 7.26 (dd, 1H, J = 7.54, 1.52 Hz, 3′, 5′- H), 7.34–7.41 (m, 3H, 2′, 4′, 6′-H), 7.96 (d, 1H, J = 8.88 Hz, 5-H). ^{13}C NMR (DMSO-d_6) δ 24.1 (–CH_2), 24.9 (–CH_2), 28.0 (–CH_2), 33.1 (–CH_2), 33.4 (–CH_2), 68.5 (–CH_2–O–), 101.0 (C-8), 115.8 (C-6), 116.7 (C-10), 123.9 (C-1′), 126.9 (C-3), 127.8 (C-3′, 5′), 127.9 (C-5), 129.8 (C-2′, 6′), 131.7 (C-4′), 151.7 (C-2), 156.8 (C-9), 162.3 (>C=O), 163.8 (C-7), 173.3 (C-4), 175.5 (C-4). Anal. ($C_{22}H_{20}O_7$) for C, H. calcd 66.67, 5.09; found: 66.89, 5.14.

7-Hydroxy-4′-fluoroisoflavone (11a). A mixture of 1.54 g of 4-fluorophenylacetic acid (90.5 mmol), 11.0 g of resorcinol (99.9 mmol) and 50 mL of boron trifluoride diethyl etherate was stirred at 80 °C for 5 h. The reaction mixture was cooled to room temperature and then washed first with aqueous K_2CO_3 and then with water until the pH of the water layer approached 7. The product was extracted with ethyl acetate and concentrated. The residue was dissolved and loaded onto a Sephadex-LH-20 column (chloroform/methanol, 7:3). Fractions that contained pure **11a** were pooled and dried to give 3.5 g of crude 2, 4-dihydroxyphenyl-4′-fluorobenzylketone (**I**). To 3.5 g of **I** in 20 mL of 2-propanol was added 1.0 mL of morpholine and 2.5 mL of triethyl orthoformate. The mixture was stirred at 80 °C for 7 h. Solvents were removed and residue was dissolved in 30 mL of methanol and stirred at 50 °C for 30 min. The solution was cooled to room temperature and kept at 4 °C overnight. White crystals were collected by filtration, washed with small portions of methanol, and dried to give 1.53 g of **11a**, a yield of 43.7% (w/w): mp 235–236 °C. ^{1}H NMR (DMSO-d_6) δ 6.88 (d, 1H, J = 1.73 Hz, 8-H), 6.95 (dd, 1H, J = 8.80, 1.85 Hz, 6-H), 7.25 (t, 2H, J = 8.82 Hz, 3′, 5′-H), 7.61(dd, 2H, J = 2.52, 8.21 Hz, 2′, 6′-H), 7.97 (d, 1H, J = 8.80 Hz, 5-H), 8.40 (s, 1H, 2-H), 10.8 (s. 1H, 7-OH). ^{13}C NMR (DMSO-d_6) δ

102.2 (C-8), 114.9 (C-6), 115.0 (C-3′, 5′), 116.5 (C-10), 122.5 (C-1′), 127.3 (C-3), 128.4 (C-5), 130.9 (C-2′, 6′), 153.8 (C-2), 157.5 (C-9), 160.8 (C-4′), 162.7 (C-7), 174.3 (C-4). MS (*m*/*z*) 257.2 $(M+H)^+$, 279.3 $(M+Na)^+$, 255.4 $(M-H)^-$. Anal. ($C_{15}H_9O_3F$) for C, H. Calcd: 70.31, 3.54; found: 70.56, 3.51.

