Increased Anti-P-glycoprotein Activity of Baicalein by Alkylation on the A Ring

Yashang Lee,[†,||] Hosup Yeo,[†,‡,||] Shwu-Huey Liu,[§] Zaoli Jiang,[§] Ruben M. Savizky,[‡] David J. Austin,[‡] and Yung-chi Cheng*[,†]

Department of Pharmacology, Yale University School of Medicine, Department of Chemistry, Yale University, and PhytoCeutica, Inc., New Haven, Connecticut 06520

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The aqueous extract of *Scutellariae baicalensis Georgi* has inhibitory activity against P-gp 170, a multiple drug resistant gene product. Baicalein, one of the major flavones, was found to be responsible for this activity. The hydroxyl groups of the A ring of baicalein were systematically alkylated in order to assess the effect of such modifications on the activity against P-gp 170. The impact of the baicalein modifications on activity against the growth of a human nasopharyngeal cancer cell line KB and its P-gp 170 overexpressing cell line KB/MDR were also examined. The results indicate that alkylation of R5 of baicalein does not have a major impact on the interaction with P-gp 170, whereas alkylation of R6 or R7 alone or both, could enhance the interaction of baicalein with P-gp 170 as well as the amount of intracellular accumulation of vinblastine, a surrogate marker for the activity of P-gp 170 pump of KB/MDR cells. In this case, the optimal linear alkyl functionality is a propyl side chain. These modifications could also alter the activity of compounds inhibiting cell growth. Among the different compounds synthesized, the most potent molecule against P-gp 170 is 5-methoxy-6,7-dipropyloxyflavone (**23**). Its inhibitory activity against P-gp 170 is approximately 40 times better, based on EC_{50} (concentration of the compound enhancing 50% of the intracellular vinblastine accumulation in the KB/MDR cells) and 3 times higher, based on A_{max} (the intracellular vinblastine accumulation of the KB/MDR cells caused by the compound) as compared to baicalein. Compound **23** is also a more selective inhibitor than baicalein against P-gp 170, because its cytotoxicity is less than that observed for baicalein. The growth inhibitory IC_{50} of compound **23** against KB and KB/MDR cells are about the same, suggesting that compound **23** is unlikely to be a substrate of P-gp 170 pump. Acetylation of R6, R7 or both could also decrease EC_{50} and increase A_{max}. Acetylated compounds are more toxic than baicalein, and their potency against cell growth is compromised by the presence of P-gp 170, suggesting that these compounds are substrates of P-gp 170. Benzylation of R6 or R7 but not both also enhanced anti-P-gp170 activity and potency against cell growth; however, the presence of P-gp 170 in cells did not have an impact on their sensitivity to these molecules, suggesting that the benzylated compounds are inhibitors but not substrates of P-gp 170, and perhaps have a different mechanism of action. In conclusion, the substitutions of R6 and R7 hydroxyl groups by alkoxy groups, acetoxy groups, or benzyloxy groups could yield compounds with different modes of action against P-gp 170 with different mechanisms of action against cell growth.

Introduction

P-glycoprotein 170 (P-gp 170), a member of the ABC (ATP Binding Cassette) family, acts as an ATP-dependent drug efflux pump, preventing intracellular accumulation of miscellaneous drugs.[1,2] Overexpression of this protein is one of the mechanisms of multidrug resistance (MDR) of cancer cells. This protein is expressed in a cell- and tissue-specific manner, with high levels detectable in the kidney, liver, blood−brain barrier, and lining of the intestine.[3] Studies using *mdr1* knockout mice and P-gp 170 tissue distribution in humans suggested several physiological roles of P-gp 170, including protection against toxic xenobiotics by blocking absorption by the intestine, excretion of chemicals into the bile duct or kidney tubules, prevention of chemicals taken into the brain through the blood−brain barrier, and efflux of steroid hormones and cholesterol from feces.[2,4,5] Developing drugs to inhibit P-gp 170 activity is an important area of drug discovery. Such drugs could have use in facilitating the oral absorption of drugs through the intestine, or the uptake of chemicals that are substrates of P-gp, into the brain. In addition, these compounds could also potentiate the action of antitumor drugs, which are substrates of P-gp 170 in cancer cells that overexpress the P-gp 170 protein.

