Flavonoid Dimers as Bivalent Modulators for P-Glycoprotein-Based Multidrug Resistance: Synthetic Apigenin Homodimers Linked with Defined-Length Poly(ethylene glycol) Spacers Increase Drug Retention and Enhance Chemosensitivity in Resistant Cancer Cells

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Much effort has been spent on searching for better P-glycoprotein- (P-gp-) based multidrug resistance (MDR) modulators. Our approach was to target the binding sites of P-gp using dimers of dietary flavonoids. A series of apigenin-based flavonoid dimers, linked by poly(ethylene glycol) chains of various lengths, have been synthesized. These flavonoid dimers modulate drug chemosensitivity and retention in breast and leukemic MDR cells with the optimal number of ethylene glycol units equal to 2−4. Compound **9d** bearing four ethylene glycol units increased drug accumulation in drug-resistant cells and enhanced cytotoxicity of paclitaxel, doxorubicin, daunomycin, vincristine, and vinblastine in drug-resistant breast cancer and leukemia cells in vitro, resulting in reduction of IC_{50} by 5−50 times. This compound also stimulated P-gp's ATPase activity by 3.3-fold. Its modulating activity was presumably by binding to the substrate binding sites of P-gp and disrupting drug efflux.

Introduction

Multidrug resistance (MDR)[a] is a major problem in cancer chemotherapy. The best characterized resistance mechanism is the one mediated by overexpression of the drug efflux transporter, permeability-glycoprotein (P-gp), which pumps a variety of anticancer drugs out of the cells, resulting in lowered intracellular drug accumulation. P-gp belongs to the ATP binding cassette (ABC) transporter superfamily, which is an ATP-dependent membrane-spanning multidrug transporter, with a remarkably broad specificity for a variety of compounds.[1] P-gp has 1280 amino acids that are organized into two repeating units of 610 amino acids joined by a linker region of about 60 amino acids.[2] In each unit, there are six transmembrane domains (TMD) and a hydrophilic nucleotide binding domain (NBD).[3] The NBDs, which are peripherally located at the cytoplasmic face of the membrane, bind ATP and couple ATP hydrolysis to the drug transport process. Recently, the three-dimensional structure of P-gp has been determined by electron crystallography at a resolution limit of about 2 nm.[4] These results revealed that P-gp has a pseudodimeric structure. In its nucleotide-free state, the TMDs form a barrel 5−6 nm in diameter and about 5 nm deep with a central pore that is open to the extracellular surface. Upon binding nucleotide, the TMDs appeared to reorganize to open the central pore in a manner that could allow access of the transport substrate from the lipid bilayer to the central pore of the P-gp.[5−7]

Development of reversing or modulating agents against P-gp has attracted interests from both academia and industry. Tsuruo et al.[8] first reported that verapamil (VP; $C_{27}H_{38}N_2O_4$), a calcium channel blocker, could reverse resistance by inhibiting P-gp-mediated drug efflux. Since then, there are considerable in vitro data suggesting that MDR due to P-gp can be effectively modulated by a range of compounds including calcium channel blockers,[9,10] calmodulin inhibitors,[11,12] indole alkaloids,[13,14] cyclosporine,[15−18] quinolines,[19] and steroid.[20−22] However, their unacceptable toxicity precluded them from clinical use. Searches for the congeners of these first-generation MDR modulators resulted in less toxic and more potent agents such as dexverapamil,[23] dexniguldipine,[24] PSC 833 (valspodar; $C_{63}H_{111}N_{11}O_{12}$),[22,25] and VX-710 (biricodar; $C_{34}H_{41}N_3O_7$).[26−28] Although these so-called second-generation MDR modulators showed some encouraging results, their uses are limited by their unpredictable pharmacokinetic interactions with the anticancer drugs.[29] The third-generation MDR modulators developed by structure−activity relationships and combinatorial chemistry approaches include zosuquidar LY335979, tariquidar XR9576, laniquidar R101933, the acridonecarboxamide GF120918, and the substituted diarylimidazole ONT-090.[30−32]

Another promising family of P-gp modulators is the flavonoids.[33] Flavonoids are polyphenolic compounds that are naturally abundant in fruits, vegetables, nuts, stems, flowers, wine, and tea.[34] They are common components of normal human food and are known to be associated with a wide range of beneficial properties for human health, such as antioxidizing, free-radical scavenging, antiinflammatory, antiviral, and anticancer properties.[35] In the past decade, flavonoids have been implicated in the modulation of P-gp-type MDR in various types of cancers through inhibiting drug efflux.[36−42] There are a number of possibilities of how flavonoids assert their modulating effect on P-gp. Some flavonoids were good inhibitors for a variety of ATP-binding proteins including protein kinases such as cyclic AMP-dependent protein kinase,[43,44] protein kinase C,[45] serine/threonine kinases,[46] tyrosine kinase,[47,48] topoisomerase II,[49] and myosin[50] as well as various membrane ATPases such

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[a] Abbreviations: MDR, multidrug resistance; ABC, ATP binding cassette; NBD, nucleotide binding domain; TMD, transmembrane domain; PEG, poly(ethylene glycol); RF, relative fold; VP, verapamil; ADR, adriamycin; DOX, doxorubicin.

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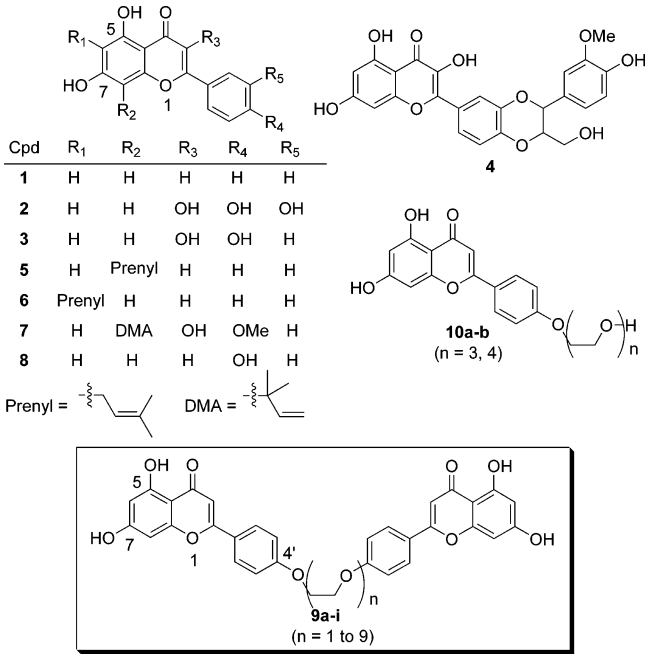


Figure 1. Structure of some flavonoids, target compounds **9**, and monovalent apigenins **10**.

as mitochondrial H^+-ATPase,[51] Na^+/K^+-ATPase,[52] Ca^{2+}-ATPase,[53] or H^+/K^+-ATPase.[54]

The mechanism by which the flavonoids interact with P-gp has also been studied. Chrysin (**1**), quercetin (**2**), kaempferol (**3**), and dehydrosilybin (**4**) (Figure 1) were reported to bind directly to the NBD2 cytosolic domain of mouse P-gp.[55] Increased hydrophobicity through the introduction of prenyl or other alkyl groups into the flavonoid structure often produced more efficient inhibitors. 8- or 6-Prenylchrysin (**5** or **6**) (Figure 1) inhibited P-gp-mediated drug efflux within leukemic K562/R7 cells,[37] whereas 8-dimethylallylkaempferide (**7**) was a better modulator than either cyclosporine A or VP in the inhibition of Ltrmdr1.[56] Extensive structure activity relationships led to the proposal that unsubstituted flavonols (**1**, **2**, and **3**) interact bifunctionally with cytosolic NBDs of P-gp, whereas prenylation at either position 6 or 8 of the A-ring (**5**, **6**, and **7**) would increase hydrophobic interactions with both the steroid interacting region and the drug binding site of TMDs.[55]

Even with their low toxicity, the current generation of flavonoid modulators has limitations.[57] The first is that their activities tend to be moderate. Second, they have a broad spectrum of biological activities including anti-estrogen and inhibition of other ATPases. High-dosage application of flavonoids as MDR modulators is likely to lead to side effects. An approach to improve the potency and selectivity of flavonoids is to take advantage of the pseudodimeric nature and the multiple binding sites of P-gp by using polyvalent interactions. Polyvalent interactions in biological systems are characterized by the simultaneous binding of multiple ligands on one biological entity.[58] In the literature, successful bivalent[59] and multivalent[60,61] ligands to different receptor systems have been achieved, with a level of potency 10^3-10^5 times higher than that of the corresponding monovalent ligands. In the present series of investigations, we have synthesized flavonoids linked together by poly(ethylene glycol) (PEG) chains of various lengths of general structure **9** as potential bivalent ligands for P-gp (Figure 1). The PEG chain spacer has the advantages of high degree of freedom, biocompatibility, and simple synthetic

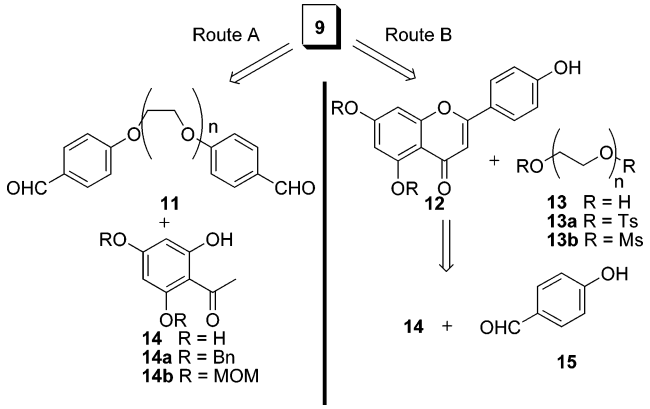


Figure 2. Retrosynthetic analysis of the synthesis of **9** via two pathways.

procedure. We have chosen apigenin (**8**) as the parent monoligand because it has been reported to be a modulator of MDR in colon HCT-15 cancer cells.[38,43,55] The C4′ position has been chosen as the point of attachment of the linker because substitution at this position has been shown to have little effect on the activity of the molecules.[57] There is only one previous publication that attempts to modulate the activity of P-gp through the employment of bivalent interaction. Ambudkar and coworkers[62] demonstrated that functional derivatives of stipiamide dimer with optimal spacer increase by 11-fold the affinity of stipiamide, which is thought to modulate MDR by binding to the TMD of P-gp.

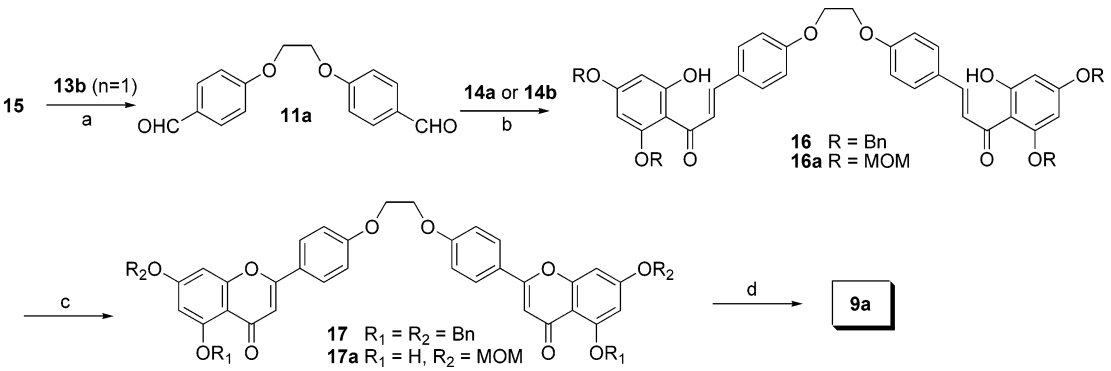
Herein we describe the synthesis of flavones **9** via two synthetic pathways. For comparison purposes, we have also prepared some monovalent apigenin derivatives of general structure **10** (Figure 1).

Chemistry[86]

There are two synthetic pathways that could be exploited to achieve the synthesis of PEG-linked flavonoids **9**, as shown in Figure 2. The first approach (route A) involves the employment of a series of PEG-linked bisaldehydes **11**, which were synthesized from aldehyde **15** and corresponding ethylene glycol ditosylate **13a** or dimesylate **13b** according to the literature.[63] Then aldol condensation of the bisaldehyde **11** with trihydroxyacetophenone **14**, followed by oxidative cyclization of bischalcone to flavone, should furnish **9**. The other pathway (route B) involved the synthesis of selectively protected flavonoid **12**, which is then coupled with activated PEG chains **13a** or **13b**. The flavonoid **12** can in turn be derived from trihydroxyacetophenone **14** and benzaldehyde **15**.

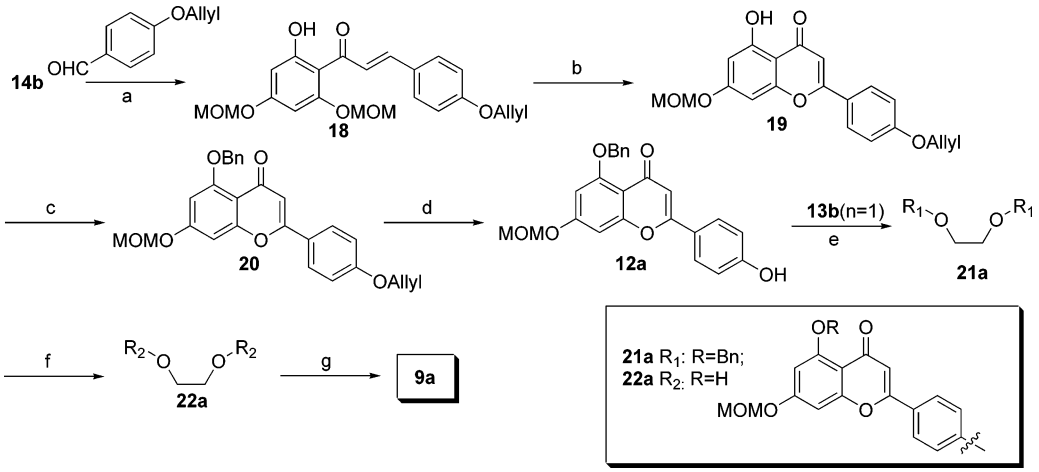
PEG chains **13** are commercially available up to $n = 6$. PEGs with $n > 6$ are not readily available commercially and hence they had to be synthesized. We have developed a convenient approach to synthesize the PEG chains **13** under phase transfer conditions.[64] PEGs with $n > 6$ are obtained in excellent yields and high purity. Ethylene glycol ditosylates **13a** (for $n = 2, 3$) and dimesylate **13b** (for $n = 1, 4-9$) were prepared from the corresponding PEG chains **13**, tosyl chloride or methanesulfonyl chloride, and triethylamine in dichloromethane at ice-bath temperature.[65] Both compounds trihydroxyacetophenone **14** and benzaldehyde **15** are commercially available. Protected 2-hydroxyacetophenones **14a**[66] and **14b**[67] were prepared in high yields according to the literature. With the requisite starting materials in hand, the synthesis of our target compounds was pursued. Prior to attempting the synthesis of other target

Scheme 1[a]



[a] (a) K_2CO_3, Acn/H_2O, reflux, 14 h; (b) for **16** from **14a**, 60% KOH, room temperature, 14 h; for **16a** from **14b**, 3 M KOH in EtOH, room temperature, 14 h; (c) for **17**, cat. I_2, DMSO, reflux, 14 h; for **17a**, DDQ, PhMe/dioxane, reflux, 14 h; (d) from **17**, H_2, Pd/C, THF/H_2O, room temperature, 14 h; from **17a**, 80% AcOH, reflux, 14 h.