7-*O*-Ethoxycarbonylpentyl-4′-fluoroisoflavone (11b). This compound was prepared by the same method described for **3b** except that the starting materials were **11a** and ethyl 6-bromohexanoate. From 1.28 g of **11a**, 964 mg of **11b** (crystalline needles) was obtained, a yield of 75.3% (w/w): mp 91–93 °C. ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3), 1.43 (m, 2H, –CH_2–), 1.60 (m, 2H, –CH_2–), 1.76 (m, 2H, –CH_2–), 2.31 (t, 2H, –CH_2–), 4.05 (q, 2H, –CH_2–O–), 4.10 (t, 2H, –CH_2–O–), 7.05 (d, 1H, *J* = 8.88, Hz, 6-H), 7.14 (d, 1H, *J* = 1.97 Hz, 8-H), 7.26 (d, 2H, *J* = 8.78 Hz, 3′, 5′-H), 7.63 (t, 2H, *J* = 8.78 Hz, 2′, 6′-H), 8.01 (d, 1H, *J* = 8.84 Hz, 5-H), 8.46 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (–CH_3), 24.2 (–CH_2–), 24.9 (–CH_2–), 28.1 (–CH_2–), 33.4 (–CH_2–), 59.6 (–CH_2–O–), 68.3 (–CH_2–O–), 101.0 (C-8), 114.9 (C-6), 115.0 (C-3′, 5′), 117.4 (C-10), 122.7 (C-1′), 126.9 (C-3), 128.3 (C-5), 130.9 (C-2′, 6′), 154.1 (C-2), 157.4 (C-9), 160.9 (C-4′), 163.1 (C-7), 172.8 (>C=O), 174.3 (C-4). MS (*m*/*z*) 399.4 $(M+H)^+$, 421.4 $(M+Na)^+$. Anal. ($C_{23}H_{23}O_5F$) for C, H. calcd 69.34, 5.82; found: 69.23, 5.79.

7-Hydroxy-4′-bromoisoflavone (12a). The synthesis was similar to that described for **11a** except that 4-fluorophenylacetic acid was replaced with 4-bromophenylacetic acid. This synthesis resulted in 1.41 g of **12a**, a yield of 42% (based on resorcinol, w/w): mp 266–268 °C. ^{1}H NMR (DMSO-d_6) δ 6.87 (d, 1H, *J* = 1.65 Hz, 8-H), 6.94 (dd, 1H, *J* = 8.80, 1.78 Hz, 6-H), 7.54 (d, 2H, *J* = 8.43 Hz, 3′, 5′-H), 7.61 (d, 2H, *J* = 8.43 Hz, 2′, 6′-H), 7.97 (d, 1H, *J* = 8.63 Hz, 5-H), 8.42 (s, 1H, 2-H), 10.8 (s, br. 1H, 7-OH). ^{13}C NMR (DMSO-d_6) δ 102.2 (C-8), 115.4 (C-6), 116.4 (C-10), 121.0 (C-1′), 122.3 (C-3), 127.3 (C-5), 130.9 (C-3′, 5′), 131.0 (C-2′, 6′), 131.4 (C-4′), 154.0 (C-2), 157.5 (C-9), 162.7 (C-7), 174.1 (C-4). MS (*m*/*z*) 317.1 $(M)^+$, 340.8 $(M+Na)^+$. Anal. ($C_{15}H_9O_3Br$) for C, H. calcd 56.81, 2.86; found: 56.56, 2.85.