A large number of compounds with major structural differences have been found to act as inhibitors or substrates of P-gp 170: these include verapamil (VRM), a calcium channel antagonist; trifluoperazine, a calmodulin inhibitor; cyclosporin A (CSA), an immunosuppressant; and progesterone, a steroid hormone. Verapamil has been examined clinically in combination

* Corresponding author. Dr. Yung-chi Cheng, Department of Pharmacology, Yale University School of Medicine, 333 Cedar St., SHM B254, New Haven, CT 06520. Tel: (203)-785-7119. Fax: (203)-785-7129. E-mail: cheng.lab@yale.edu.

[†] Department of Pharmacology, Yale University School of Medicine.

[‡] Department of Chemistry, Yale University.

[§] PhytoCeutica, Inc.

[||] The first two authors contributed equally to this manuscript.

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with cancer chemotherapy.[6,7] However, the results were rather unsatisfactory due to high plasma drug levels, required to effectively reverse the MDR phenotype of cancer cells, which could cause cardiac toxicity. Compounds with higher potency against and selectivity for P-gp 170 are needed. A second generation of MDR reversal agents has emerged and is based on the chemical modification of the first generation of inhibitors. Among these, dexniguldipine[8] and dexverapamil[9] were found to be more selective against P-gp 170, but they did not display improved potency. The acridonecarboxamide derivative GF120918 (GG918)[10] and the cyclosporin A analogue PSC833[11] both displayed an activity that was 10−30 times more potent than the first generation of modulators, such as verapamil, tamoxifen, and cyclosporin A.[12] A number of these compounds are currently under clinical evaluation.

Flavonoids are important class of natural products found in plants. With its polyphenolic structure, this class of compounds has multiple actions, including interacting with estrogen receptor, serving as a free-radical scavenger, and inhibiting protein kinase, NF-κB and P-gp 170, among others.[13] The biological activity found in herbal preparations is often attributed to its flavonoids. For example, the coadministration of grapefruit juice with various drugs has led to an increase in the plasma concentration of the drugs, which was attributed to the bioflavonoids found in the grapefruit juice.[14,15]

Flavonoids have also been shown to act on multiple targets with different specificity. For example, the flavonoid that binds to estrogen receptor requires hydroxyl groups at positions 2 and 3 of the B-ring, a double bond at positions 2−3 of the C-ring, and the absence of any hydrophobic prenylated substituent.[16] This is markedly different than the flavonoids that inhibit various ATPases or protein kinases. Recognition of the ATP binding pocket of these proteins requires the presence of three hydroxyl groups at positions 5 and 7 on the A-ring and position 3 of the C-ring, which favors some flavonols.[17] Moreover, some protein kinases exhibit different structural requirements for binding: an isoflavone structure has been demonstrated to inhibit tyrosine kinase activity,[18] and a flavone substituted at position 8 of A-ring with 4-(3-hydroxy-1-methylpiperidinyl) group has demonstrated activity against CDK2.[19] The inhibition of P-gp 170 by flavonoids has also been investigated and two binding modes have been postulated. The studies were performed using a truncated form of P-gp 170 in a membrane preparation from P-gp 170 overexpressing cells. The structural requirements for flavonoid activity have recently been summarized,[20] and it appears that different classes of flavonoids have different structural requirements for inhibitory activity against P-gp 170.

In our previous study, we found that *Radix scutellariae* (Scute), a well-known Chinese herb, has inhibitory activity against P-gp 170. The active component of the Scute herb was found to be the natural product baicalein. However, the 7-glucuronyl form of baicalein, which is the most abundant component of Scute, did not show anti-P-gp 170 activity. Other abundant Scute components, such as wogonoside and wogonin, were also found to lack inhibitory activity against P-gp 170 efflux action. Since baicalein (a 5,6,7-trihydroxyflavone) and wogonin (a 5,7-dihydroxy-8-methoxyflavone) have a very similar structure, this raised the possibility of an interesting structure−activity relationship of the flavone natural products for P-gp 170 inhibition.