Scheme 2[a]



[a] (a) 3 M KOH in EtOH, room temperature, 16 h; (b) DDQ, PhMe/dioxane, reflux, 7 h; (c) K_2CO_3, BnBr, DMF, reflux, 2 h; (d) cat. $Pd(PPh_3)_4$, K_2CO_3, MeOH, reflux, 2 h; (e) K_2CO_3, DMF, reflux, 2 h; (f) H_2, Pd/C, $CHCl_3$, room temperature, 12 h; (g) 80% AcOH, reflux, 14 h.

compounds, flavonoid **9a** ($n = 1$) was chosen as model study to ascertain the optimal conditions for synthesis.

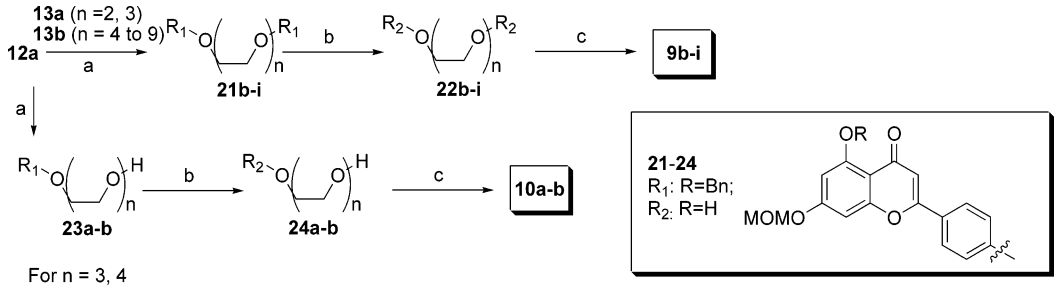
Results

Synthesis of 9a ($n = 1$) via Route A. The synthesis of **9a** was investigated according to route A. The results are summarized in Scheme 1. *p*-Hydroxybenzaldehyde (**15**) was coupled with ethylene glycol dimesylate (**13b**) in the presence of potassium carbonate in 50% acetonitrile (ACN) in water at refluxing temperature to furnish bisaldehyde **11a** in high yield. Then the bischalcone **16** was prepared from aldol condensation of the bisaldehyde **11a** with dibenzyl-protected trihydroxyacetophenone **14a** under basic medium. The bischalcone **16** has a characteristic golden yellow color. The large coupling constant of the olefinic protons ($J = 16$ Hz) indicated that the carbon−carbon double bond should be in the *E* configuration. Cyclization of bischalcone **16** to bisflavonoid **17** proceeded smoothly in one pot via a cyclization−elimination route with a catalytic amount of iodine in dimethyl sulfoxide (DMSO) at 130 °C.[68] Among the methods used for deprotection of the benzyl group in **17** are hydrogen transfer hydrogenolysis with Pd/C[69] or with a catalytic amount of $Pd(OH)_2$ on charcoal[70] or with Pd/C under an atmosphere of hydrogen.[71] However, these were not successful and only starting material was ever recovered. After many variations of reaction condition, flavonoid **9a** was finally achieved in very low yield by employing a large amount of 10% Pd/C in a tetrahydrofuran (THF)/water mixture. The use of benzyl group as protecting group thus seems problematic and the overall yield was poor. Hence the methoxymethyl (MOM) group was chosen to replace benzyl group and the whole synthetic pathway was repeated from bisaldehyde **11a** (Scheme 1).

Bischalcone **16a** was obtained in high yield by aldol condensation of bisaldehyde **11a** with diMOM-protected trihydroxyacetophenone **14b** by use of 3 M KOH solution in EtOH. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone- (DDQ-) mediated oxidative cyclization under thermal conditions proceeded to furnish **17a** with the cleavage of one MOM group, presumably due to the slightly acidic nature of the reduced DDQ.[72] Tedious chromatographic purification of the reaction mixture resulted in low yield of **17a**. Conversion of **17a** to the flavonoid **9a** was achieved by acidic medium deprotection of MOM group. These results suggested that the use of MOM group for protection was superior to the benzyl group, since the MOM group can be cleaved readily under mild conditions. However, the yield of the overall conversion of **15** to **9a** was still low.

Synthesis of 9a ($n = 1$) via Route B. The synthesis of **9a** was then investigated according to route B. The results are summarized in Scheme 2. The acetophenone **14b** was condensed with *p*-allyloxybenzaldehyde under basic medium to yield

Scheme 3[a]



[a] (a) K_2CO_3, DMF, reflux; (b) H_2, Pd/C, $CHCl_3$, room temperature; (c) for $n = 2$ or 3, 80% AcOH, reflux; for $n = 4-9$, 6 M HCl, THF, room temperature.

chalcone **18** in high yield. DDQ-mediated oxidative cyclization of **18** proceeded to furnish **19** with the cleavage of one MOM group. Protection of the hydroxyl group in **19** with benzyl bromide by use of potassium carbonate in *N*,*N*-dimethylformamide (DMF) gave **20** in good yield. The allyl protecting group of **20** was cleaved with a catalytic amount of $Pd(PPh_3)_4$ and potassium carbonate in methanol to furnish **12a** in high yield.[73] The intermolecular nucleophilic substitution of dimesylate **13b** ($n = 1$) by the *p*-phenoxy moiety of **12a** under basic conditions gave **21a**. The dimeric nature of **21a** was evident from the high-resolution mass spectrum. Palladium-catalyzed deprotection of benzyl groups followed by acidic deprotection of MOM groups gave flavonoid **9a** in high yield.

Synthesis of Flavones 9b−i via Route B. Having established the optimal conditions for the synthesis of **9a** via route B, other flavonoid dimers with different PEG chains were synthesized in a similar manner. The results are summarized in Scheme 3. For the shorter chains ($n = 2$ and 3), the PEG ditosylates (**13a**) were used, whereas for the longer chains ($n = 4-9$), the PEG dimesylates (**13b**) were used. In all cases, the flavonoid dimers **9a−i** were prepared in reasonable overall yields, in the range of 30−50% based on **12a**. In general, the flavonoid dimers with longer PEG chains ($n = 5$ or more) were obtained as oil. For flavonoid dimers with shorter PEG chain lengths ($n = 4$ or less), they were obtained as solid with melting point decreasing from 352 °C ($n = 1$) to 131 °C ($n = 4$).

Synthesis of Monovalent Flavonoids 10a and 10b. In the course of subsequent biological studies, it became evident that monovalent flavonoids **10** were required for the purpose of control experiments. Fortuitously, in the coupling of **12a** with the ditosylate **13a** ($n = 3$) or the dimesylate **13b** ($n = 4$), the monocoupled product **23a** ($n = 3$) or **23b** ($n = 4$) was obtained as a minor side product presumably because of the hydrolysis of one of the tosylate or mesylate groups during the reaction. The monomeric nature of **23a** and **23b** was evident from the high-resolution mass spectra. Subsequent palladium-catalyzed deprotection of the benzyl groups followed by acid deprotection of the MOM groups gave the monovalent flavonoids **10a** and **10b** (Scheme 3).

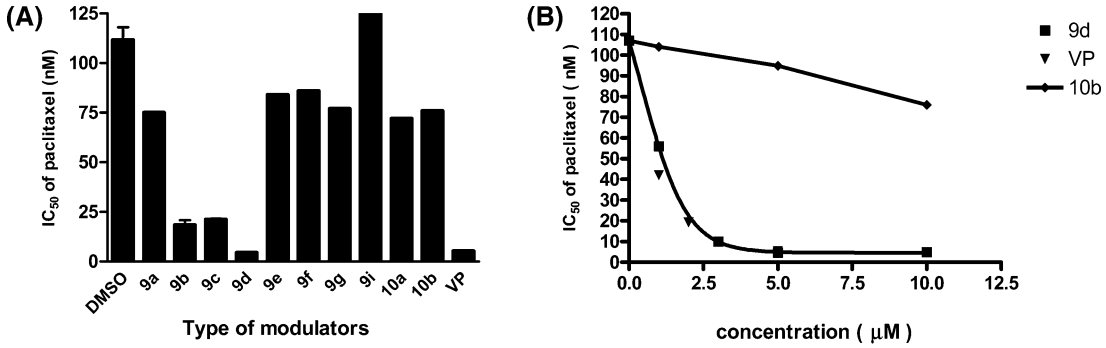
Thus, we have synthesized a series of apigenin-based flavonoid dimers, which were linked together by PEG chain of various lengths via two synthetic routes. The use of the MOM group for protection was found to be superior to the benzyl group, since the MOM group can be cleaved readily under mild conditions. This may be applied in the synthesis of other flavonoid compounds.

Biological Studies

Effect of Flavonoid Dimers on Reversing Paclitaxel ($C_{47}H_{51}NO_{14}$) Resistance in MDA435/LCC6 MDR Cells. We have studied the ability of the apigenin dimers in modulating drug resistance in MDR cancer cells. Compounds **9a−i** alone had no cytotoxicity on the tested MDR cell lines at a concentration below 5 μM, as well as at 10 μM for the monomers **10a** and **10b**. Therefore, 5 μM apigenin dimers and 10 μM monomers were chosen to evaluate their chemosensitizing effect in the following assays.

MDA435/LCC6 is an estrogen-independent human breast cancer cell line. Its MDR subtype (MDA435/LCC6 MDR) was generated by transducing a retroviral vector directing the constitutive expression of the human MDR1 cDNA, producing a cell line with a classical MDR1 resistance pattern.[74] We tested the ability of our synthetic apigenin dimers to reverse paclitaxel resistance in MDA435/LCC6 MDR cells. Paclitaxel is one of the first-line drugs of choice for treating breast cancer,[75] and it has been shown that paclitaxel resistance is mediated by P-gp.[76−78] VP at 5 μM was used as a positive control. Apigenin monomers **10a** and **10b** at 10 μM were used as the negative controls. As shown in Figure 3A, different dimers potentiated the toxicity of paclitaxel to different extents. Compound **9d**, with a spacer length of four ethylene glycols, exhibited the most dramatic reversal activity. The reversal activity is measured by the relative fold (RF), defined as the ratio of (IC_{50} without modulator) to (IC_{50} with modulator). The RF change of IC_{50} of paclitaxel was about 26, changing from 115 to 4.4 nM. Its efficacy was comparable to that of VP ($IC_{50} = 5.2$ nM). Compounds **9b** and **9c**, with spacer lengths of two and three ethylene glycols, also significantly reversed paclitaxel resistance. The relative folds (RF) of IC_{50} were about 5.8 and 5.4, to 19.9 and 21.5 nM, respectively. However, dimers with spacers shorter than two ethylene glycols (**9a**) or longer than five ethylene glycols (**9e**, **9f**, **9g**, **9i**) showed little or no reversing effect at 5 μM. We used the apigenin monomers with three and four ethylene glycols (**10a** and **10b**) as the negative controls in these experiments to determine whether the anticancer resistance reversal activity is due solely to the dimeric nature of the synthetic modulators. As found, the monomers **10a** and **10b** have little reversing effect even when used at double the concentration (10 μM) of the dimers **9c** and **9d** (5 μM) with the same number of ethylene glycol units. This result highly suggests that the modulating activity of **9d**, **9c**, and **9b** is due to their bivalent structures and not due to the simple increase in the apigenin moieties present.

To quantitatively compare the reversal potency of **9d** with VP and the monomer **10b**, their EC_{50} values were compared. EC_{50} values are referred to as the "reversal potency" for the modulators, which describe the concentration of modulators required to reduce the IC_{50} by half compared to control without modulators. It was found that **9d** ($EC_{50} = 0.9$ μM) produced a pronounced effect on reversing paclitaxel cytotoxicity in MDA435/LCC6 MDR cells, with comparable reversal potency to VP ($EC_{50} = 0.7$ μM) (Figure 3B). Compound **10b** has a much



	9d	VP	10b
EC_{50} (μM)	0.9	0.7	>10

Figure 3. (A) Effects of apigenin monomers and dimers on paclitaxel cytotoxicity in MDA435LCC6 MDR cells. The results are presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays with or without modulators. Concentration of apigenin dimers (**9a**−**i**) and monomers (**10a,b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Each experiment was performed in triplicate. Experiments involving compound **9b**−**d** have been repeated three times and the averaged result used. (B) Concentration-dependent effect of **9d**, VP, and **10b** on paclitaxel cytotoxicity in MDA435LCC6 MDR cells, presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays in the presence of different concentrations of modulators (0−10 μM). Presented is a typical experiment performed in triplicate. EC_{50} values for **9d** and VP are calculated and shown. EC_{50} for **10b** cannot be accurately determined because the IC_{50} of paclitaxel was reduced to only about 71% that of no-modulator control at the highest concentration used (10 μM).

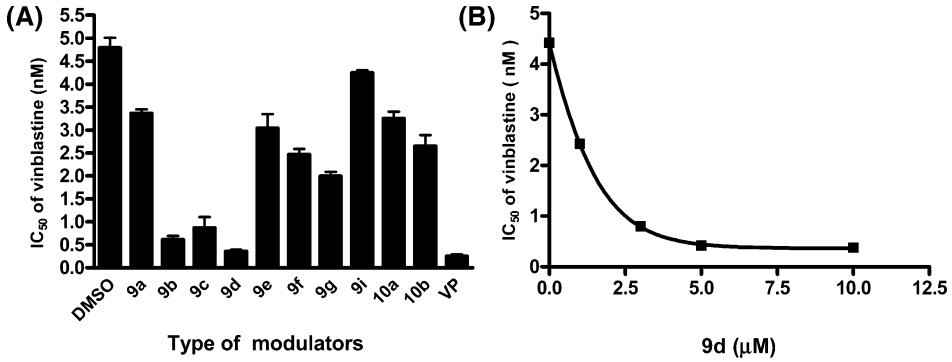


Figure 4. (A) Effects of apigenin monomers and dimers on vinblastine cytotoxicity in MDA435LCC6 MDR cells. The results are presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays with or without modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomers (**10a,b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Each experiment was performed in triplicate. Experiments involving compound **9b**−**d** have been repeated three times and the averaged result used. (B) Concentration-dependent effect of **9d** on vinblastine cytotoxicity in MDA435LCC6 MDR cells, presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays in the presence of different concentrations of **9d** (0−10 μM). Presented is a typical experiment performed in triplicate.

higher EC_{50} (>10 μM), suggesting that the activity of **9d** is indeed due to its bivalent structure.

Effect of Apigenin Dimers on Reversing Resistance to Other Cytotoxic Drugs in MDA435/LCC6 MDR Cells. A similar trend of chemosensitizing effects by different apigenin dimers in vinblastine ($C_{46}H_{58}N_4O_9$) resistance was observed (Figure 4A). Figure 4A shows that **9d** exhibited the greatest efficacy in potentiating the cytotoxicity of vinblastine, giving a RF in IC_{50} values of about 13, from 4.8 to 0.36 nM. The efficacy of **9d** was similar to that of VP (IC_{50} = 0.25 nM). Compounds **9b** and **9c** have lower but still very high activities in reducing the IC_{50} by 7.9 and 5.5 RF to 0.61 and 0.87 nM, respectively. Other dimers with shorter (**9a**) or longer spacers (**9e**, **9f**, **9g**, **9i**) have little or no activity. Monomers **10a** and **10b**, at double the concentration used (10 μM), were also ineffective. Compound **9d** also exhibited a dose-dependent effect in potentiating vinblastine cytotoxicity (Figure 4B). Similarly, **9d** is more effective than others in potentiating doxorubicin (DOX; $C_{27}H_{29}NO_{11}$) cytotoxicity, reducing the IC_{60} by about 6 RF from 4.7 to 0.73 μM (Figure 5). Compounds **9c** (IC_{60} = 1.3 μM) and **9b** (IC_{60} = 1.3 μM) also showed high efficacy in changing the IC_{60} by about 3.6 and 3.1 RF, respectively. Apigenin dimers with shorter (**9a**) or longer PEGs (**9e**, **9f**, **9g**, and **9i**) gave very little or no DOX sensitization. Monomers (**10a** and **10b**) were ineffective reversers as well.