7-*O*-Ethoxycarbonylpentyl-4′-bromoisoflavone (12b). This compound was prepared by same method described for **11b** except **12a** and ethyl 6-bromohexanoate were the starting materials. From 2.8 g of **12a**, 1.62 g of **12b** was obtained, a yield of 57.9% (w/w): mp 122–124 °C. ^{1}H NMR (DMSO-d_6) δ, 1.17 (t, 3H, –CH_3), 1.43 (m, 2H, –CH_2–), 1.60 (m, 2H, –CH_2–), 1.76 (m, 2H, –CH_2–), 2.31 (m, 2H, –CH_2–), 4.04 (q, 2H, –CH_2–O–), 4.11 (t, 2H, –CH_2–O–), 7.06 (dd, 1H, *J* = 8.88, 2.56 Hz, 6-H), 7.15 (d, 1H, *J* = 1.93 Hz, 8-H), 7.55 (d, 2H, *J* = 8.68 Hz, 3′, 5′-H), 7.62 (d, 2H, *J* = 8.68 Hz, 2′, 6′-H), 8.01 (d, 1H, *J* = 8.87 Hz, 5-H), 8.50 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (–CH_3), 24.1 (–CH_2–), 24.9 (–CH_2–), 28.0 (–CH_2–), 33.4 (–CH_2–), 59.6 (–CH_2–O–), 68.4 (–CH_2–O–), 101.0 (C-8), 115.2 (C-6), 117.4 (C-10), 121.1 (C-1′), 122.5 (C-3), 126.9 (C-5), 130.9 (C-3′, 5′), 131.0 (C-2′, 6′), 131.2 (C-4′), 154.3 (C-2), 157.4 (C-9), 163.2 (C-7), 172.8 (>C=O), 174.1 (C-4). MS (*m*/*z*), 460.0 $(M+H)^+$. Anal. ($C_{23}H_{23}O_5Br$) for C, H. calcd 60.14, 5.05; found: 59.82, 5.04.

7-Hydroxy-4′-nitroisoflavone (13a). This compound was prepared by the same method described for **11a** except that the starting material 4-fluorophenylacetic acid was replaced by 4-nitrophenyl acetic acid. A total of 1.35 g of **13a** was obtained, a yield of 38.6% (w/w): mp 270 °C (decomposes). ^{1}H NMR (DMSO-d_6) δ 6.86 (d, 1H, *J* = 2.0 Hz, 8-H), 6.94 (dd, 1H, *J* = 8.84, 1.93 Hz, 6-H), 7.88 (d, 2H, *J* = 8.76, 1.48 Hz, 2′, 6′-H), 7.97 (d, 1H, *J* = 8.79 Hz, 5-H), 8.27 (d, 2H, *J* = 8.78, 1.70 Hz, 3′, 5′-H), 8.56 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 102.3 (C-8), 115.9 (C-6), 116.0 (C-10), 121.5 (C-3), 123.2 (C-2′, 6′), 127.3 (C-5), 129.9 (C-3′, 5′), 139.4 (C-1′), 146.7 (C-4′), 155.3 (C-2), 157.5 (C-9), 163.8 (C-7), 173.8 (C-4). MS (*m*/*z*) 283.9 (M^+), 306.3 $(M+Na)^+$, 282.4 $(M-H)^-$. Anal. ($C_{15}H_9O_5N$) for C, H, N. calcd 63.61, 3.20, 4.95; found: 63.48, 3.21, 4.91.

7-*O*-Ethoxycarbonylpentyl-4′-nitroisoflavone (13b). This compound was prepared by the same method described for **11b** except that **13a** was used as the starting material. A total of 270 mg **13b** was obtained from this synthesis, a yield of 13.5% (w/w): mp 173–175 °C. ^{1}H NMR (DMSO-d_6) δ1.17 (t, 3H, –CH_3), 1.44 (m, 2H, –CH_2–), 1.59 (m, 2H, –CH_2–), 1.60 (–CH_2–), 1.77 (–CH_2–), 2.32 (–CH_2–), 4.04 (–CH_2–O–), 4.13 (–CH_2–O–), 7.10 (dd, 1H, *J* = 8.85, 1.94 Hz, 6-H), 7.20 (d, 1H, *J* = 2.32 Hz, 8-H), 7.92 (d, 2H, *J* = 8.73 Hz, 2′, 6′-H), 8.04 (d, 1H, *J* = 8.87 Hz, 5-H), 8.29 (d, 2H, *J* = 8.74 Hz, 3′, 5′-H), 8.68 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (–CH_3), 24.2 (–CH_2–), 24.9 (–CH_2–), 28.0 (–CH_2–), 33.4 (–CH_2–), 59.7 (–CH_2–O–), 68.4 (–CH_2–O–), 101.2 (C-8), 115.4 (C-6), 117.4 (C-10), 121.8 (C-3), 123.2 (C-2′, 6′), 126.9 (C-5), 129.9 (C-3′, 5′), 139.1 (C-1′), 146.7 (C-4′), 155.7 (C-2), 157.4 (C-9), 163.4 (C-7), 172.8 (–C=O), 173.9 (C-4). Anal. ($C_{23}H_{23}O_7N$) for C, H, N. calcd 64.93, 5.54, 3.29; found: 64.63, 5.48, 3.31.