In this study, we synthesized and evaluated a series of baicalein analogues, focusing on the substitution pattern at positions 5, 6, and 7 of the A-ring. Our results show that alkoxy groups on the A-ring of the flavone greatly increase the anti-P-gp 170 activity and alter their selectivity for the efflux pump.

Chemistry

The synthesis of *O*-substituted baicalein derivatives from commercially available reagents was carried out using the general synthetic approach shown in Schemes 1−4. Acetylation of baicalein (**1**) with acetic anhydride was performed in pyridine to give mono-, di-, and triacetylated derivatives **2**−**4**. Baicalein was treated with benzyl bromide in dry acetone with potassium carbonate to provide mono- and dibenzylated analogues **15** and **16**. The *O*-methylated derivatives **6** and **8** of baicalein were readily prepared by reaction with trimethylsilyldiazomethane ($TMSCHN_2$) in a methanolic THF solution at room temperature. Since the hydroxyl function on the C-5 position of baicalein makes an intramolecular hydrogen bond with the 4-keto group, it is resistant to alkylation, and benzylation of baicalein occurred in the following order: **6** > **7** > **5**.

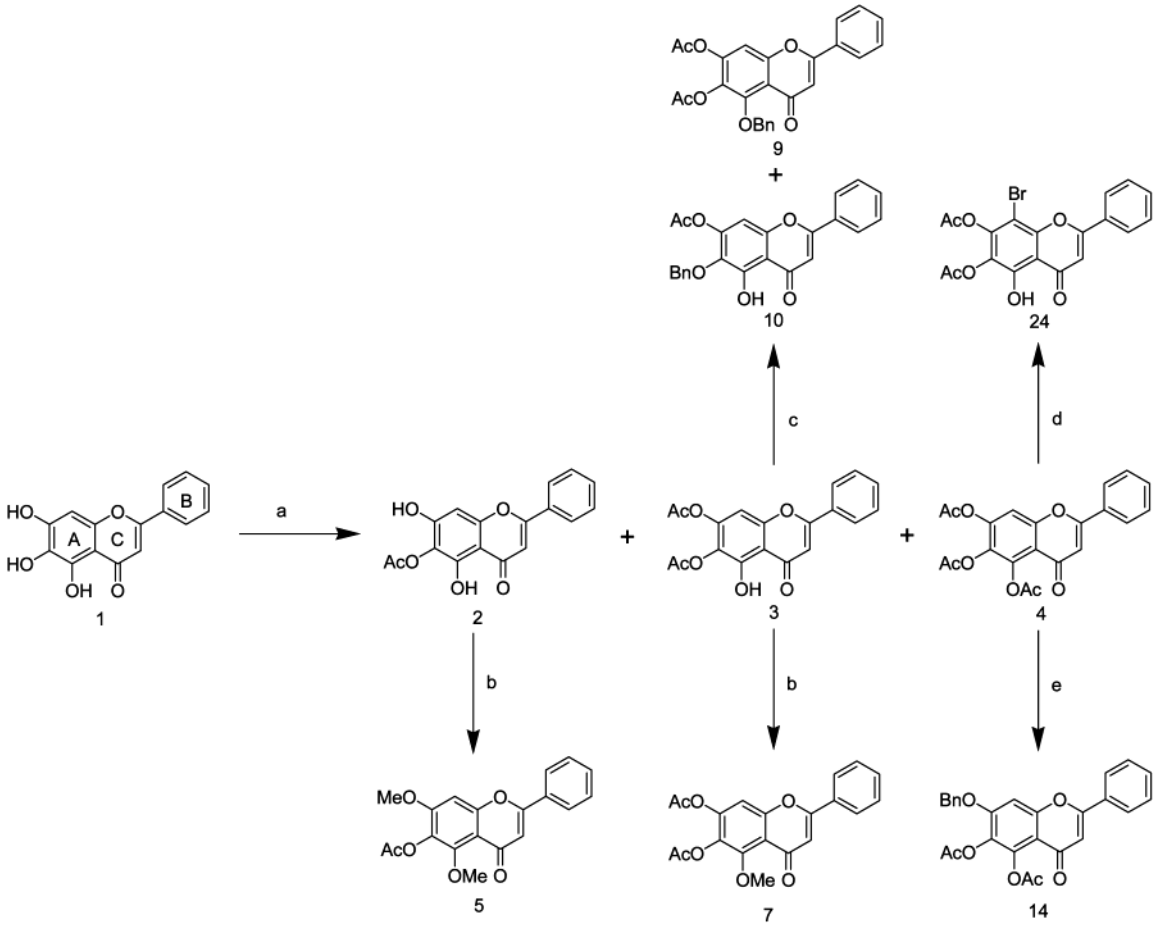
The *O*-methylated products **6** and **8** of baicalein exhibited more potent anti-P-gp activities than that of baicalein itself. This finding led us to design and synthesize a series of alkylated baicalein analogues in order to examine their anti-P-gp activity. A variety of alkyl chains were attached to the hydroxyl groups in the baicalein A ring, and related flavonoids, through phenol alkylation with a number of alkyl halides. The reaction of catechols on the baicalein A ring and flavonoids with bromochloromethane in DMF at 50 °C in the presence of cesium carbonate provided the corresponding methylenedioxy derivative **19**. Similarly, compound **13** was readily prepared by heating baicalein at 170 °C for 1 h with dichlorodiphenylmethane and then reaction with $TMSCHN_2$ in methanolic THF at room temperature.