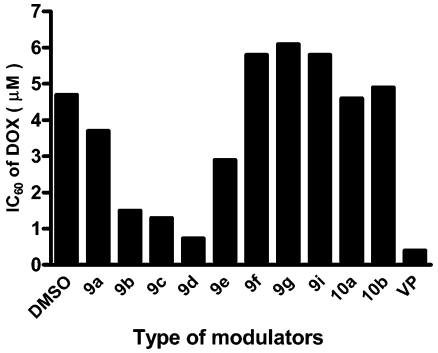


Figure 5. Effects of apigenin monomers and dimers on DOX cytotoxicity in MDA435LCC6 MDR cells. The results are presented as IC_{60} values calculated from dose−response curves of MTS cytotoxicity assays with or without modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomers (**10a,b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Presented is a typical experiment performed in triplicate.

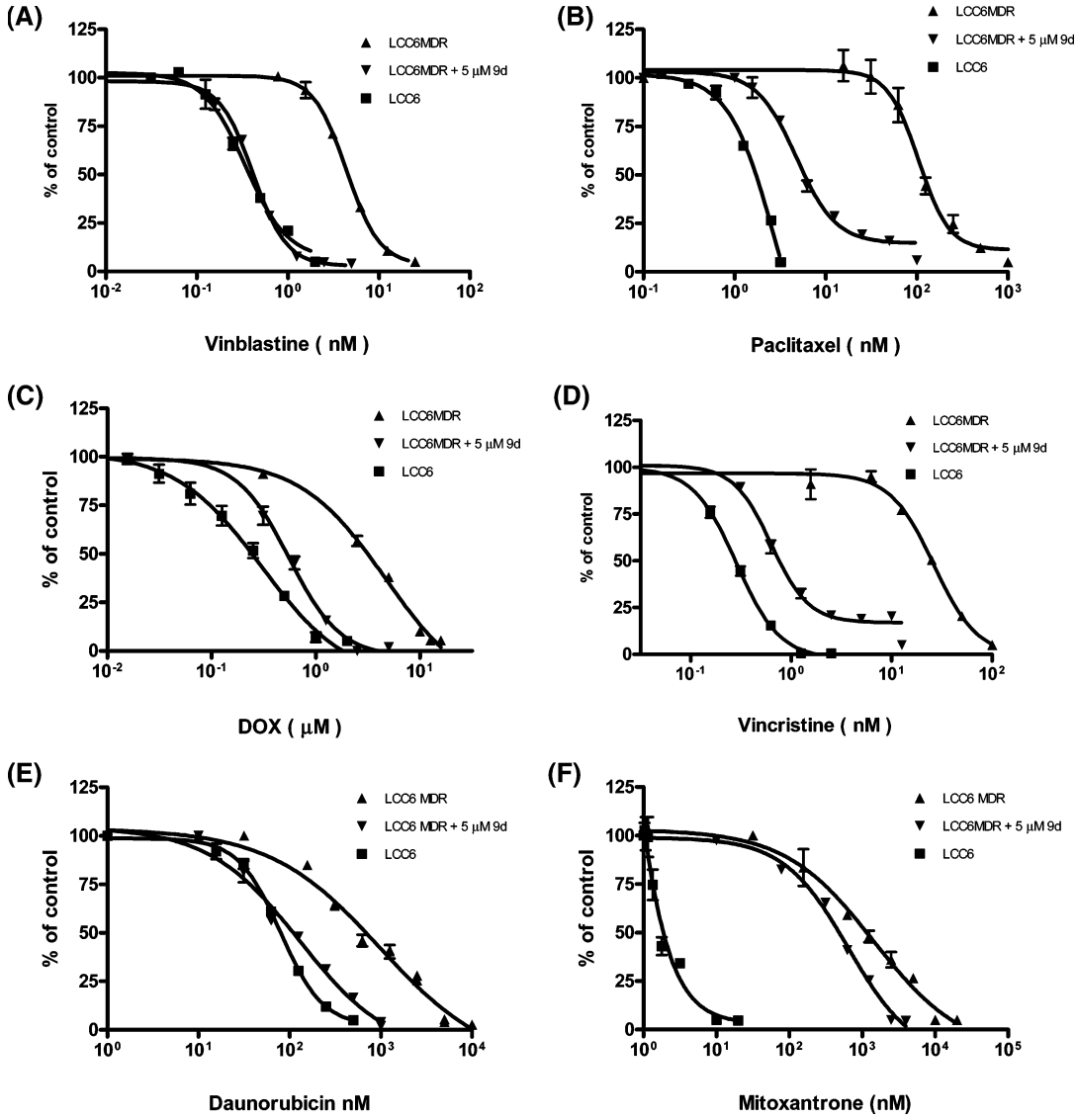


Figure 6. Proliferation of MDA435LCC6 MDR and MDA435LCC6 cells in the presence of anticancer drugs (A) vinblastine, (B) paclitaxel, (C) DOX, (D) vincristine, (E) daunorubicin, and (F) mitoxantrone with or without 5 μM **9d**. The data are expressed as a percentage of optical density at 490 nm (MTS activity) relative to untreated cell populations and represent the mean ± SD of three or more replicates. (▲) MDA435LCC6 MDR; (▼) MDA435LCC6 MDR + 5 μM **9d**; (■) MDA435LCC6.

Compound 9d Can Reverse MDR of MDA435/LCC6 MDR to Almost Parental Level. Since compound **9d** consistently showed the highest modulating activity against paclitaxel, vinblastine, and DOX, we therefore focused on investigating whether 5 μM **9d** can reverse the resistance of MDA435/LCC6 MDR back to that of the parental level (MDA435/LCC6). Figure 6A−E indicated that 5 μM **9d** can reverse resistance of MDA435/LCC6 MDR to vinblastine, paclitaxel, DOX, vincristine, and daunorubicin ($C_{27}H_{29}NO_{10}$) to a level close to the parental (MDA435/LCC6) level. No effect was observed for mitoxantrone ($C_{22}H_{28}N_4O_6$) (Figure 6F). The reversing ability, as determined by the relative fold changes in the IC_{50} of drugs with or without **9d**, is summarized in Table 1. It ranges from 7.6 to 41 RF. The reversing activity for vinblastine and paclitaxel are particularly impressive as the IC_{50} can be lowered to almost the same level as in the sensitive counterparts.

Effect of Apigenin Dimers on Cellular Accumulation of DOX in MDA435/LCC6 and MDA435/LCC6 MDR Cells. To understand whether the modulating activity for various anticancer drugs by different dimers is due to their different ability to modulate P-gp-mediated drug efflux, their effects on the accumulation of DOX in both MDA435/LCC6 sensitive and resistant cells were investigated. DOX is a fluorescent drug substrate of P-gp and was used in this experiment to monitor the P-gp-mediated drug efflux. Accumulation of DOX in these cells was determined in the presence or absence of apigenin dimers (10 μM) and monomer (20 μM). VP (5 μM) was used as a positive control.

The results are shown in Figure 7A. Accumulation of DOX in LCC6, with a basal level of P-gp expression, was unaffected by treatment with either solvent control (DMSO), apigenin monomer **10b**, various dimers, or VP. For LCC6 MDR cells, the accumulation level of DOX, when treated with DMSO control, was found to be around 20% that of LCC6. This is due to the P-gp-mediated DOX efflux found in LCC6 MDR cells. Such a low level of accumulation, however, was completely reversed by cotreatment with **9d**. At 10 μM, **9d** enhanced DOX accumulation of LCC6 MDR cells by 5.8 RF. The accumulation of DOX was almost the same (97%) as that of the **9d**-treated LCC6 cells. This efficacy was comparable to that of VP (6.2 RF). Compounds **9b** and **9c**, which also have drug resistance reversing activity on paclitaxel, vinblastine, and DOX in LCC6

Table 1. Summary of **9d** Effects on the Cytotoxicity of Chemotherapeutic Drugs in MDR Cells[a]

		cell lines					
		LCC6 MDR		LCC6	P388/ADR		P388
agent	treatment	IC_{50} (nM)	RF[b]	IC_{50} (nM)	IC_{50} (nM)	RF	IC_{50} (nM)
vinblastine	drug	4.4	1	0.34	95	1	4.1
vinblastine	drug + **9d**	0.42	10		4.3	22	
vinblastine	drug + VP	0.29	15		ND[c]		
paclitaxel	drug	105	1	2.9	1636	1	22
paclitaxel	drug + **9d**	4.8	22		30	55	
paclitaxel	drug + VP	5.2	20		ND		
DOX	drug	4690	1	300	1738	1	22
DOX	drug + **9d**	550	9		123	14	
DOX	drug + VP	300	16		ND		
vincristine	drug	26	1	0.29	299	1	2.2
vincristine	drug + **9d**	0.63	41		4.5	66	
vincristine	drug + VP	ND			ND		
daunorubicin	drug	977	1	79	2111	1	25
daunorubicin	drug + **9d**	129	7.6		106	20	
daunorubicin	drug + VP	ND			40	53	
mitoxantrone	drug	1442	1	0.35	395	1	4.3
mitoxantrone	drug + **9d**	646	2.2		194	2	
mitoxantrone	drug + VP	ND			ND		

[a] The IC_{50} value was determined for each cell line after exposure to a series of drug concentration with/without 5 μM **9d** or VP, as described under Materials and Methods. RF represents fold-change in drug sensitivity. [b] RF, relative fold = ratio of (IC_{50} without modulator) to (IC_{50} with modulator). This is used as an indicator of the strength of the reversing activity of the modulator. [c] ND, not determined.

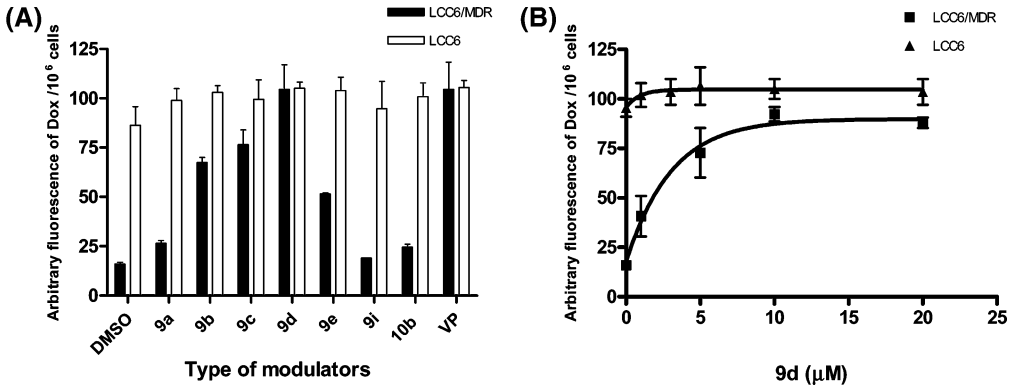


Figure 7. (A) Intracellular accumulation of DOX in MDA435LCC6 MDR and MDA435LCC6 cells treated with different modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomer **10b** used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Results are presented as mean ± SD from three independent experiments. (B) Intracellular accumulation of DOX in MDA435LCC6 MDR and MDA435LCC6 cells treated with different concentrations of **9d** (0−20 μM). Results are presented as mean ± SD from three independent experiments.

MDR, also enhanced DOX accumulation by 4.5 and 4 RF in comparison with control, respectively. In general, modulators' reversal efficacy of DOX accumulation is closely paralleled by their potencies in reversing DOX resistance in LCC6 MDR.

We then investigated the dose-dependent effect of **9d** on the accumulation of DOX in P-gp positive and negative cells (Figure 7B). It was found that **9d** significantly increased DOX accumulation in MDA435/LCC6 MDR cells in a dose-dependent manner but not in sensitive MDA435/LCC6 cells. The intracellular DOX concentration was gradually increased from 17% to 88% of the LCC6 when the concentration of **9d** was increased from 0 to 10 μM.

Effect of Apigenin Dimers on Reversing the Drug Cytotoxicity in P388/ADR Cells. The above data suggest that apigenin dimers, particularly **9d**, are promising in reversing drug resistance in human breast cancer cells. If these apigenin dimers can modulate MDR by inhibiting the P-gp efflux, they should be able to modulate other MDR cancers as well. To prove this, we investigated another well-characterized cancer MDR system, the P388/ADR-murine leukemia cell line, which is resistant to ADR (adriamycin, brand name of DOX). P388/ADR has been widely used as a standard for preclinical evaluation of MDR modulators.

Consistent with the previous observations, the apigenin dimers with different spacer lengths exhibited different modulatory activities in P388/ADR cells (Figures 8A and 9A). Again, 5 μM **9d** is the most potent modulator, reducing the DOX and daunorubicin IC_{50} by about 10 and 21 RF from 1.5 and 2.1 μM to 0.15 and 0.10 μM, respectively (Figures 8A and 9A). Compound **9d** also showed a dose-dependent effect on reversing the resistance toward DOX (Figure 8B) and daunorubicin (Figure 9B), with the saturating concentration at about 5 μM. Modest inhibition was noted with **9c** and **9b** with shorter spacer lengths, reducing the IC_{50} of DOX to about 3 and 2 RF and IC_{50} of daunorubicin to about 4.6 and 2.5 RF, respectively. Modulators with spacers longer than **9d** or shorter than **9b** have little or no effect on potentiating the DOX and daunorubicin cytotoxicity in P388/ADR cells. Both monomers **10a** and **10b**, at 10 μM, gave little modulatory activity. Nevertheless, unlike MDA435/LCC6 MDR cells, the reversing activity of **9d** was

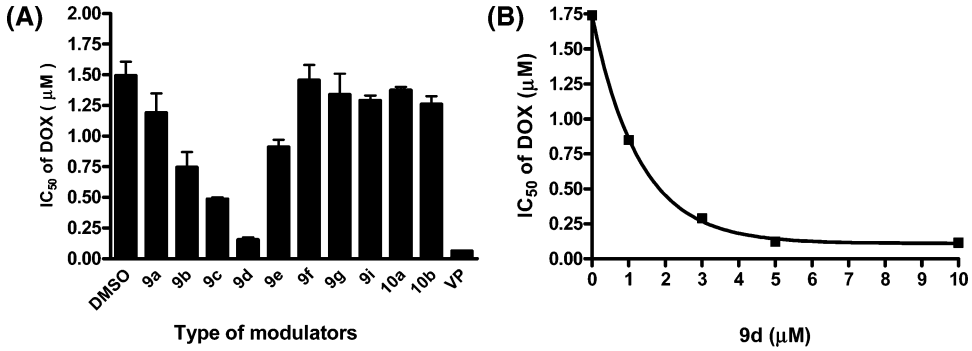


Figure 8. (A) Effects of apigenin monomers and dimers on DOX cytotoxicity in P388/ADR cells. The results are presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays with or without modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomers (**10a,b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Each experiment contains data points averaged from triplicates. Data are presented as mean ± SD from three independent experiments. (B) Concentration-dependent effect of **9d** on DOX cytotoxicity in P388/ADR cells, presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays in the presence of different concentrations of **9d** (0−10 μM). Presented is a typical experiment performed in triplicate.

not as good as VP, which almost completely reversed the DOX and daunorubicin resistance in P388/ADR cells (IC_{50} = 0.06 and 0.04 μM, respectively). The above results suggest that apigenin dimers are inhibiting the P-gp in both LCC6 MDR and P388/ADR cells. The correlation between drug resistance reversing activity and the spacer length of apigenin dimers is almost identical in these two cell lines.

Compound 9d Can Reverse Drug Resistance of P388/ADR Cells to Almost the Level of the Sensitive Parent Cell Line P388. Compound **9d** also potentiated the action of other P-gp substrates on P388/ADR cells, including DOX, daunorubicin, paclitaxel, vincristine, and vinblastine, to different extents (Figure 10A−E). In the cases of paclitaxel, vincristine, and vinblastine, 5μM **9d** completely reversed the resistance of P388/ADR to almost the sensitive level (Figure 10C−E), indicating complete inhibition of efflux of the anticancer drugs by **9d**. There was nearly no effect on mitoxantrone resistance. This suggests P388/ADR harbors an additional MDR mechanism for mitoxantrone that is insensitive to **9d**. The reversing ability, as determined by the relative fold changes in IC_{50}, is summarized in Table 1. It varies from 14 to 66 RF.