7-Hydroxy-4′-methylisoflvone (14a). This compound was prepared by a similar method described for **11a** except that 4-flurophenylacetic acid was replaced by *p*-tolylacetic acid. A total of 780 mg **14a** was obtained from this procedure, a yield of 21.3% (based on resorcinol, w/w): mp 241–243 °C. ^{1}H NMR (DMSO-d_6) δ 2.33 (s, 3H, –CH_3), 6.87 (d, 1H, *J* = 1.94 Hz, 8-H), 6.94 (dd, 1H, *J* = 8.72, 2.68 Hz, 6-H), 7.21 (t, 2H, *J* = 7.92 Hz, 3′, 5′-H), 7.45 (d, 2H, *J* = 8.28 Hz, 2′, 6′-H), 7.97 (d, 1H, *J* = 8.85 Hz, 5-H), 8.33 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 20.8 (–CH_3), 102.1 (C-8), 115.2 (C-6), 116.6 (C-10), 123.4 (C-1′), 127.3 (C-3), 128.7 (C-3′, 5′), 128.7 (C-2′, 6′), 129.1 (C-5), 137.0 (C- 4′), 153.4 (C-2), 157.4 (C-9), 162.6 (C-7), 174.5 (C-4). MS (*m*/*z*) 253.4 $(M+H)^+$, 275.2 $(M+Na)^+$, 251.4 $(M-H)^-$. Anal. ($C_{16}H_{12}O_3$) for C, H. Calcd: 76.18, 4.79; found: 75.93, 4.82.

7-*O*-Ethoxycarbonylpentyl-4′-methylisoflavone (14b). This compound was prepared by the same method described for **3b** except that starting materials were **14a** and ethyl 6-bromohexanoate. The synthesis resulted in 900 mg of **14b**, a yield of 35.71% (w/w): mp 141–142 °C. ^{1}H NMR (DMSO-d_6) δ 1.16 (t, 3H, –CH_3), 1.44 (m, 2H, –CH_2–), 1.59 (m, 2H, –CH_2–), 1.76 (m, 2H, –CH_2–), 2.31 (m, 2H, –CH_2–), 2.33 (–CH_3), 4.04 (q, 2H, –CH_2–O–), 4.11 (t, 2H, –CH_2–O–), 7.06 (dd, 1H, *J* = 8.79, 2.53 Hz, 6-H),

7.14 (d, 1H, $J=1.89$ Hz, 8-H), 7.23 (d, 2H, $J=8.04$ Hz, 3′, 5′-H), 7.47 (d, 2H, $J=7.63$ Hz, 2′, 6′-H), 8.01 (d, 1H, $J=8.86$ Hz, 5-H), 8.42 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (–CH_3), 20.8 (–CH_3), 24.1 (–CH_2–), 24.9 (–CH_2–), 28.0 (–CH_2–), 33.4 (–CH_2–), 59.6 (–CH_2–O–), 68.3 (–CH_2–O–), 101.0 (C-8), 115.0 (C-6), 117.5 (C-10), 123.6 (C-1′), 126.9 (C-3), 128.7 (C-3′, 5′), 128.7 (C-2′, 6′), 129.0 (C-5), 137.1 (C-4′), 153.7 (C-2), 157.4 (C-9), 163.1 (C-7), 172.8 (>C=O), 174.5 (C-4). Anal. ($C_{24}H_{26}O_5$) for C, H. calcd 73.08, 6.64; found: 73.11, 6.61.