The selective brominations at the C-8 position of baicalein and baicalein derivatives were performed directly with *N*-bromosuccinimide in the presence of a catalytic amount of concentrated sulfuric acid at room temperature.

Biological Results

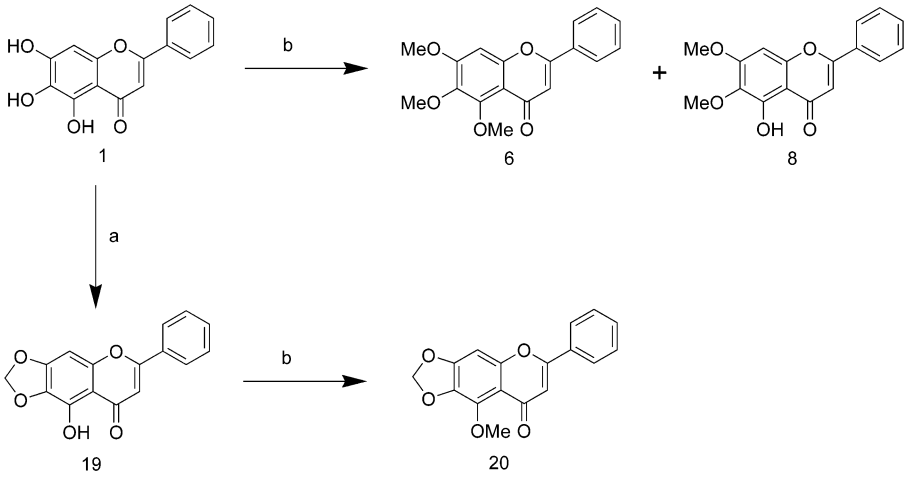
In recent studies, we found that the anti-P-gp activity of *Scutellaria baicalensis Georgi* could inhibit P-gp 170 and have attributed this activity to the high quantity of baicalein (compound **1**) found in the extract. We therefore synthesized a number of baicalein-related compounds in order to evaluate their structure−activity relationship against P-gp 170 activity. Since flavonoids could have multiple sites of action that affect cell growth, we also evaluated their growth inhibitory activity against KB, a human cancer cell line. If a compound exhibits cytotoxicity through the inhibition of cell function, in addition to serving as a substrate of

Scheme 1[a]



[a] Reagents and conditions: (a) Ac_2O, pyridine, rt; (b) $TMSCHN_2$, THF:MeOH (2:1), rt; (c) K_2CO_3, BnBr, acetone, reflux; (d) NBS, THF, concd H_2SO_4, rt; (e) K_2CO_3, KI, BnBr.