Effect of Apigenin Dimer on Cellular Accumulation of DOX in P388 and P388/ADR Cells. We examined the ability of apigenin dimers to affect DOX accumulation in both P388 and P388/ADR cells. In the DMSO-treated control, accumulation of DOX in P388/ADR cells was about 33% that of P388 cells, indicating efflux of DOX (Figure 11A). Addition of different apigenin dimers inhibited P-gp efflux of DOX in the MDR cells to different extents. Compounds **9b**−**d** showed the highest efficacy, causing an increase of the DOX accumulation to about 2-fold that of the control. On the other hand, the monomer **10b** (with double concentration of the dimers) or other apigenin dimers with longer (**9e**, **9i**) or shorter (**9a**) spacers gave little or no activity at all. The correlation between drug resistance reversing activity in P388/ADR and spacer lengths of apigenin dimers is similar to what we observed in LCC6 MDR cells.

When cells were preincubated with various concentrations of **9d** for 30 min, **9d** significantly increased DOX accumulation in P388/ADR cells in a dose-dependent manner, but not in sensitive P388 cells (Figure 11B). Compound **9d**, however, cannot restore cellular DOX levels in resistant P388/ADR cells to the level of sensitive cells, whereas VP can (Figure 11A). This suggests that **9d** does not completely inhibit the P-gp efflux of DOX in P388/ADR. This is consistent with its cytotoxicity modulating effect being not as high as VP (Figure 8A).

Effects of 9d on P-gp ATPase Activity. To further investigate the interaction between **9d** and P-gp, we examined the effect of **9d** (100 μM) on both P-gp ATPase activity and VP-induced ATPase activity. Interestingly, **9d** (100 μM) can increase P-gp ATPase activity over the basal level by 3.3-fold ($P < 0.0001$) (Figure 12). As expected, VP (a well-known P-gp ATPase stimulator acting by binding to the substrate binding site) can increase P-gp ATPase activity over the basal level by 7.4-fold ($P < 0.0001$). Such VP-induced P-gp ATPase activity was lowered from 7.4-fold to 6.1-fold when **9d** was also present ($P < 0.0001$). This result suggested that **9d**, like VP, can stimulate P-gp ATPase activity and it probably works by binding to the same site of P-gp as VP does. Both VP and **9d** (100 μM) had no significant effects on non-P-gp ATPase activity (data not shown).

Discussion

So far, P-gp remains the most frequent cause of clinical MDR in cancers. Searches for MDR-reversing compounds have also come a long way. However, success in overcoming MDR by reversing agents has been limited, due to the toxicities, lack of specificity, or a low affinity for the drug binding site(s). The present research aims to develop efficient P-gp modulators by using dimers of dietary flavonoids of low toxicity linked together by a suitably biocompatible oligomer, PEG. The apigenin dimers **9** were evaluated for their efficacy to modulate the drug resistance of (1) human breast cancer MDR cells (LCC6 MDR) and (2) murine leukemic MDR cells (P388/ADR). We found a correlation between the length of ethylene glycol linker and the drug resistance reversing effects of the apigenin dimers in both LCC6 MDR and P388/ADR cells. The most potent apigenin dimers have two to four ethylene glycol linkers (compounds **9b**−**d**), beyond which there is an inverse correlation between the number of ethylene glycols and the drug resistance reversing effects. This "U"-shaped relationship was also found when we studied the DOX accumulation in LCC6 MDR and P388/ADR cells. This suggests that (1) apigenin dimers are working in a similar mechanism in both LCC6 MDR and P388/ADR cells, namely, inhibiting the P-gp mediated resistance and drug efflux, and (2) there is an optimal length of ethylene glycol linkers (n = 2−4) that can inhibit P-gp. Our results suggest that there is a relatively rigid distance between the two apigenin-binding sites. It certainly seems that it is the bivalent nature of the apigenin dimers that is responsible for the enhanced modulating effect, with **9c** and **9d** displaying consistently higher activity

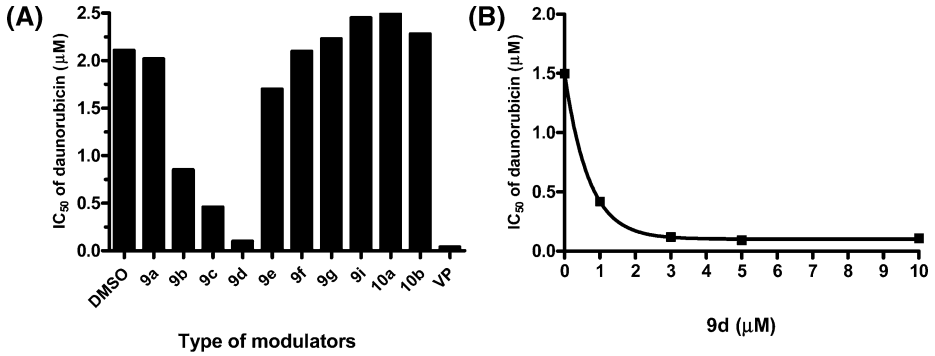


Figure 9. (A) Effects of apigenin monomers and dimers on daunorubicin cytotoxicity in P388/ADR cells. The results are presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays with or without modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomers (**10a,b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as positive and solvent control, respectively. Presented is a typical experiment performed in triplicate. (B) Concentration-dependent effect of **9d** on daunorubicin cytotoxicity in P388/ADR cells. The results are presented as IC_{50} values calculated from dose−response curves of MTS cytotoxicity assays in the presence of different concentrations of **9d** (0−10 μM). Presented is a typical experiment performed in triplicate.

than their ethylene glycol-ligated counterparts **10a** and **10b**. This does not appear to be a statistical effect (i.e., effectively doubling the concentration of the flavonoid). Among all the dimers, compound **9d** is particularly effective in reversing MDR of a number of drugs in both breast cancer LCC6 MDR cells and leukemia P388/ADR cells. For paclitaxel, compound **9d** showed 22 and 55 RF change in drug sensitivity for those two resistant cell lines, respectively. Compound **9d** may therefore have potential clinically.

What is not clear from the studies thus far is the mode of action of modulation or the location of the binding. Previously, it has been suggested that the hydrogen-bonding pattern of the C4 carbonyl and C7 phenolic functionalities of apigenin mimics that of N1 and N7 of ATP and the binding occurs at the NBD of P-gp.[43,44] Alternatively, it has been suggested that alkyl-substituted flavonoids may bind to the hydrophobic steroidal interacting region or the drug binding sites of TMD.[54] Figure 13 shows three possible interactions of the flavonoid dimers with P-gp. Figure 13A shows that the flavonoid dimers can compete with ATP at both NBDs, effectively disrupting the process of drug efflux. It has been suggested that the hydrolysis of ATP at one of the NBDs promotes binding of ADP at the second NBD and that the two NBDs are in close proximity during this process. The fact that only the apigenin dimers with certain linker lengths showed considerable activity could well indicate that our dimers are interacting with both NBDs, with the longer or shorter ethylene glycol-linked dimers displaying similar activity to apigenin. If that is the case, ATP hydrolysis would be inhibited. In Figure 13B, one apigenin moiety is attached to the NBD while the second sits in the drug binding cavity (or steroid binding region). This would possibly be even more effective at reversing MDR, as both NBD and the drug binding site are blocked. In such a case, it is not clear whether ATP hydrolysis would be inhibited upon addition of the apigenin dimer. In the case of Figure 13C, both flavonoid moieties are bound to the drug binding site and/or steroidal binding pocket close to the membrane. In such a case, one would not expect the ATP hydrolysis to have been inhibited. P-gp has been demonstrated to be an ATPase,[79] and compounds that interact with P-gp can be either stimulators or inhibitors of its ATPase activity. Compounds that are substrates for transport by P-gp typically stimulate its ATPase activity.[80] For example, the P-gp inhibitor VP, being a substrate of P-gp, is also one of the best stimulators of P-gp ATPase.[81,82] Flavonoids have been reported to modulate P-gp by interacting bifunctionally with the vicinal ATP-binding site and the steroid binding site.[83] Thus the inhibition of P-gp ATPase by binding to the ATP-binding site is proposed to be one potential mechanism responsible for the flavonoid inhibition of P-gp-mediated efflux. However, it has also been reported that the interactions of flavonoids with P-gp may differ, with some flavonoids (such as silymarin) significantly inhibiting VP-induced P-gp ATPase activity while others (such as biochanin A and phloretin) could stimulate P-gp ATPase activity.[41]

The experimental observation that the dimer **9d** in fact enhanced ATPase activity of P-gp (Figure 12) is more consistent with scenarios in Figure 13 panel B or C. Compound **9d** can significantly stimulate P-gp ATPase activity by 3.3-fold, although not as effectively as VP (7.4-fold). When present together, **9d** slightly inhibited the VP-induced P-gp ATPase activity by 17%. The stimulatory effect of **9d** suggests that **9d** probably does not bind to the NBD of P-gp, arguing against the scenario in Figure 13A. Rather, it is possibly binding to the P-gp's substrate-binding site. If this is true, it could prevent binding of VP to the drug binding site, and since it is a weaker stimulator than VP, it can therefore inhibit the P-gp ATPase activity induced by VP.

Our observation demonstrated that mitoxantrone is a substrate of P-gp, as LCC6 MDR was cross-resistant to mitoxantrone (IC_{50} = 1442 μM as compared to 0.35 μM for LCC6). Others have also reported that mitoxantrone can also be a substrate of P-gp. [84] Compound **9d**, however, has no modulating activity on mitoxantrone resistance in either LCC6 MDR or P388/ADR cells (Figures 6F and 10F). This observation suggests that **9d** is blocking P-gp in a substrate-specific manner (supporting models in Figure 13B,C) where the flavonoid moieties are binding to one or both substrate binding sites. In such a case, there exist two classes of substrate-binding sites, with one specific toward mitoxantrone whereas the other is specific toward paclitaxel, vinblastine, DOX, vincristine, and daunorubicin. We have also tested the efficacy of **9d** on reversing resistance to other non-P-gp substrates such as cisplatin [85] and found that **9d** did not have any modulating effect on LCC6MDR's sensitivity to cisplatin (data not shown). This suggests that **9d** may not affect other non-P-gp functions.

The observation that dimers **9b**, **9c**, and **9d** are more or less comparable in potencies in modulating MDR, but dimers with shorter or longer spacers are less active, is consistent with divalent binding. There is presumably limited conformational flexibility in the binding between P-gp and the divalent ligand. It is also possible that the increased activity in the case of certain dimers is brought about by a combination of all three or more

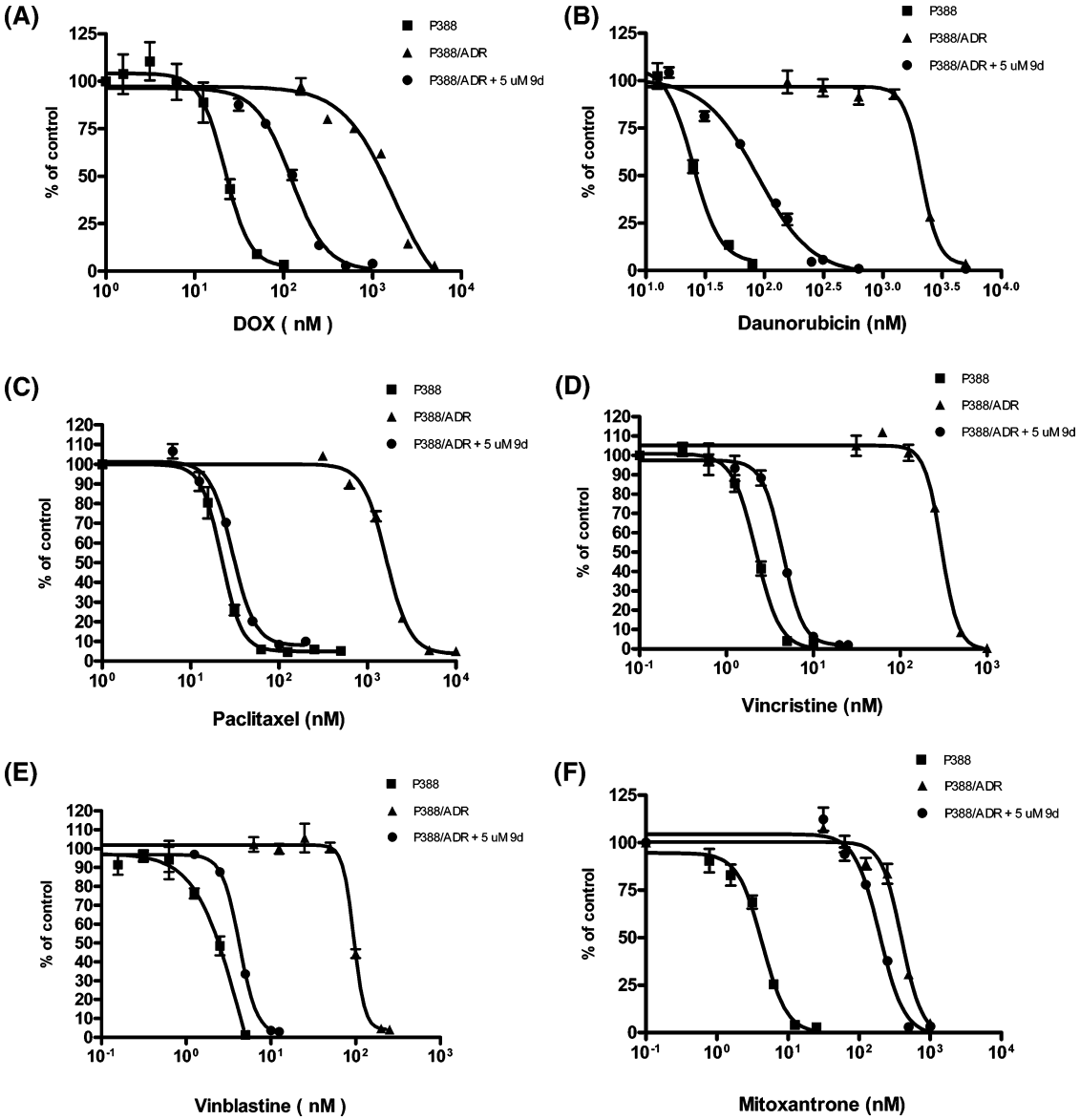


Figure 10. Proliferation of P388/ADR and P388 cells in the presence of anticancer drugs (A) DOX (B) daunorubicin, (C) paclitaxel, (D) vincristine, (E) vinblastine,and (F) mitoxantrone with or without 5 μM **9d**. The data are expressed as a percentage of optical density at 490 nm (MTS activity) relative to untreated cell populations and represent the mean ± SD of three or more replicates.