7, 4′-Dimethoxyisoflavone (15). To a solution of 1.28 g of daidzein (5.0 mmol) in 40 mL of DMSO was added 3.84 g of NaOH pellets. The mixture was stirred at RT for 6 min and 3 mL of iodomethane (48.2 mmol) was added dropwise. The mixture was stirred at RT for another 6 min and poured into ice water. Product in the water was extracted with chloroform and dried. Residue was fractionated on a Sephadex LH-20 column (chloroform/methanol, 7:3). Fractions that contained pure product were pooled, concentrated, and recrystallized from acetone to give 450 mg of **15** (crystalline needles), a yield of 35.2 (w/w): mp 162–163 °C. ^{1}H NMR (DMSO-d_6) δ, 3.79 (–OCH_3), 3.90 (–OCH_3), 6.99 (d, 2H, $J=8.36$ Hz, 3′, 5′-H), 7.07 (dd, 1H, $J=8.88$, 2.51 Hz, 6-H), 7.14 (d, 1H, $J=1.82$ Hz, 8-H), 7.52 (d, 2H, $J=8.67$ Hz, 2′, 6′-H), 8.02 (d, 1H, $J=8.88$ Hz, 5-H), 8.40 (s, 1H, 2-H). Anal. ($C_{17}H_{14}O_4$) for C, H. calcd 72.33, 4.99; found: 72.47, 4.95.

7-Hydroxy-4′-aminoisoflavone (16a). This compound was prepared by the reduction of **13a**. To a suspension of 500 mg of **13a** and 50 mL of ethanol was added 1.0 g of zinc powder. The mixture was stirred at 50 °C while 10 mL of glacial acetic acid was added slowly through a span of 30 min. After **13a** was completely reduced, reaction mixture was filtered and filtrate was concentrated. The concentrate was suspended in 20 mL of water and extracted with ethyl acetate. The organic layer was evaporated to dryness and residue was recrystallized from methanol/chloroform/petroleum ether (30–60 °C) (1:5:20) to give 153 mg of yellow amorphous powder of **16a**, a yield of 30.6% (w/w): mp 250 °C (decomposes). ^{1}H NMR (DMSO-d_6) δ 6.59 (d, 2H, $J=8.62$ Hz, 3′, 5′-H), 6.81 (d, 1H, $J=2.63$ Hz, 8-H), 6.89 (dd, 1H, $J=8.93$, 2.56 Hz, 6-H), 7.24 (d, 2H, $J=8.08$ Hz, 2′, 6′-H), 7.94 (d, 1H, $J=8.78$ Hz, 5-H), 8.21 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 102.1 (C-8), 113.4 (C-3′, 5′), 115.2 (C-6), 117.5 (C-10), 119.0 (C-1′), 123.9 (C-3), 127.2 (C-5), 129.5 (C-2′, 6′), 148.5 (C-4′), 152.3 (C-2), 157.4 (C-9), 162.9 (C-7), 174.9 (C-4). MS (*m*/*z*) 254.3 $(M+H)^+$, 276.2 $(M+Na)^+$, 252.6 $(M-H)^-$. Anal. ($C_{15}H_{10}O_3N$) for C, H, N. calcd 71.14, 4.38, 5.53; found: 70.89, 4.40, 5.49.