Scheme 2[a]

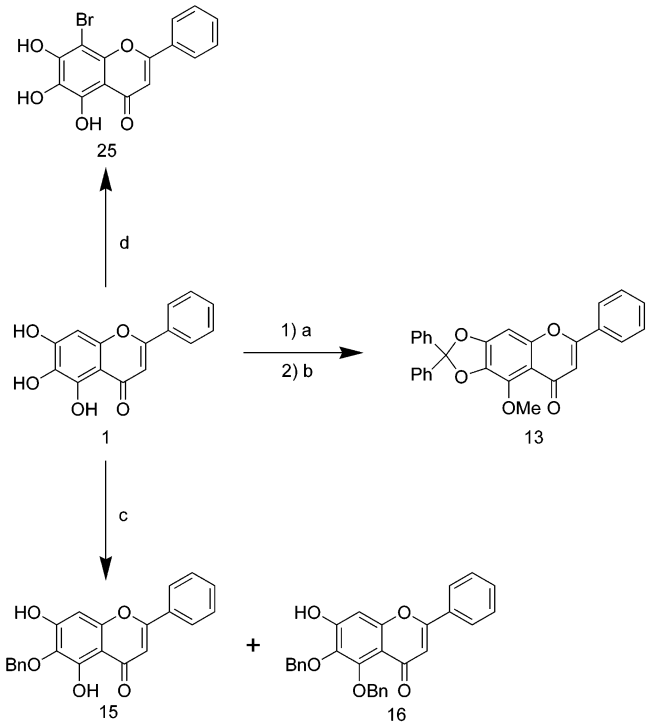


[a] Reagents and conditions: (a) Cs_2CO_3, $BrCH_2Cl$, DMF, 50 °C; (b) $TMSCHN_2$, THF:MeOH (2:1), rt.

P-gp 170, then the compound would be expected to demonstrate less activity against a cell line overexpressing P-gp 170 than the parent cell line. Therefore, we employed a multi-drug resistant cell line (KB/MDR) in addition to the parent KB cell line, to assess the susceptibility of a compound to act as a substrate or inhibitor of the P-gp 170 efflux pump. Our modification of the natural products focused primarily on the functional groups of the baicalein A ring, especially positions 5, 6, and 7, since our initial studies indicated that these positions might play a crucial role in P-gp 170 inhibition.

The KB/MDR cells, which overexpress human P-gp 170 protein, were used to evaluate the anti-P-gp 170 activity of drugs. In this manner, the intracellular amount of vinblastine, a substrate of P-gp 170 pump, was measured in the presence of the known P-gp inhibitors cyclosporin A and verapamil and compared to our synthetic flavones. The concentration of com-

Scheme 3[a]



[a] Reagents and conditions: (a) Ac_2O, pyridine, rt; (b) $TMSCHN_2$, THF:MeOH (2:1), rt; (c) K_2CO_3, BnBr, acetone, reflux; (d) NBS, THF, concd H_2SO_4, rt.

pound required to achieve 50% maximum accumulation of vinblastine is presented as the EC_{50} value. The maximum accumulation of intracellular vinblastine caused by the compounds in 1 h is expressed as A_{max} (picomoles/10^6 cells). The cell growth inhibitory activity of compounds is presented as the concentration required to inhibit 50% growth of KB or KB/MDR cell lines (IC_{50}) following 3 days of compound treatment.