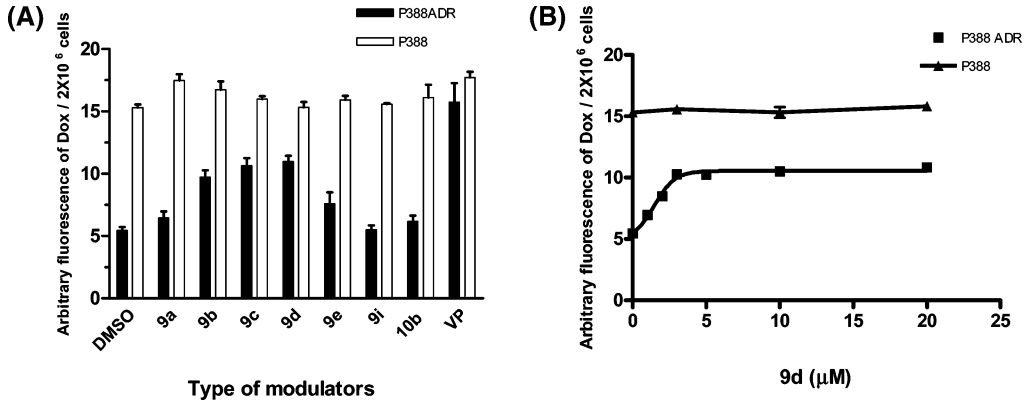


Figure 11. (A) Intracellular accumulation of DOX in P388/ADR and P388 cells by different modulators. Concentrations of apigenin dimers (**9a**−**i**) and monomer (**10b**) used were 5 μM and 10 μM, respectively. VP (5 μM) and DMSO (0.05%) were included as a positive and solvent control, respectively. Results are presented as mean ± SD from three independent experiments. (B) Intracellular accumulation of DOX in P388/ADR and P388 cells by different concentrations of **9d** (0−20 μM). Results are presented as mean ± SD from three independent experiments.

binding events, and that B and C would provide the most optimal conditions for reversing MDR. Of course there are other possibilities not shown here that may involve binding between the flavonoid dimers and P-gp through allosteric interactions. Consideration of the different binding modes opens up a new dimension to this study. Rather than treating P-gp as a divalent

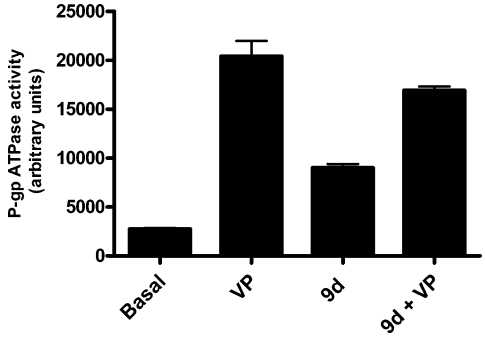


Figure 12. Effects of **9d** on P-gp ATPase activity. The effects of **9d** on P-gp ATPase activity and VP-induced P-gp ATPase activity were studied as described under Materials and Methods. P-gp ATPase activity was presented as drop in luminescence (due to ATP/luciferase-generated signal) of samples compared to that treated with Na_3VO_4 (a specific P-gp ATPase inhibitor). Data are expressed as mean ± SD from three independent experiments.

receptor, we should in fact treat it as a multivalent receptor with several different binding regions. We can therefore consider other alternatives, using nonsymmetrical dimeric flavonoids that are optimized to promote one binding event over another. However, this is future work and further discussion is outside the scope of this report.

In conclusion, our studies have clearly demonstrated that the flavonoid dimers linked by optimal spacer length have great potential to be developed as codrugs for the chemotherapeutic treatment of cancer. We proved this principle by identifying an apigenin dimer with optimal spacer length (**9d**) that, when used at 5 μM, displayed 6−50-fold increase of cytotoxicity of anticancer drugs in both breast and leukemia MDR cells in vitro and by dramatically enhancing their intracellular drug accumulation. Additional mechanistic and in vivo studies are needed to understand **9d** as a chemosensitizer in cancer therapy.

Experimental Section

General. All NMR spectra were recorded on a Bruker MHz DPX400 spectrometer at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C. All NMR measurements were carried out at room temperature and the chemical shifts are reported as parts per million (ppm) in δ units relative to the resonance of $CDCl_3$ (7.26 ppm in the ^{1}H, 77.0 ppm for the central line of the triplet in the ^{13}C modes). Low- and high-resolution mass spectra were obtained on a Micromass Q-TOF-2 in electron spray ionization (ESI) mode or on a Finnigan MAT95 ST in electron ionization (EI) mode. Melting points were measured on an Electrothermal IA9100 digital melting point apparatus and were uncorrected. All reagents and solvents were reagent-grade and were used without further purification unless otherwise stated. The plates used for thin-layer chromatography (TLC) were E. Merck silica gel $60F_{254}$ (0.25-mm thickness) and they were visualized under short-wavelength (254-nm) UV light. Chromatographic purifications were carried out on MN silica gel 60 (230−400 mesh).

***trans*-3-(4-Allyloxyphenyl)-1-[2,4-bis(methoxymethoxy)-6-hydroxyphenyl]propenone (18).** A round-bottom flask was charged with 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone **14b** (4.39 g, 17.1 mmol), 4-allyloxybenzaldehyde (2.90 g, 17.9 mmol), and KOH solution (3 M solution in 96% EtOH, 30 mL). The solution turned brown immediately and was stirred at room temperature for 16 h. When TLC indicated complete consumption of acetophenone, the reaction mixture was poured into a separating funnel containing 0.5 M HCl solution (180 mL). The mixture was extracted with CH_2Cl_2 (40 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude brown oil, which was subjected to flash column chromatography (20% EtOAc in hexane) on silica gel (70 g) to furnish chalcone **18** (6.53 g, 95%)

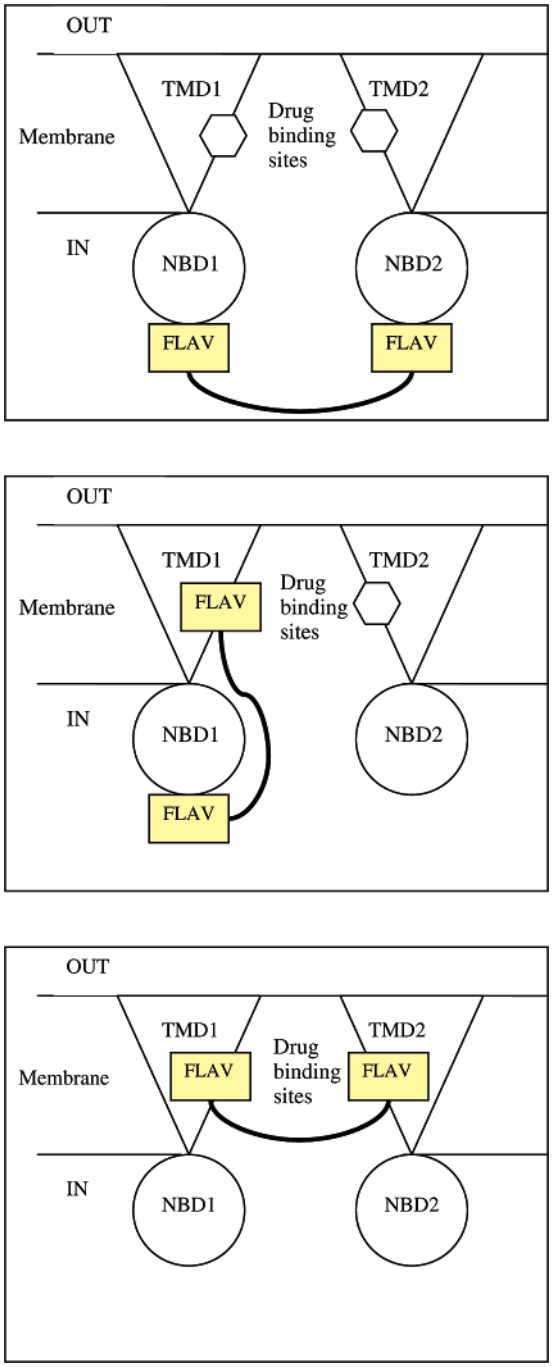


Figure 13. Three possible interactions of flavonoid dimers with P-gp. (A) The two flavonoid moieties (shown as shaded boxes labeled FLAV) are binding to the two NBDs of P-gp (shown as circles labeled NBD1 and NBD2). The two transmembrane domains (TMD1 and TMD2) are represented by two inverted triangles. The putative drug binding sites present on TMD1 and TMD2 are represented by two hexagons. (B) One of the flavonoid moieties (FLAV) is binding to the NBD while the other FLAV is binding to the drug binding site formed by one of the transmembrane domains (TMD1 or TMD2). (C) Both flavonoid moieties (FLAV) are binding to the drug binding site.

as a yellow solid: mp 70−71 °C; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 3H), 3.53 (s, 3H), 4.57 (d, J = 5.2 Hz, 2H), 5.18 (s, 2H), 5.28 (s, 2H), 5.31 (d, J = 10.4 Hz, 1H), 5.42 (dd, J = 1.2, 17.2 Hz, 1H), 6.02−6.04 (m, 1H), 6.24 (d, J = 2.0 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 6.93 (d, J = 8.8 Hz, 2H), 7.54 (d, J = 8.8 Hz, 2H), 7.76 (A of AB, J = 15.4 Hz, 1H), 7.83 (B of AB, J = 15.4 Hz, 1H), 13.9 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.4, 56.8, 68.8, 94.0, 94.7, 95.1, 97.5, 107.5, 115.1, 118.0, 125.0, 128.3, 130.0, 132.7, 142.6, 159.8, 160.4, 163.2, 167.2, 192.8; LRMS (ESI) m/z 401 (M^+ + H, 100),

423 (M^+ + Na, 22); HRMS (ESI) calcd for $C_{22}H_{25}O_7$ (M^+ + H) 401.1600, found 401.1604.

5-Hydroxy-7-methoxymethoxy-2-(4′-allyloxyphenyl)-4*H*-chromen-4-one (19). A round-bottom flask was charged with chalcone **18** (6.53 g, 16.3 mmol), DDQ (5.56 g, 24.5 mmol), and a dry solvent of 25% dioxane in toluene (100 mL). The solution turned deep brown immediately and was stirred under nitrogen atmosphere at refluxing temperature for 7 h. When TLC indicated complete consumption of chalcone **18**, the reaction mixture was cooled to room temperature and the solvents were evaporated to dryness. After addition of CH_2Cl_2 (150 mL), the insoluble brown solid was removed by suction filtration. The deep brown filtrate was washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, evaporated, and subjected to flash column chromatography (15% EtOAc in hexane) on silica gel (130 g) to furnish compound **19** (2.10 g, 36%) as a pale yellow solid: mp 100−101 °C; ^{1}H NMR ($CDCl_3$) δ 3.49 (s, 3H), 4.59 (d, J = 5.2 Hz, 2H), 5.22 (s, 2H), 5.32 (d, J = 10.8 Hz, 1H), 5.43 (d, J = 17.2 Hz, 1H), 6.00−6.09 (m, 1H), 6.44 (d, J = 1.8 Hz, 1H), 6.54 (s, 1H), 6.25 (d, J = 1.8 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 12.74 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.4, 68.9, 94.2, 94.2, 100.0, 104.2, 106.1, 115.1, 118.2, 123.5, 127.9, 132.4, 157.5, 161.6, 161.9, 162.8, 163.9, 182.4; LRMS (ESI) *m*/*z* 355 (M^+ + H, 36); HRMS (ESI) calcd for $C_{20}H_{19}O_6$ (M^+ + H) 355.1182, found 355.1164.

5-Benzyloxy-7-methoxymethoxy-2-(4′-allyloxyphenyl)-4*H*-chromen-4-one (20). A round-bottom flask was charged with compound **19** (2.24 g, 6.3 mmol), benzyl bromide (1.70 g, 9.9 mmol), K_2CO_3 (1.80 g, 13.0 mmol), and DMF (15 mL). The reaction mixture was stirred at refluxing temperature for 2 h. When TLC indicated complete consumption of **19**, the reaction mixture was poured into a separating funnel containing water (200 mL). The mixture was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a brown oil, which was subjected to flash column chromatography with gradient elution (from 30% to 60% EtOAc in hexane) on silica gel (50 g) to furnish compound **20** (2.01 g, 72%) as an off-white solid: mp 120−122 °C; ^{1}H NMR ($CDCl_3$) δ 3.68 (s, 3H), 4.78 (d, J = 5.2 Hz, 2H), 5.41 (s, 2H), 5.43 (s, 2H), 5.51 (d, J = 10.8 Hz, 1H), 5.62 (d, J = 17.2 Hz, 1H), 6.21−6.26 (m, 1H), 6.69 (d, J = 2.0 Hz, 1H), 6.77 (s, 1H), 6.94 (d, J = 2.0 Hz, 1H), 7.18 (d, J = 8.6 Hz, 2H), 7.45 (t, J = 7.6 Hz, 1H), 7.58 (dd, J = 7.2, 7.6 Hz, 2H), 7.82 (d, J = 7.2 Hz, 2H), 7.99 (d, J = 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 56.4, 68.9, 7.07, 94.3, 96.0, 98.7, 107.6, 110.2, 115.0, 118.1, 123.9, 126.6, 127.6, 128.5, 128.7, 132.6, 136.4, 159.4, 159.6, 160.7, 161.0, 161.2, 177.4; LRMS (ESI) *m*/*z* 445 (M^+ + H, 100), 467 (M^+ + Na, 15); HRMS (ESI) calcd for $C_{27}H_{25}O_6$ (M^+ + H) 445.1651, found 445.1641.

5-Benzyloxy-7-methoxymethoxy-2-(4′-hydroxyphenyl)-4*H*-chromen-4-one (12a). A round-bottom flask was charged with compound **20** (2.01 g, 4.5 mmol), a catalytic amount of $Pd(PPh_3)_4$ (0.1 g), K_2CO_3 (2.50 g, 18.1 mmol), and MeOH (80 mL). The reaction mixture was stirred at refluxing temperature for 2 h. When TLC indicated complete consumption of **20**, the reaction mixture was poured into a beaker containing water (200 mL). The solution was acidified to pH 4 with 1 M HCl solution and abundant off-white solid was formed, which was collected by suction filtration. The collected solid was dissolved in 50% EtOAc in MeOH and the insoluble dark charcoal was removed by filtration. The brown filtrate was evaporated under reduced pressure and compound **12a** (1.42 g, 78%) slowly precipitated out as a white solid: mp 202−204 °C; ^{1}H NMR (DMSO-d_6) δ 3.59 (s, 3H), 5.40 (s, 2H), 5.51 (s, 2H), 6.77 (s, 1H), 6.85 (d, J = 1.8 Hz, 1H), 7.07 (d, J = 1.8 Hz, 1H), 7.09 (d, J = 8.8 Hz, 2H), 7.49 (t, J = 7.6 Hz, 1H), 7.58 (dd, J = 7.2, 7.6 Hz, 2H), 7.79 (d, J = 7.2 Hz, 2H), 8.06 (d, J = 8.8 Hz, 2H), 10.41 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 56.5, 70.3, 94.4, 96.2, 99.1, 106.6, 109.6, 116.3, 121.7, 127.3, 127.9, 128.3, 128.7, 137.3, 159.2, 159.4, 160.7, 161.0, 161.2, 176.1; LRMS (ESI) *m*/*z* 405 (M^+ + H, 100), 427 (M^+ + Na, 19); HRMS (ESI) calcd for $C_{24}H_{21}O_6$ (M^+ + H) 405.1338, found 405.1336.

General Procedure for Synthesis of Flavonoid Dimers 21a−i from 12a. A round-bottom flask was charged with compound **12a** (1.6 equiv), dimesylate **13b** (for n = 1, 4−9) or ditosylate **13a** (for n = 2, 3) (1 equiv), K_2CO_3 (8 equiv), and DMF. The reaction mixture was stirred at refluxing temperature for 2−3 h. During heating, the reaction mixture turned slowly from pale brown to milky in color. When TLC indicated complete consumption of **12a**, the reaction mixture was poured into a separating funnel containing water (200 mL). The mixture was extracted with CH_2Cl_2 (20 mL × 3). If the mixture could not be separated into two layers, 1 M HCl (20 mL) was added. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude reaction mixture. Purification of the flavonoid dimer was performed by crystallization or flash column chromatography as indicated.