7-Ethoxycarbonylpentyl-4′-aminoisoflavone (16b). This compound was prepared by reducing **13b** under the same conditions described in **16a.** A total of 863 mg of **16b** was obtained, a yield of 43.2% (w/w): mp 147–148 °C. ^{1}H NMR (DMSO-d_6) δ1.17 (t, 3H, –CH_3), 1.44 (m, 2H, –CH_2–), 1.60 (–CH_2–), 1.74 (–CH_2–), 2.31 (–CH_2–), 4.04 (–CH_2–O–), 4.13 (–CH_2–O–), 6.60 (d, 2H, $J=8.74$ Hz, 3′, 5′-H), 7.03 (dd, 1H, $J=8.93$, 2.56 Hz, 6-H), 7.09 (d, 1H, $J=2.63$ Hz, 8-H), 7.26 (d, 2H, $J=6.52$, 1.61 Hz, 2′, 6′-H), 7.99 (d, 1H, $J=8.88$ Hz, 5-H), 8.29 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 14.1 (-CH_3), 24.2 (–CH_2–), 24.9 (–CH_2–), 28.0 (–CH_2–), 33.4 (–CH_2–), 59.7 (–CH_2–O–), 68.3 (–CH_2–O–), 100.8 (C-8), 113.4 (C-3′, 5′), 114.8 (C-6), 117.5 (C-10), 118.8 (C-1′), 124.1 (C-3), 126.9 (C-5), 129.5 (C-2′, 6′), 148.5 (C-4′), 152.3 (C-2), 157.3 (C-9), 162.9 (C-7), 174.9 (C-4). MS (*m*/*z*) 396.5 $(M+H)^+$, 418.4 $(M+Na)^+$, 394.1 $(M-H)^-$. Anal. ($C_{23}H_{25}O_5N$) for C, H, N. calcd 69.86, 6.37, 3.54; found: 69.34, 6.29, 3.53.

7, 8-Dimethoxyisoflavone (17). This compound was prepared as described in the synthesis of **11a**. A mixture of 2.76 g of phenylacetic acid (20.08 mmol), 5.0 g of 2,3-dimethoxylphenol (32.43 mmol) and 50 mL of boron trifluoride diethyl etherate was stirred at 80 °C for 19 h. Reaction mixture was then cooled to rt and washed with aqueous K_2CO_3 and water sequentially until pH of the filtrate approached neutrality. Product was extracted with ethyl acetate and concentrated to dryness. The residue, which contained mostly 2-hydroxy-3, 4-dimethoxyphenylbenzylketone, was dissolved in 50 mL of 2-propanol and 3.0 mL of morpholine and 6.0 mL of triethyl orthoformate (36.07 mmol) were added. The mixture was stirred at 80 °C for 20 h and evaporated to dryness. The residue was fractionated on a silica gel (chloroform-methanol system) and fractions that contained pure product were pooled, dried, and recrystallized from acetone to give 300 mg of **17** (crystalline plates), a yield of 6% (based on 2, 3-dimethoxylphenol, w/w): mp 143–144 °C. ^{1}H NMR (DMSO-d_6) δ 3.89 (8-OCH_3), 3.96 (7-OCH_3), 7.30 (d, 1H, $J=9.03$, Hz, 6-H), 7.39 (t, 1H, $J=6.94$ Hz, 4′-H), 7.43 (t, 2H, $J=7.06$, 7.72 Hz, 3′, 5′-H), 7.57 (dd, 2H, $J=7.26$, 1.44 Hz, 2′, 6′-H), 7.87 (d, 1H, $J=8.88$ Hz, 5-H), 8.50 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ 56.5 (–OCH_3), 60.9 (–OCH_3), 111.0 (C-6), 118.5 (C-10), 120.9 (C-3), 123.3 (C-1′), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 131.9 (C-4′), 136.1(C-8), 149.9(C-7), 154.1 (C-2), 156.2 (C-9), 174.6 (C-4). MS (*m*/*z*) 283.3 $(M+H)^+$, 305.4 $(M+Na)^+$, 281.9 $(M-H)^-$. Anal. ($C_{17}H_{14}O_4$) for C, H. calcd 72.33, 4.99; found: 72.21, 4.97.