As shown in Table 1, the number of acetoxy groups on the baicalein A ring altered the EC_{50} of anti-P-gp activity. Compounds with one (**2**) or two (**3**) acetoxy groups on position 6 and 7 of the A ring exhibit an EC_{50} that is one-fourth that of the parental compound. The activity of the flavone with three acetoxyl groups (**4**) at position 5, 6, and 7 did not differ from compound **2** or **3**. The A_{max} values of these three compounds were also similar, but higher than that found for baicalein. The flavones with acetoxy groups were more toxic to KB cells than the KB/MDR cells, indicating that the substitutions of hydroxyl groups by acetoxy groups could render the flavone a better substrate for P-gp 170 efflux pump. Substitution of the acetoxy groups in compound **4** with one (**7**) or two (**5**) methoxy groups did not alter the EC_{50} or A_{max} substantially, but increased the IC_{50} value against cell growth. We also evaluated the impact of a bromo group on position 8 on anti-P-gp 170 efflux activity. Compound **25** (8-bromobaicalein) decreased the EC_{50} to 15 μM, but the A_{max} did not change, as compared to baicalein. Compound **24** (the 8-bromo derivative of compound **3**) also showed less favorable activity against P-gp 170 activity than compound **3**. Both of the compounds with an 8-bromo group were toxic to KB and KB/MDR cells and showed the same IC_{50} as compound **3** to KB cells, but lower than that for the KB/MDR cells. This suggests that the bromo substitution prevents compound **3** from acting as a substrate of P-gp 170.

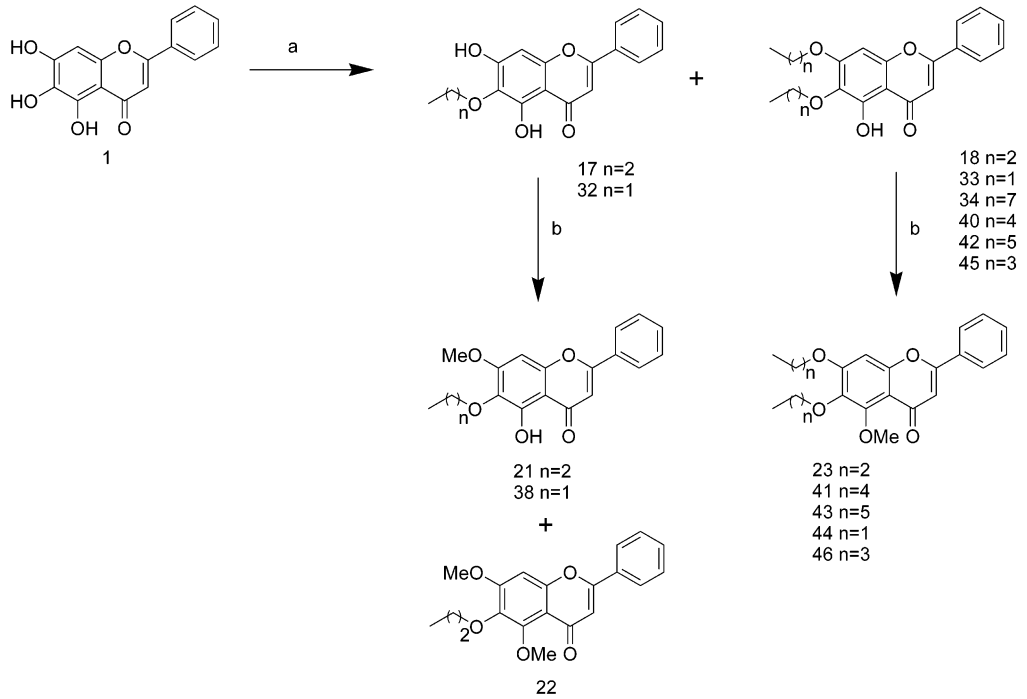
By adding one benzyloxy group to the A ring of baicalein (Table 1), the flavone became a very potent inhibitor of P-gp 170, irrespective of the position or the presence of other functional groups. All the flavones 6,7-diacetoxy-5-benzyloxyflavone (**9**), 7-acetoxy-6-benzyloxy-5-hydroxyflavone (**10**), 5,6-diacetoxy-7-benzyloxyflavone (**14**), and 6-benzyloxy-5,7-dihydroxyflavone (**15**) exhibited a low EC_{50} but higher A_{max} in comparison with baicalein. On the other hand, the benzyloxy group transformed the flavone into a more toxic compound toward both KB and KB/MDR cells than baicalein. This suggests that these compounds have a different site of action in addition to P-gp 170, for which activity is required to maintain cell growth. The fact that these compounds have the same IC_{50} for KB and KB/MDR cells, suggests that they are inhibitors but not substrates of the P-gp 170 efflux pump. When the A ring of baicalein is substituted with two benzyloxy groups, the flavone shows decreased cytotoxicity and anti-P-gp activity, without regard to being cyclic (**13**) or not (**16**).