1,4-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4-dioxabutane (21a). This compound was prepared from **12a** (230 mg, 0.57 mmol), ethylene glycol dimesylate (75 mg, 0.34 mmol), K_2CO_3 (380 mg), and DMF (8 mL) by the general procedure for synthesis of flavonoid dimers. After crystallization from EtOAc, the title compound (150 mg, 63%) was obtained as white solid: mp 173−175 °C; ^{1}H NMR ($CDCl_3$) δ 3.50 (s, 6H), 4.40 (s, 4H), 5.23 (s, 4H), 5.25 (s, 4H), 6.51 (d, J = 1.6 Hz, 2H), 6.57 (s, 2H), 6.77 (d, J = 1.6 Hz, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.30 (t, J = 7.2 Hz, 2H), 7.39 (dd, J = 7.2, 7.6 Hz, 4H), 7.63 (d, J = 7.6 Hz, 4H), 7.83 (d, J = 8.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 66.5, 70.7, 94.3, 96.0, 98.8, 107.7, 110.2, 114.9, 124.3, 126.6, 127.6, 127.7, 128.5, 136.4, 159.4, 159.6, 160.6, 160.9, 161.3, 177.4; LRMS (ESI) *m*/*z* 835 (M^+ + H, 100), 857 (M^+ + Na, 68); HRMS (ESI) calcd for $C_{50}H_{42}O_{12}Na$ (M^+ + Na) 857.2574, found 857.2571.

1,7-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7-trioxaheptane (21b). This compound was prepared from **12a** (200 mg, 0.50 mmol), diethylene glycol ditosylate (130 mg, 0.31 mmol), K_2CO_3 (360 mg), and DMF (8 mL) by the general procedure for synthesis of flavonoid dimers. After crystallization from EtOAc, the title compound (88 mg, 40%) was obtained as white solid: mp 110−111 °C; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 6H), 3.96 (t, J = 4.6 Hz, 4H), 4.22 (t, J = 4.6 Hz, 4H), 5.26 (s, 8H), 6.47 (d, J = 1.8 Hz, 2H), 6.58 (s, 2H), 6.73 (d, J = 1.8 Hz, 2H), 6.99 (d, J = 8.6 Hz, 4H), 7.30 (t, J = 7.6 Hz, 2H), 7.40 (dd, J = 7.2, 7.6 Hz, 4H), 7.62 (d, J = 7.2 Hz, 4H), 7.78 (d, J = 8.6 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.6, 69.8, 70.7, 94.3, 95.9, 98.7, 107.5, 110.1,114.9, 123.9, 126.6, 127.6, 128.5, 136.4, 159.4, 159.5, 160.7, 161.1, 161.3, 177.4; LRMS (ESI) *m*/*z* 879 (M^+ + H, 7); HRMS (ESI) calcd for $C_{52}H_{47}O_{13}$ (M^+ + H) 879.3017, found 879.3032.

1,10-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10-tetraoxadecane (21c) and 9-[4′-((5-Benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-3,6,9,-trioxanonan-1-ol (23a). These compounds were prepared from **12a** (200 mg, 0.50 mmol), triethylene glycol ditosylate (140 mg, 0.33 mmol), K_2CO_3 (380 mg), and DMF (8 mL) by the general procedure for synthesis of flavonoid dimers. After crystallization from EtOAc, compound **21c** (96 mg, 42%) was obtained as a white solid: mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 6H), 3.77 (s, 4), 3.89 (t, J = 4.8 Hz, 4H), 4.17 (t, J = 4.8 Hz, 4H), 5.20 (s, 8H), 6.46 (d, J = 1.6 Hz, 2H), 6.56 (s, 2H), 6.72 (d, J = 1.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 4H), 7.30 (t, J = 7.2 Hz, 2H), 7.39 (t, J = 7.2 Hz, 4H), 7.62 (d, J = 7.2 Hz, 4H), 7.76 (d, J = 8.6 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.6, 70.6, 70.9, 94.3, 95.9, 98.7, 107.4, 110.1, 114.9, 123.8, 126.6, 127.5, 128.5, 136.4, 159.4, 159.5, 160.7, 161.2, 161.2, 177.3; LRMS (ESI) *m*/*z* 923 (M^+ + H, 18), 946 (M^+ + Na, 50); HRMS (ESI) calcd for $C_{54}H_{50}O_{14}Na$ (M^+ + Na) 945.3098, found 945.3103. Then the mother liquid was further evaporated and subjected to flash column chromatography with gradient elution (20% to 50% acetone in CH_2Cl_2) on silica gel (20 g) to furnish compound **23a** (56 mg, 21%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.64 (br s, 1H), 3.47 (s, 3H), 3.60 (t, J = 4.2 Hz, 2H), 3.67−3.73 (m, 6H), 3.86 (t, J = 4.7 Hz, 2H), 4.16 (t, J = 4.7 Hz, 2H), 5.20 (s, 2H), 5.22 (s, 2H), 6.48 (d, J = 2.0 Hz, 1H), 6.57 (s, 1H), 6.73 (d, J = 2.0 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 7.28 (t, J = 7.4 Hz, 1H), 7.37 (dd, J = 7.4, 7.8 Hz, 2H), 7.61 (d, J = 7.8 Hz, 2H), 7.77 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$)

δ 56.3, 61.6, 67.4, 69.4, 70.2, 70.6, 70.7, 72.4, 94.2, 95.9, 98.6, 107.4, 110.0, 114.8, 123.8, 126.5, 127.5, 127.5, 128.4, 136.3, 159.3, 159.5, 160.7, 161.1, 161.2, 177.3.

1,13-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13-pentaoxatridecane (21d) and 12-[4′-((5-Benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)-phenyl]-3,6,9,12-tetraoxadodecan-1-ol (23b). These compounds were prepared from **12a** (1.33 g, 3.3 mmol), tetraethylene glycol dimesylate (0.72 g, 2.1 mmol), K_2CO_3 (2.27 g), and DMF (30 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (2% MeOH in CH_2Cl_2) on silica gel (40 g), the titled compound **21d** (0.93 g, 58%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.44 (s, 6H), 3.67 (t, J = 1.6 Hz, 4H), 3.69 (t, J = 1.6 Hz, 4H), 3.83 (t, J = 4.4 Hz, 4H), 4.09 (t, J = 4.0 Hz, 4H), 5.15 (s, 8H), 6.42 (d, J = 1.8 Hz, 2H), 6.49 (s, 2H), 6.67 (d, J = 1.8 Hz, 2H), 6.92 (d, J = 8.8 Hz, 4H), 7.25 (t, J = 7.2 Hz, 2H), 7.36 (dd, J = 7.2, 7.6 Hz, 4H), 7.60 (d, J = 7.6 Hz, 4H), 7.71 (d, J = 8.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.5, 70.6, 70.6, 70.8, 94.3, 95.9, 98.6, 107.4, 110.0, 114.8, 123.7, 126.6, 127.5, 127.5, 128.5, 136.5, 159.3, 159.5, 160.6, 161.2, 177.2; LRMS (ESI) m/z 967 (M^+ + H, 18), 989 (M^+ + H, 100); HRMS (ESI) calcd for $C_{56}H_{55}O_{15}$ (M^+ + H) 967.3541, found 967.3568. The title compound **23b** (0.27 g, 14%) was obtained as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.00 (br s, 1H), 3.44 (s, 3H), 3.56 (t, J = 4.2 Hz, 2H), 3.62−3.69 (m, 10H), 3.82 (t, J = 4.5 Hz, 2H), 4.13 (t, J = 4.5 Hz, 2H), 5.17 (s, 2H), 5.18 (s, 2H), 6.44 (d, J = 1.8 Hz, 1H), 6.53 (s, 1H), 6.70 (d, J = 1.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.35 (dd, J = 7.4, 7.6 Hz, 2H), 7.59 (d, J = 7.6 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 56.2, 61.4, 67.3, 69.3, 70.0, 70.3, 70.4, 70.4, 70.6, 72.4, 94.1, 95.8, 98.5, 107.2, 109.9, 114.7, 123.6, 126.4, 127.4, 128.3, 136.2, 159.2, 159.3, 160.6, 161.0, 161.1, 177.2.

1,16-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16-hexaoxahexadecane (21e). This compound was prepared from **12a** (300 mg, 0.74 mmol), pentaethylene glycol dimesylate (170 mg, 0.43 mmol), K_2CO_3 (480 mg), and DMF (10 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (2% MeOH in CH_2Cl_2) on silica gel (15 g), the title compound (160 mg, 43%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.44 (s, 6H), 3.63−3.68 (m, 12H), 3.81 (t, J = 4.2 Hz, 4H), 4.09 (t, J = 4.2 Hz, 4H), 5.15 (s, 8H), 6.42 (d, J = 1.6 Hz, 2H), 6.49 (s, 2H), 6.67 (d, J = 1.6 Hz, 2H), 6.93 (d, J = 8.6 Hz, 4H), 7.26 (t, J = 6.8 Hz, 2H), 7.36 (dd, J = 6.8, 7.4 Hz, 4H), 7.59 (d, J = 7.4 Hz, 4H), 7.71 (d, J = 8.6 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.5, 70.5, 70.8, 94.3, 95.9, 98.6, 107.4, 110.0, 114.8, 123.7, 126.6, 127.5, 127.5, 128.5, 128.6, 136.4, 159.3, 159.5, 160.6, 161.2, 177.3; LRMS (ESI) m/z 1011 (M^+ + H, 4), 1033 (M^+ + Na, 26); HRMS (ESI) calcd for $C_{58}H_{59}O_{16}$ (M^+ + H) 1011.3803, found 1011.3793.

1,19-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19-heptaoxanonadecane (21f). This compound was prepared from **12a** (230 mg, 0.57 mmol), hexaethylene glycol dimesylate (160 mg, 0.37 mmol), K_2CO_3 (400 mg), and DMF (10 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (2% MeOH in CH_2Cl_2) on silica gel (15 g), the title compound (160 mg, 53%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 6H), 3.64−3.71 (m, 16H), 3.85 (t, J = 4.4 Hz, 4H), 4.15 (t, J = 4.4 Hz, 4H), 5.19 (s, 4H), 5.21 (s, 4H), 6.47 (d, J = 2.0 Hz, 2H), 6.54 (s, 2H), 6.72 (d, J = 2.0 Hz, 2H), 6.97 (d, J = 8.8 Hz, 4H), 7.27 (t, J = 7.2 Hz, 2H), 7.38 (dd, J = 7.2, 7.6 Hz, 4H), 7.61 (d, J = 7.6 Hz, 4H), 7.76 (d, J = 8.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.5, 70.5, 70.6, 70.6, 70.8, 94.3, 95.9, 98.7, 107.5, 110.1, 114.9, 123.8, 126.6, 127.5, 128.5, 136.4, 159.4, 159.5, 160.6, 161.2, 177.3; LRMS (ESI) m/z 1055 (M^+ + H, 11), 1077 (M^+ + Na, 47); HRMS (ESI) calcd for $C_{60}H_{62}O_{17}Na$ (M^+ + Na) 1077.3885, found 1077.3883.

1,22-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22-octaoxadocosane (21g), This compound was prepared from **12a** (220 mg, 0.54 mmol), heptaethylene glycol dimesylate (160 mg, 0.33 mmol), K_2CO_3 (370 mg), and DMF (10 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (4% MeOH in CH_2Cl_2) on silica gel (15 g), the title compound (160 mg, 54%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.44 (s, 6H), 3.61−3.69 (m, 20H), 3.82 (t, J = 4.2 Hz, 4H), 4.12 (t, J = 4.2 Hz, 4H), 5.17 (s, 4H), 5.18 (s, 4H), 6.44 (d, J = 1.6 Hz, 2H), 6.51 (s, 2H), 6.69 (d, J = 1.6 Hz, 2H), 6.94 (d, J = 8.6 Hz, 4H), 7.25 (t, J = 6.8 Hz, 2H), 7.36 (dd, J = 6.8, 7.0 Hz, 4H), 7.60 (d, J = 7.0 Hz, 4H), 7.34 (d, J = 8.6 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.5, 70.5, 70.5, 70.8, 94.3, 95.9, 98.6, 107.4, 110.1, 114.8, 123.7, 126.6, 127.5, 128.5, 128.7, 136.4, 159.3, 159.5, 160.6, 161.2, 177.3; LRMS (ESI) m/z 1099 (M^+ + H, 7), 1121 (M^+ + Na, 31); HRMS (ESI) calcd for $C_{62}H_{66}O_{18}Na$ (M^+ + Na) 1121.4147, found 1121.4132.

1,25-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25-nonaoxapentacosane (21h). This compound was prepared from **12a** (250 mg, 0.62 mmol), octaethylene glycol dimesylate (200 mg, 0.38 mmol), K_2CO_3 (420 mg), and DMF (10 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (4% MeOH in CH_2Cl_2) on silica gel (15 g), the title compound (170 mg, 48%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.43 (s, 6H), 3.59−3.67 (m, 24 H), 3.80 (t, J = 4.8 Hz, 4H), 4.10 (t, J = 4.8 Hz, 4H), 5.15 (s, 4H), 5.16 (s, 4H), 6.43 (d, J = 2.0 Hz, 2H), 6.50 (s, 2H), 6.68 (d, J = 2.0 Hz, 2H), 6.92 (d, J = 9.2 Hz, 4H), 7.25 (t, J = 7.6 Hz, 2H), 7.34 (dd, J = 7.6, 7.2 Hz, 4H), 7.59 (d, J = 7.2 Hz, 4H), 7.72 (d, J = 9.2 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.5, 69.4, 70.5, 70.5, 70.8, 94.3, 95.9, 98.6, 107.4, 110.1, 114.8, 123.7, 126.5, 127.5, 128.5, 136.4, 159.3, 159.5, 160.6, 161.2, 161.2, 177.2; LRMS (ESI) m/z 1144 (M^+ + H, 3), 1166 (M^+ + Na, 21); HRMS (ESI) calcd for $C_{64}H_{70}O_{19}Na$ (M^+ + Na) 1165.4409, found 1165.4424.

1,28-Bis[4′-((5-benzyloxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25,28-decaoxaoctacosane (21i). This compound was prepared from **12a** (240 mg, 0.59 mmol), nonaethylene glycol dimesylate (210 mg, 0.37 mmol), K_2CO_3 (410 mg), and DMF (10 mL) by the general procedure for synthesis of flavonoid dimers. After flash column chromatography (4% MeOH in CH_2Cl_2) on silica gel (15 g), the title compound (180 mg, 51%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.42 (s, 6H), 3.58−3.66 (m, 28H), 3.80 (t, J = 4.6 Hz, 4H), 4.10 (t, J = 4.6 Hz, 4H), 5.14 (s, 4H), 5.15 (s, 4H), 6.42 (d, J = 2.0 Hz, 2H), 6.48 (s, 2H), 6.67 (d, J = 2.0 Hz, 2H), 6.91 (d, J = 8.8 Hz, 4H), 7.23 (t, J = 7.6 Hz, 2H), 7.33 (t, J = 7.6 Hz, 4H), 7.59 (d, J = 7.6 Hz, 4H), 7.71 (d, J = 8.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 56.3, 67.5, 69.4, 70.5, 70.5, 70.8, 94.2, 95.9, 98.6, 107.4, 110.1, 114.8, 123.7, 126.5, 127.5, 128.5, 136.4, 159.3, 159.5, 160.6, 161.2, 161.2, 177.2; LRMS (ESI) m/z 1188 (M^+ + H, 3), 1210 (M^+ + Na, 23); HRMS (ESI) calcd for $C_{66}H_{75}O_{20}$ (M^+ + H) 1187.4852, found 1187.4825.

General Procedure for Hydrogenolysis of Compounds 21a−i and 23a,b. A round-bottom flask was charged with compound **21** or **23**, a catalytic amount of 10% Pd on activated charcoal, and chloroform. The reaction mixture was stirred vigorously under hydrogen atmosphere at balloon pressure and room temperature for 12 h. When TLC indicated complete consumption of the starting material, the charcoal was removed by suction filtration. The pale yellow filtrate was purified by passage through a short pad of silica gel to furnish the deprotected products.