7-Ethylisoflavone (18). This compound was prepared by the same method described for the synthesis of **11a**. To a solution of 6.51 g of phenylacetic acid (47.81 mmol) in 50 mL of boron trifluroide diethyl etherate was added 6.5 mL of 3-ethylphenol (53.20 mmol). The mixture was stirred at 80 °C for 21 h, cooled to RT, and then washed with aqueous K_2CO_3 and water until the pH of wash approached neutrality. The product was extracted with ethyl acetate and concentrated. Residue was dissolved in 50 mL of 2-propanol and 8 mL of triethyl formate (48.1 mmol) and 3 mL of morpholine were added. The mixture was stirred at 80 °C for 7 h and solvent was removed by flash evaporation. The syrup obtained was dissolved in 30 mL of methanol, stirred at 50 °C for 30 min, cooled to room temperature, and kept at 4 °C overnight. Crystals were collected by filtration and washed with small portions of acetone to give 790 mg of **18**, a yield of 12.2% (based on 3-ethylphenol, w/w): mp 103–104 °C. ^{1}H NMR (DMSO-d_6) δ 1.35 (t, 3H, –CH_3),

2.78 (q, 2H, –CH_2–), 7.38 (m, 4′-H), 7.39 (dd, 1H, J = 8.10, 1.91 Hz, 6-H), 7.44 (t, 2H, J = 7.77 Hz, 3′, 5′-H), 7.52 (d, 1H, J = 2.0 Hz, 8-H), 7.59 (dd, 2H, J = 7.35, 1.38 Hz, 2′, 6′-H), 8.05 (d, 1H, J = 8.54 Hz, 5-H), 8.51 (s, 1H, 2-H). ^{13}C NMR (DMSO-d_6) δ14.9 (–CH_3), 28.2 (–CH_2–), 116.7 (C-6, 8), 121.8 (C-10), 123.8 (C-3), 125.4 (C-1′), 125.8 (C-7), 127.8 (C-5), 128.1 (C-3′, 5′), 128.9 (C-2′, 6′), 131.9 (C-4′), 154.4 (C-2), 155.9 (C-9), 174.9 (C-4). MS (m/z), 251.4 $(M+H)^+$, 273.2 $(M+Na)^+$, 249.3 $(M-H)^-$. Anal. ($C_{17}H_{14}O_2$) for C, H. calcd 81.58, 6.91; found: 80.92, 6.87.

7-Ethoxylcarbonylpentoxypuerarin (19). This compound was prepared by the same method described for **3b**. To a solution of 3.5 g of puerarin (8.41 mmol) in 60 mL of DMF, 2.76 g of anhydrous K_2CO_3 (20.0 mmol) and 6.0 mL of ethyl 6-bromohexanoate (33.6 mmol) were added. The mixture was stirred at 80 °C for 6 h, poured into ice water, and extracted with ethyl acetate. Solvent was evaporated and residue was fractionated on a on Silica gel column (chloroform/methanol, 8:2) followed by a Sephadex LH-20 column (chloroform/methanol, 7:3). Final product was recrystallized from petroleum ether/chloroform/methanol (10:0.5:0.1) to give 3.19 g of **19** (white amorphous powder), a yield of 91.14% (w/w): mp 165–168 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (–CH_3), 1.47 (–CH_2–), 1.58 (–CH_2–), 1.77 (–CH_2–), 2.32 (–CH_2–), 4.07 (–CH_2–O–), 4.12 (–CH_2–O–), 3.08–5.23 (m, H from glucose), 6.81 (dd, 2H, J = 7.29, 2.11 Hz, 3′, 5′-H), 7.22 (d, 1H, J = 8.57, 6-H), 7.41 (dd, 2H, J = 8.6, 2.1 Hz, 2′, 6′-H), 8.08 (d, 1H, J = 9.0 Hz, 5-H), 8.41 (s, 1H, 2-H), 9.53 (s, 1H, 4′-OH), ^{13}C NMR (DMSO-d_6) δ14.1(–CH_3), 24.1 (–CH_2–), 25.0 (–CH_2–), 28.3 (–CH_2–), 33.5 (–CH_2–), 59.7 (–CH_2–O–), 61.7 (C-6″), 68.4 (–CH_2–O–), 70.2 (C-4″), 70.6 (C-2″), 73.0 (C-1″), 78.8 (C-3″), 81.8 (C-5″), 111.3 (C-8), 114.8 (C-6), 114.9 (C-3′, 5′), 117.2 (C-10), 122.4 (C-1′), 123.1 (C-3), 126.8 (C-5), 130.0 (C-2′, 6′), 155.1 (C-2), 157.1 (C-9), 157.4 (C-4′), 162.1 (C-7), 172.9 (–C=O), 175.0 (C-4). MS (m/z) 559.6 $(M+H)^+$, 581.5 $(M+Na)^+$, 557.9 $(M-H)^-$. Anal. ($C_{29}H_{34}O_{11}$) for C, H. calcd 62.36, 6.14; found: 61.92, 6.17.