Experimental results for the alkylated baicalein compounds are shown in Table 2. The introduction of a methoxy group onto the A ring decreased the EC_{50} and increased the A_{max}. The presence of two (**8**) or three (**6**) methoxy groups on the A ring of baicalein dramatically increased the anti-P-gp activity, as evidenced by their EC_{50} of 4.6 μM and 5.5 μM respectively, without a further increase in the A_{max}. Cyclizing the R6 and R7 position of the A ring decreased the cytotoxic activity and A_{max}, but did not alter the EC_{50} against P-gp 170 activity as compared to compounds **8** and **6**, regardless of whether the substitution at R5 is a hydroxy (**19**) or methoxy (**20**) group.

Alkyl substitution (methoxy) turned out to be a far more favorable substitution than either the acetoxy or benzyloxy group for flavone anti-P-gp efflux activity. This is most likely due to the fact that this substitution prevents the compound from acting as a substrate for P-gp 170 or renders it more specific against P-gp 170. For this reason, we further explored the potential of this type of substitution in a search for an optimal linear alkoxy group. The ethoxy group was shown to be better than the methoxy group. The EC_{50} values of 6-ethoxy-5,7-dihydroxyflavone (**32**) and 6,7-diethoxy-5-hydroxyflavone (**33**) were 2.3 μM and 1.8 μM, respectively, which is about the same as that of CSA. In addition, the ethoxy compound **33** had a higher A_{max} and less cytotoxicity than either CSA or compound **32**. While there is no significant difference between one and two ethoxy groups on the EC_{50}, there is a big difference in their cytotoxicity. Substitution of the R5 hydroxyl group in compound **33** with methoxy (**44**) did not change the EC_{50} significantly. Substitution of the R7 ethoxy group of compound **33** with a methoxy (**38**) did not alter the IC_{50} but did decrease the A_{max}.

Similar to the benzyloxy group substitution, the presence of the propyloxy substitution on any position of the A ring has a significant effect on the potency and degree of anti-P-gp 170 efflux activity. One propyloxy group on R6 (**17**) decreases the EC_{50} to 2 μM and increases the A_{max} 9-fold higher than that of the control; two propyloxy groups on R6 and R7 (**18**) decreases the

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, $CH_3(CH_2)_nX$ (X = I or Br, n = 1 for **32**, **33**, n = 2 for **17**, **18**, n = 3 for **45**, n = 4 for **40**, n = 5 for **42**, n = 7 for **34**), acetone, reflux; (b) $TMSCHN_2$, THF:MeOH (2:1), rt.