1,4-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4-dioxabutane (22a). This compound was prepared from **21a** (64 mg, 0.08 mmol), 10% Pd on charcoal (15 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis described above. The title compound (43 mg, 86%) was obtained as a white solid: mp 206−207 °C; ^{1}H NMR ($CDCl_3$) δ 3.51 (s, 6H), 4.44 (s, 4H), 5.24 (s, 4H), 6.47 (d, J = 2.0 Hz, 2H), 6.59 (s, 2H), 6.66 (d, J = 2.0 Hz, 2H), 7.07 (d, J = 8.8 Hz, 4H), 7.86 (d, J = 8.8 Hz, 4H), 12.73 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.4, 66.5, 94.2, 94.3, 100.1, 104.5, 106.2, 115.1, 124.0, 128.1, 157.5, 161.5, 162.0, 162.9, 163.9, 182.5; LRMS (ESI) m/z 655 (M^+ + H, 14),

677 (M^+ + Na, 8); HRMS (ESI) calcd for $C_{36}H_{31}O_{12}$ (M^+ + H) 655.1816, found 655.1845.

1,7-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7-trioxaheptane (22b). This compound was prepared from **21b** (88 mg, 0.10 mmol), 10% Pd on charcoal (18 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (60 mg, 86%) was obtained as a white solid: mp 171−172 °C; ^{1}H NMR ($CDCl_3$) δ 3.50 (s, 6H), 3.98 (t, J = 4.4 Hz, 4H), 4.24 (t, J = 4.4 Hz, 4H), 5.23 (s, 4H), 6.44 (d, J = 1.6 Hz, 2H), 6.55 (s, 2H), 6.62 (d, J = 1.6 Hz, 2H), 7.00 (d, J = 9.0 Hz, 4H), 7.79 (d, J = 9.0 Hz, 4H), 12.63 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 56.5, 68.0, 69.3, 94.3, 94.9, 99.8, 104.1, 105.6, 115.5, 123.0, 128.8, 157.4, 161.5, 162.1, 162.9, 164.1, 182.4; LRMS (ESI) *m*/*z* 699 (M^+ + H, 5), 721 (M^+ + Na, 3); HRMS (ESI) calcd for $C_{38}H_{35}O_{13}$ (M^+ + H) 699.2078, found 699.2079.

1,10-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10-tetraoxadecane (22c). This compound was prepared from **21c** (96 mg, 0.10 mmol), 10% Pd on charcoal (15 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (62 mg, 80%) was obtained as a pale yellow solid: mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 6H), 3.62 (d, J = 4.0 Hz, 4H), 3.76 (t, J = 4.6 Hz, 4H), 4.17 (t, J = 4.6 Hz, 4H), 5.28 (s, 4H), 6.37 (d, J = 2.0 Hz, 2H), 6.76 (d, J = 2.0 Hz, 2H), 6.87 (s, 2H), 7.06 (d, J = 8.8 Hz, 4H), 7.96 (d, J = 8.8 Hz, 4H), 12.85 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 56.3, 67.8, 69.0, 70.3, 94.2, 94.7, 99.6, 103.9, 105.4, 115.2, 122.8, 128.6, 157.2, 161.4, 161.9, 162.7, 163.8, 182.2; LRMS (ESI) *m*/*z* 743 (M^+ + H, 9); HRMS (ESI) calcd for $C_{40}H_{39}O_{14}$ (M^+ + H) 743.2340, found 743.2343.

1,13-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13-pentaoxatridecane (22d). This compound was prepared from **21d** (930 mg, 0.96 mmol), 10% Pd on charcoal (88 mg), and chloroform (20 mL) by the general procedure for hydrogenolysis. The title compound (710 mg, 94%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 6H), 3.55−3.59 (m, 8H), 3.76 (t, J = 4.6 Hz, 4H), 4.13 (d, J = 4.6 Hz, 4H), 5.28 (s, 4H), 6.37 (d, J = 2.0 Hz, 2H), 6.75 (d, J = 2.0 Hz, 2H), 6.85 (s, 2H), 7.04 (d, J = 8.8 Hz, 4H), 7.95 (d, J = 8.8 Hz, 4H), 12.84 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 56.3, 56.3, 67.8, 69.0, 70.2, 94.2, 94.7, 99.6, 103.9, 105.4, 115.2, 122.8, 128.6, 157.1, 161.4, 161.9, 162.7, 163.8, 182.2; LRMS (ESI) *m*/*z* 787 (M^+ + H, 57), 809 (M^+ + Na, 60); HRMS (ESI) calcd for $C_{42}H_{43}O_{15}$ (M^+ + H) 787.2602, found 787.2591.

1,16-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16-hexaoxahexadecane (22e). This compound was prepared from **21e** (75 mg, 0.07 mmol), 10% Pd on charcoal (12 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (52 mg, 84%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 6H), 3.66-3.73 (m, 12H), 3.87 (t, J = 4.6 Hz, 4H), 4.17 (t, J = 4.6 Hz, 4H), 5.22 (s, 4H), 6.42 (d, J = 2.0 Hz, 2H), 6.52 (s, 2H), 6.61 (d, J = 2.0 Hz, 2H), 6.97 (d, J = 9.0 Hz, 4H), 7.77 (d, J = 9.0 Hz, 4H), 12.72 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.4, 67.0, 67.6, 69.5, 70.6, 70.8, 94.1, 94.2, 100.0, 104.2, 106.1, 115.0, 123.4, 127.9, 157.4, 161.7, 161.9, 162.8, 163.9, 182.4; LRMS (ESI) *m*/*z* 831 (M^+ + H, 35), 853 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{44}H_{46}O_{16}$-Na (M^+ + Na) 853.2684, found 853.2677.

1,19-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19-heptaoxanonadecane (22f). This compound was prepared from **21f** (76 mg, 0.07 mmol), 10% Pd on charcoal (19 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (52 mg, 83%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 6H), 3.64−3.72 (m, 16H), 3.85 (t, J = 4.6 Hz, 4H), 4.15 (t, J = 4.6 Hz, 4H), 5.20 (s, 4H), 6.40 (d, J = 2.0 Hz, 2H), 6.49 (s, 2H), 6.58 (d, J = 2.0 Hz, 2H), 6.95 (d, J = 8.8 Hz, 4H), 7.74 (d, J = 8.8 Hz, 4H), 12.70 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.3, 66.9, 67.5, 69.4, 70.4, 70.5, 70.7, 94.1, 94.1, 99.9, 104.1, 106.0, 114.9, 123.3, 127.8, 157.3, 161.7, 161.8, 162.7, 163.8, 182.3; LRMS (ESI) *m*/*z* 875 (M^+ + H, 28), 897 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{46}H_{50}O_{17}$-Na (M^+ + Na) 897.2946, found 897.2936;

1,22-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22-octaoxadocosane (22g). This compound was prepared from **21g** (102 mg, 0.09 mmol), 10% Pd on charcoal (21 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (78 mg, 91%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 6H), 3.61−3.70 (m, 20H), 3.84 (t, J = 4.6 Hz, 4H), 4.12 (t, J = 4.6 Hz, 4H), 5.18 (s, 4H), 6.38 (d, J = 2.0 Hz, 2H), 6.47 (s, 2H), 6.56 (d, J = 2.0 Hz, 2H), 6.93 (d, J = 9.0 Hz, 4H), 7.72 (d, J = 9.0 Hz, 4H), 12.70 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.3, 67.6, 69.4, 70.5, 70.5, 70.8, 94.1, 94.2, 99.9, 104.1, 106.0, 114.9, 123.3, 127.9, 157.4, 161.8, 161.8, 162.8, 163.9, 182.3; LRMS (ESI) *m*/*z* 919 (M^+ + H, 5), 941 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{48}H_{54}O_{18}Na$ (M^+ + Na) 941.3208, found 941.3188.

1,25-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25-nonaoxapentacosane (22h). This compound was prepared from **21h** (89 mg, 0.08 mmol), 10% Pd on charcoal (16 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (62 mg, 83%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.50 (s, 6H), 3.68−3.75 (m, 24H), 3.87 (t, J = 4.6 Hz, 4H), 4.18 (t, J = 4.6 Hz, 4H), 5.23 (s, 4H), 6.44 (d, J = 2.0 Hz, 2H), 6.54 (s, 2H), 6.62 (d, J = 2.0 Hz, 2H), 6.98 (d, J = 8.8 Hz, 4H), 7.78 (d, J = 8.8 Hz, 4H), 12.72 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.4, 56.4, 67.6, 69.5, 70.2, 70.3, 70.4, 70.7, 94.2, 94.3, 100.0, 104.3, 106.1, 115.0, 123.5, 128.0, 157.4, 161.7, 161.9, 162.9, 163.9, 182.4; LRMS (ESI) *m*/*z* 963 (M^+ + H, 50), 985 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{50}H_{59}O_{19}$ (M^+ + H) 963.3651, found 963.3637.

1,28-Bis[4′-((5-hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25,28-decaoxaoctacosane (22i). This compound was prepared from flavone **21i** (120 mg, 0.10 mmol), 10% Pd on charcoal (28 mg), and chloroform (10 mL) by the general procedure for hydrogenation of bisflavones. The title compound (92 mg, 90%) was obtained as a white foam: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 6H), 3.53−3.63 (m, 28H), 3.77 (t, J = 4.6 Hz, 4H), 4.04 (t, J = 4.6 Hz, 4H), 5.11 (s, 4H), 6.28 (d, J = 1.8 Hz, 2H), 6.37 (s, 2H), 6.47 (d, J = 1.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 4H), 7.62 (d, J = 8.8 Hz, 4H), 12.63 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 56.0, 57.2, 69.1, 70.1, 70.2, 70.4, 93.8, 93.8, 99.5, 103.6, 105.6, 114.6, 122.8, 127.5, 156.9, 161.4, 161.5, 162.4, 163.5, 181.9; LRMS (ESI) *m*/*z* 1007 (M^+ + H, 10), 1029 (M^+ + Na, 58); HRMS (ESI) calcd for $C_{52}H_{62}O_{20}Na$ (M^+ + Na) 1029.3732, found 1029.3696.

9-[4′-((5-Hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-3,6,9-trioxanonan-1-ol (**24a**). This compound was prepared from **23a** (48 mg, 0.09 mmol), 10% Pd on charcoal (8 mg), and chloroform (10 mL) by the general procedure for hydrogenolysis. The title compound (32 mg, 80%) was obtained as a pale yellow solid: mp 57−59 °C; ^{1}H NMR ($CDCl_3$) δ 3.49 (s, 3H), 3.62 (t, J = 4.2 Hz, 2H), 3.70−3.75 (m, 6H), 3.89 (t, J = 4.7 Hz, 2H), 4.20 (t, J = 4.7 Hz, 2H), 5.23 (s, 2H), 6.45 (d, J = 2.0 Hz, 1H), 6.56 (s, 1H), 6.64 (d, J = 2.0 Hz, 1H), 7.00 (d, J = 8.8 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 56.2, 61.5, 67.4, 69.3, 70.1, 70.7, 72.3, 94.0, 94.1, 99.9, 104.1, 106.0, 114.8, 123.4, 127.8, 157.3, 161.6, 161.8, 162.3, 163.9, 182.3; LRMS (EI) *m*/*z* 446 (M^+, 100); HRMS (EI) calcd for $C_{23}H_{26}O_9$ (M^+) 446.1577, found 446.1570.

12-[4′-((5-Hydroxy-7-methoxymethoxy)-4*H*-chromen-4-on-2-yl)phenyl]-3,6,9,12-tetraoxadodecan-1-ol (**24b**). This compound was prepared from **23b** (150 mg, 0.26 mmol), 10% Pd on charcoal (22 mg), and chloroform (20 mL) by the general procedure for hydrogenolysis. The title compound (122 mg, 96%) was obtained as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.44 (s, 3H), 3.56 (t, J = 4.2 Hz, 2H), 3.62−3.69 (m, 10H), 3.82 (t, J = 4.5 Hz, 2H), 4.13 (t, J = 4.5 Hz, 2H), 5.18 (s, 2H), 6.44 (d, J = 1.8 Hz, 1H), 6.53 (s, 1H), 6.70 (d, J = 1.8 Hz, 1H), 6.94 (d, J = 8.8 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 56.2, 61.4, 67.3, 69.3, 70.0, 70.3, 70.4, 70.4, 70.6, 72.4, 94.1, 95.8, 98.5, 107.2, 109.9, 114.7, 123.6, 126.4, 127.4, 128.3, 136.2, 159.2, 159.3, 160.6, 161.0, 161.1, 177.2; LRMS (EI) *m*/*z* 490 (M^+, 100); HRMS (EI) calcd for $C_{25}H_{30}O_{10}$ (M^+) 490.1839, found 490.1828.

General Procedure for Deprotection of MOM Group of 22a−i: Method A. A round-bottom flask was charged with compound **22** and 75% AcOH. The reaction mixture was stirred at refluxing temperature for 14 h. When TLC indicated complete consumption of **22**, the reaction mixture was cooled to 0 °C and ice water was added. The off-white solid that was formed was collected by suction filtration.

General Procedure for Deprotection of MOM Group of 22a−i: Method B. A round-bottom flask was charged with compound **22**, 6 M HCl solution, and THF. The reaction mixture was stirred at room temperature for 15 min. When TLC indicated complete consumption of **22**, the reaction mixture was poured into a separating funnel containing water. The mixture was extracted with EtOAc (20 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give a crude mixture. Purification of the crude mixture by passage through a short pad of silica gel furnished the desired product.

1,4-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4-dioxabutane (9a). This compound was prepared from compound **22a** (43 mg, 0.07 mmol) and 75% acetic acid (20 mL) by method A. The title compound (26 mg, 70%) was obtained as a pale green solid: mp 352-355 °C; ^{1}H NMR (DMSO-d_6) δ 4.46 (s, 4H), 6.19 (d, J = 1.6 Hz, 2H), 6.50 (d, J = 1.6 Hz, 2H), 6.88 (s, 2H), 7.17 (d, J = 8.4 Hz, 4H), 8.04 (d, J = 8.4 Hz, 4H), 10.85 (s, 2H), 12.90 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 67.0, 94.5, 99.3, 104.1, 104.2, 115.5, 123.4, 128.8, 157.8, 161.7, 161.9, 163.6, 164.7, 182.2; LRMS (EI) *m*/*z* 566 (M^+, 11); HRMS (ESI) calcd for $C_{32}H_{23}O_{10}$ (M^+ + H) 567.1291, found 567.1268.

1,7-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7-trioxaheptane (9b). This compound was prepared from compound **22b** (57 mg, 0.08 mmol) and 75% acetic acid (25 mL) by method A. The title compound (42 mg, 84%) was obtained as an off-white solid: mp 268−270 °C; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 4H), 4.22 (s, 4H), 6.16 (d, J = 1.8 Hz, 2H), 6.46 (d, J = 1.8 Hz, 2H), 6.84 (s, 2H), 7.09 (d, J = 8.8 Hz, 4H), 7.98 (d, J = 8.8 Hz, 4H), 10.82 (s, 2H), 12.98 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 68.0, 69.3, 94.4, 99.3, 103.9, 104.2, 115.4, 123.3, 128.7, 157.7, 161.9, 161.9, 163.6, 164.6, 182.2; LRMS *m*/*z* 611 (M^+ + H, 8), 633 (M^+ + Na, 3); HRMS calcd for $C_{34}H_{27}O_{11}$ (M^+ + H) 611.1553, found 611.1542.