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References and Notes

1. Secretary of Health and Human Services. *Drinking in the United States: Main Findings from the 1992 National Longitudinal Alcohol Epidemiologic Survey (NLAES), US Alcohol Epidemiologic Data Reference Manual*; 1998; Vol. 6.
2. Harwood, H. *Updating Estimates of the Economic Costs of Alcohol Abuse in the United States: Estimates, Update Methods and Data*. Report prepared by the Lewin Group for the National Institute on Alcohol Abuse and Alcoholism, 2000.
3. Keung, W. M.; Vallee, B. L. *Phytochemistry* **1998**, *47*, 499.
4. Keung, W. M.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10008.
5. Keung, W. M.; Vallee, B. L. In *Toward a Molecular Basis of Alcohol Use and Abuse;* Jansson, B., Jornvall, H., Rydbery, U., Terenius, L., Vallee, B. L., Eds.; Birkhauser: Basel, 1994; Vol. 71, p 371.
6. Overstreet, D. H.; Lee, Y. W.; Rezvani, A. H.; Pei, Y. H.; Criswell, H. E.; Janowsky, D. S. *Alcohol. Clin. Exp. Res.* **1996**, *20*, 221.
7. Heyman, G. M.; Keung, W. M.; Vallee, B. L. *Alcohol. Clin. Exp. Res.* **1996**, *20*, 1083.
8. Lin, R. C.; Guthrie, S.; Xie, C. I.; Mai, K.; Lee, D. Y.; Lumeng, L.; Li, T. K. *Alcohol. Clin. Exp. Res.* **1996**, *20*, 659.
9. Overstreet, D. H.; Lee, D. Y. W.; Chan, Y. T.; Rezvani, A. H. *Perfusion* **1998**, *11*, 381.
10. Keung, W. M.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2198.
11. Rooke, N.; Li, D. J.; Li, J. Q.; Keung, W. M. *J. Med. Chem.* **2000**, *43*, 4169.
12. Keung, W. M. *Chem. Biol. Interact.* **2001**, *130*, 919.
13. Wallee, B. L.; Keung, W. M. US Patent 6121010, 2001.
14. Deitrich, R. A.; Erwin, V. G. *Annu. Rev. Pharmacol.* **1980**, *30*, 55.
15. Gao, G. Y.; Li, D. J.; Keung, W. M. *J. Med. Chem.* **2001**, *44*, 3320.
16. Baker, W.; Robinson, R. *J. Chem. Soc.* **1928**, 3115.
17. Baker, W.; Chadderton, J.; Harborne, J. B.; Ollis, W. D. *J. Chem. Soc.* **1953**, 1852.
18. Kállay, T.; Lányi, G.; Ledniczky, L.; Imrei, L.; Hoffmann, G.; Sziladi, M.; Somfai, É.; Montay, T. Paent WO 91/15483, 1997.
19. Baker, W.; Chadderton, J.; Harborne, J. B.; Ollis, W. D. *J. Chem. Soc.* **1953**, 1860.
20. Li, D. Y.; Gao, Z. J.; Ji, Q. E. *Chinese J. Med. Chem.* **1991**, *1*, 38.
21. Keung, W. M.; Klyosov, A. A.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1675.
22. Keung, W. M.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1247.
23. Nilsson, G. E.; Tottmar, O. *J. Neurochem.* **1987**, *48*, 1566.