1,10-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10-tetraoxadecane (9c). This compound was prepared from compound **22c** (62 mg, 0.08 mmol) and 75% acetic acid (25 mL) by method A. The title compound (43 mg, 79%) was obtained as a pale yellow solid: mp 143−145 °C; ^{1}H NMR (DMSO-d_6) δ 3.81 (s, 4H), 3.95 (s, 4H), 4.36 (s, 4H), 6.35 (d, J = 1.0 Hz, 2H), 6.64 (d, J = 1.0 Hz, 2H), 7.01 (s, 2H), 7.27 (d, J = 8.8 Hz, 4H), 8.16 (d, J = 8.8 Hz, 4H), 11.00 (s, 2H), 13.08 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 67.8, 69.0, 70.2, 94.2, 99.1, 103.7, 104.0, 115.2, 123.0, 128.5, 157.5, 161.7, 161.8, 163.4, 164.4, 182.0; LRMS (ESI) *m*/*z* 655 (M^+ + H, 15); HRMS (ESI) calcd for $C_{36}H_{31}O_{12}$ (M^+ + H) 655.1816, found 655.1816.

1,13-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13-pentaoxatridecane (9d). This compound was prepared from compound **22d** (720 mg, 0.92 mmol), 6 M HCl solution (70 mL), and THF (50 mL) by method B. The title compound (620 mg, 97%) was obtained as a pale yellow solid: mp 131−133 °C; ^{1}H NMR (DMSO-d_6) δ 3.54−3.58 (m, 8H), 3.75 (t, J = 4.4 Hz, 4H), 4.15 (t, J = 4.4 Hz, 4H), 6.16 (d, J = 2.0 Hz, 2H), 6.45 (d, J = 2.0 Hz, 2H), 6.81 (s, 2H), 7.07 (d, J = 8.8 Hz, 4H), 7.96 (d, J = 8.8 Hz, 4H), 10.81 (s, 2H), 12.88 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 68.0, 69.2, 70.3, 70.4, 94.4, 99.3, 103.9, 104.2, 115.4, 123.2, 128.7, 157.7, 161.8, 161.9, 163.6, 164.6, 182.2; LRMS (ESI) *m*/*z* 699 (M^+ + H, 33), 721 (M^+ + Na, 58); HRMS (ESI) calcd for $C_{38}H_{35}O_{13}Na$ (M^+ + Na) 721.1897, found 721.1896.

1,16-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16-hexaoxahexadecane (9e). This compound was prepared from compound **22e** (48 mg, 0.06 mmol), 6 M HCl solution (20 mL), and THF (20 mL) by method B. The title compound (37 mg, 86%) was obtained as a pale yellow foam; ^{1}H NMR (acetone-d_6) δ 3.59−3.65 (m, 12H), 3.83 (t, J = 4.6 Hz, 4H), 4.20 (t, J = 4.6 Hz, 4H), 6.22 (d, J = 2.0 Hz, 2H), 6.51 (d, J = 2.0 Hz, 2H), 6.63 (s, 2H), 7.09 (d, J = 8.8 Hz, 4H), 7.95 (d, J = 8.8 Hz, 4H), 12.90 (s, 2H); ^{13}C NMR (acetone-d_6) δ 67.8, 69.2, 70.4, 70.5, 93.8, 98.8, 103.6, 104.4, 115.0, 123.4, 128.1, 157.8, 162.0, 164.0, 164.6, 182.2; LRMS (ESI) *m*/*z* 743 (M^+ + H, 34), 765 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{40}H_{38}O_{14}Na$ (M^+ + Na) 765.2159, found 765.2164.

1,19-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19-heptaoxanonadecane (9f). This compound was prepared from compound **22f** (45 mg, 0.05 mmol), 6M HCl solution (20 mL), and THF (20 mL) by method B. The title compound (36 mg, 89%) was obtained as a pale yellow foam: ^{1}H NMR (acetone-d_6) δ 3.56−3.65 (m, 16H), 3.81 (t, J = 4.6 Hz, 4H), 4.17 (t, J = 4.6 Hz, 4H), 6.22 (d, J = 2.0 Hz, 2H), 6.48 (d, J = 2.0 Hz, 2H), 6.57 (s, 2H), 7.02 (d, J = 8.8 Hz, 4H), 7.88 (d, J = 8.8 Hz, 4H), 12.88 (s, 2H); ^{13}C NMR (acetone-d_6) δ 67.7, 69.2, 70.3, 70.3, 70.5, 93.9, 98.8, 103.5, 104.4, 114.9, 123.3, 128.0, 157.6, 162.0, 162.3, 163.6, 163.9, 182.0; LRMS (ESI) *m*/*z* 809 (M^+ + Na, 15); HRMS (ESI) calcd for $C_{42}H_{43}O_{15}$ (M^+ + H) 787.2602, found 787.2614.

1,22-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22-octaoxadocosane (9g). This compound was prepared from compound **22g** (65 mg, 0.07 mmol), 6 M HCl solution (20 mL), and THF (20 mL) by method B. The title compound (58 mg, 99%) was obtained as a pale yellow foam: ^{1}H NMR (acetone-d_6) δ 3.54−3.65 (m, 20H), 3.81 (t, J = 4.6 Hz, 4H), 4.18 (t, J = 4.6 Hz, 4H), 6.23 (d, J = 2.0 Hz, 2H), 6.49 (d, J = 2.0 Hz, 2H), 6.59 (s, 2H), 7.04 (d, J = 9.0 Hz, 4H), 7.90 (d, J = 9.0 Hz, 4H), 12.90 (s, 2H); ^{13}C NMR (acetone-d_6) δ 67.7, 69.2, 70.3, 70.3, 70.5, 93.9, 98.8, 103.6, 104.4, 114.9, 123.3, 128.0, 157.8, 162.0, 162.0, 163.6, 163.9, 182.0; LRMS (ESI) *m*/*z* 853 (M^+ + Na, 36); HRMS (ESI) calcd for $C_{44}H_{47}O_{16}$ (M^+ + H) 831.2864, found 831.2889.

1,25-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25-nonaoxapentacosane (9h). This compound was prepared from compound **22h** (50 mg, 0.05 mmol), 6 M HCl solution (20 mL), and THF (20 mL) by method B. The title compound (42 mg, 92%) was obtained as a pale yellow foam: ^{1}H NMR (acetone-d_6) δ 3.53−3.65 (m, 24H), 3.83 (t, J = 4.6 Hz, 4H), 4.19 (t, J = 4.6 Hz, 4H), 6.23 (d, J = 2.0 Hz, 2H), 6.51 (d, J = 2.0 Hz, 2H), 6.62 (s, 2H), 7.07 (d, J = 9.0 Hz, 4H), 7.94 (d, J = 9.0 Hz, 4H), 12.88 (s, 2H); ^{13}C NMR (acetone-d_6) δ 67.8, 69.2, 70.3, 70.3, 70.5, 93.9, 98.8, 103.6, 104.4, 115.0, 123.3, 128.0, 157.6, 162.0, 162.3, 163.7, 163.9, 182.0; LRMS (ESI) *m*/*z* 875 (M^+ + H, 3), 897 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{46}H_{51}O_{17}$ (M^+ + H) 875.3126, found 875.3145.

1,28-Bis[4′-((5,7-dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-1,4,7,10,13,16,19,22,25,28-decaoxaoctacosane (9i). This compound was prepared from compound **22i** (78 mg, 0.08 mmol), 6 M HCl solution (20 mL), and THF (20 mL) by method B. The title compound (69 mg, 97%) was obtained as a pale yellow oil: ^{1}H NMR (acetone-d_6) δ 3.53-3.64 (m, 28H), 3.80 (t, J = 4.6 Hz, 4H), 4.15 (t, J = 4.6 Hz, 4H), 6.23 (d, J = 2.0 Hz, 2H), 6.48 (d, J = 2.0 Hz, 2H), 6.57 (s, 2H), 7.02 (d, J = 8.8 Hz, 4H), 7.88 (d, J = 8.8 Hz, 4H), 12.94 (s, 2H); ^{13}C NMR (acetone-d_6) δ 67.7, 69.2, 70.3, 70.3, 70.5, 93.9, 98.9, 103.5, 104.4, 114.9, 123.2, 128.0, 157.7, 162.0, 162.3, 163.6, 164.0, 182.0; LRMS (ESI) *m*/*z* 919 (M^+ + H, 4), 941 (M^+ + Na, 100); HRMS (ESI) calcd for $C_{48}H_{55}O_{18}$ (M^+ + H) 919.3388, found 919.3399.

9-[4′-((5,7-Dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-3,6,9,-trioxanonan-1-ol (10a). This compound was prepared from compound **24a** (28 mg, 0.06 mmol), 6 M HCl solution (10 mL), and THF (10 mL) by method B. The title compound (19 mg, 75%) was obtained as a pale yellow solid: mp 135−137 °C; ^{1}H NMR (DMSO-d_6) δ 3.40 (t, J = 4.8 Hz, 2H), 3.45−3.59 (m, 6H), 3.75 (t, J = 4.4 Hz, 2H), 4.18 (t, J = 4.4 Hz, 2H), 4.57 (t, J = 5.2 Hz, 1H), 6.18 (d, J = 2.0 Hz, 1H), 6.48 (d, J = 2.0 Hz, 1H), 6.86 (s, 1H), 7.10 (d, J = 8.8 Hz, 2H), 8.00 (d, J = 8.8 Hz, 2H), 10.85 (br s, 1H), 12.91 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 60.6, 68.0, 69.2, 70.2, 70.4, 72.8, 94.4, 99.3, 103.9, 104.2, 115.4, 123.2, 128.7, 157.7, 161.8, 162.0, 163.7, 164.6, 182.2; LRMS (EI) *m*/*z* 402 (M^+, 100); HRMS (EI) calcd for $C_{21}H_{22}O_8$ (M^+) 402.1315, found 402.1297.

12-[4′-((5,7-Dihydroxy)-4*H*-chromen-4-on-2-yl)phenyl]-3,6,9,-12-tetraoxadodecan-1-ol (**10b**). This compound was prepared from compound **24b** (80 mg, 0.16 mmol), 6 M HCl solution (10 mL), and THF (10 mL) by method B. The title compound (65 mg, 89%) was obtained as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 3.61 (t, J = 4.1 Hz, 2H), 3.68−3.75 (m, 10H), 3.84 (t, J = 4.4 Hz, 2H), 4.05 (t, J = 4.4 Hz, 2H), 6.21 (d, J = 2.0 Hz, 1H), 6.28 (d, J = 2.0 Hz, 1H), 6.35 (s, 1H), 6.74 (d, J = 8.8 Hz, 2H), 7.52 (d, J = 8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 61.4, 67.2, 69.4, 69.8, 70.4, 70.4, 70.4, 72.2, 94.2, 99.4, 103.1, 104.4, 114.4, 122.7, 127.3, 157.3, 161.2, 161.5, 163.3, 181.9; LRMS (EI) *m*/*z* 446 (M^+, 97); HRMS (EI) calcd for $C_{23}H_{26}O_9$ (M^+) 446.1577, found 446.1574.

Materials for Biological Studies. DMSO, VP ($C_{27}H_{38}N_2O_4$), DOX ($C_{27}H_{29}NO_{11}$), daunorubicin ($C_{27}H_{29}NO_{10}$), vinblastine ($C_{46}H_{58}N_4O_9$), paclitaxel ($C_{47}H_{51}NO_{14}$), and mitoxantrone ($C_{22}H_{28}N_4O_6$) were purchased from Sigma−Aldrich. Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, trypsin−ethylenediaminetetraacetic acid (EDTA), and penicillin/streptomycin were from Gibco BRL. Fetal bovine serum (FBS) was from HyClone Laboratories. 3-(4,5-Dimethylthiazol-2-yl)-5-[3-(carboxymethoxy)phenyl]-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS), phenazine methosulfate (PMS), and Pgp-Glo assay system with P-glycoprotein were purchased from Promega. Human breast cancer cell lines MDA435/LCC6 and MDA435/LCC6 MDR were kindly provided by Dr. Robert Clarke (Georgetown University, Washington, DC). Murine leukemia cell lines P388 and P388/ADR were obtained from the National Cancer Institute (Bethesda, MD).

Cell Culture. MDA435/LCC6 and P388 (both parent and MDR subtype) were maintained in DMEM and RPMI 1640 culture medium supplemented with 10% FBS, respectively. RPMI 1640 medium also contained 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were cultured at 37 °C in a humidified atmosphere with 5% CO_2. A solution of 0.05% trypsin−EDTA was used to detach the MDA435/ LCC6 (both wild-type and MDR subtype) cells.

Cell Proliferation Assay. MDA435/LCC6 and P388 (both parent and MDR subtype) cells were seeded at 2000 and 5000 cells/well, respectively, in 96-well plates. Varying concentrations of anticancer drugs (DOX, daunorubicin, vincristine, vinblastine, paclitaxel, and mitoxantrone) with or without the flavonoid dimers were added in a final volume of 200 μL and cells were grown for 4 and 3 days for MDA435/LCC6 and P388 (both parent and MDR subtype) cells, respectively. For MDA435/LCC6 (both parent and MDR subtype), the corresponding drugs were added after cell attachment (24 h incubation). To measure cell proliferation, the Cell Titer 96 aqueous assay (Promega) was used according to the manufacturer's instructions. Briefly, MTS (2 mg/mL) and PMS (0.92 mg/ml) were mixed in a ratio of 20:1. An aliquot (30 μL) of the MTS/PMS mixture was added into each well, and the plate was incubated for 2 h at 37 °C. Optical absorbance at 490 nm was then recorded with an enzyme-linked immunosorbent assay (ELISA) microtiter plate reader (Bio-Rad). Each experiment was done at least in triplicate and repeated twice. Cytotoxicity of the anticancer drugs was expressed as the fraction of the cells that survived relative to the untreated DMSO (0.05%) solvent controls. IC_{50} or IC_{60} of the anticancer drugs was expressed as the concentration of the drugs inhibiting cell growth by 50% or 60%, respectively.

DOX Accumulation. Aliquots (2.5 mL, 10^5 cells/mL) of MDA435/LCC6 (both parent and MDR subtype) cells were seeded in each well of 6-well plates. At confluence, culture medium was removed. Fresh DMEM with modulators (2 mL) was added, and cells were incubated for 30 min at 37 °C. DOX (final concentration of 20 μM) was then added and the mixture was incubated for 2 h at 37 °C. The cells were then harvested by trypsinization.

For P388 (both parent and MDR subtype) cells, 1 mL (10^5 cells) of confluent cells was aliquotted into an Eppendorf tube and preincubated with flavone dimers for 30 min at 37 °C. DOX (final concentration of 10 μM) was then added and the mixture was incubated for 2 h at 37 °C.The cell pellets were washed three times with cold phosphate-buffered saline (PBS) by using an Eppendorf microcentrifuge, lysed with 0.3 N HCl in 50% ethanol, and sonicated for 30 s. After centrifugation at 10 000 rpm for 3 min, the supernatant was saved. The fluorescence of DOX is measured on a spectrofluorometer (λ_{excite} = 470 nm, λ_{emit} = 585 nm).

ATPase Assay. P-gp ATPase activity was measured with the Pgp-Glo assay system with human P-gp membrane by following the manufacturer's instructions. The assay relies on the ATP dependence of the light-generating reaction of firefly luciferase. Briefly, 25 μg of P-gp membrane was incubated at 37 °C with either Na_3VO_4 (100 μM), solvent control (0.1% DMSO), **9d** (100 μM), VP (100 μM), or VP (100 μM) plus **9d** (100 μM). The ATPase reaction was initiated by addition of 5 mM MgATP and followed by incubation for 40 min at 37 °C. The reaction was stopped, and the remaining unmetabolized ATP was detected as a luciferase-generated luminescence signal by addition of ATP detection reagent. Following a 20 min room-temperature signal-stabilization period, luminescence was read on a BMG Fluostar plate luminometer. P-gp ATPase activity was presented as a drop in luminescence of samples compared to that treated with Na_3VO_4.

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Supporting Information Available: Proton and carbon NMR spectra of compounds **9a**−**i**, **10a**,**b**, **12a**, and **18**−**24**; HPLC chromatograms of **9d**; and details for synthesis of **9a** according to Scheme 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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