Table 1. Anti-P-gp Activity and Cytotoxicity of Modified Baicalein Compounds

	functional group					anti-P-gp activity[b]		cytotoxicity IC_{50}[e]	
compd	R5	R6	R7	R8	clogD[a]	EC_{50}[d]	A_{max}[c]	KB	KB/MDR
control							0.5 ± 0.1[f]		
CSA					2.9	1.2 ± 0.3	3.5 ± 0.3	0.6 ± 0.2	1.5 ± 0.7
VRM					4.5	14 ± 1.2	2.2 ± 0.1	19.6 ± 2.7	51.7 ± 4.7
1	OH	OH	OH	H	3.0	41 ± 5.1	1.7 ± 0.1	62.3 ± 3.7	87.1 ± 3.6
2	OH	OAc	OH	H	2.5	11 ± 2.1	3.1 ± 0.2	10.5 ± 1.4	61.6 ± 4.8
3	OH	OAc	OAc	H	2.4	9.7 ± 1.8	2.6 ± 0.1	12.7 ± 2.3	69.3 ± 6.4
4	OAc	OAc	OAc	H	1.2	6.8 ± 0.7	3.0 ± 0.2	14.5 ± 2.1	57.2 ± 7.3
7	OMe	OAc	OAc	H	1.7	12.3 ± 1.5	2.9 ± 0.1	>100	>100
5	OMe	OAc	OMe	H	2.3	11.5 ± 1.1	2.4 ± 0.1	85.5 ± 8.5	>100
24	OH	OAc	OAc	Br	3.1	15 ± 3.1	1.8 ± 0.3	12.4 ± 2.7	16.2 ± 2.3
25	OH	OH	OH	Br	3.7	15 ± 2.9	1.8 ± 0.2	14.1 ± 1.4	18.7 ± 3.1
9	OBn	OAc	OAc	H	3.5	3.7 ± 0.2	3.6 ± 0.2	11.3 ± 1.7	12.2 ± 1.1
10	OH	OBn	OAc	H	4.7	2.4 ± 0.1	2.8 ± 0.1	16.3 ± 2.1	18.3 ± 2.3
14	OAc	OAc	OBn	H	3.5	1.1 ± 0.1	3.4 ± 0.2	13.4 ± 2.4	13.7 ± 2.1
15	OH	OBn	OH	H	4.8	1.8 ± 0.1	3.7 ± 0.2	4.3 ± 1.6	3.2 ± 1.2
16	OH	OBn	OBn	H	7.1	70 ± 5.4	1.1 ± 0.1	>100	>100
13	OMe	$OCPh_2O$		H	6.7	11.5 ± 2.2	1.9 ± 0.1	60.5 ± 5.5	40.6 ± 3.4

[a] clogD was calculated using the following equation: $clogD = clogP - log[1 + 10^{(pH - pK_a)}]$, where clogP is found using ChemDraw ULTRA, version 6.0.1, for 1-octanol/water system, the pH of the experiment was 7, and the pK_a was assumed to be 10 (values ranging from 8 to 11 made insignificant changes to the clogD value). [b] The anti-P-gp efflux activity is represented by intracellular vinblastine accumulation in 1 h with or without drug treatment; see Experimental Section for details. [c] Maximum vinblastine accumulation (pmoles) of flavones (<100 μM) treated cells in the 10^6 cells in 1 h. [d] EC_{50} calculated as the concentration (μM) that causes 50% of maximum vinblastine accumulation in the cells in 1 h. [e] Cytotoxicity IC_{50} calculated as the concentration (μM) required for 50% inhibition of cell growth, for each respective cell line, after 72 h of drug exposure. [f] Control cells were treated with vinblastine only. All values represent the mean ± SD of at least three identical experiments.

EC_{50} to 1.4 μM, and the A_{max} is 10-fold higher than the control. In addition, the EC_{50} of 5-hydroxy-7-methoxy-6-propyloxyflavone (**21**) was further decreased to 1.2 μM and the A_{max} was 9-fold higher than that of the control. The molecule 5,7-dimethoxy-6-propyloxyflavone (**22**), with an added methoxy on the R5 hydroxyl group, showed the same EC_{50} and A_{max} as compound **21**. The cytotoxicity of 6,7-diethoxy-5-methoxyflavone (**44**) was higher than that of 6,7-diethoxy-5-hydroxyflavone (**33**), which only contains a single R5 position change from hydroxy to methoxy. The same phenomenon is found when comparing 5-hydroxy-6,7-dimethoxyflavone (**8**) to 5,6,7-trimethoxyflavone (**6**), compound **41** to **40** and compound **43** to **42**. Finally, the most potent flavone in this series was found to be 5-methoxy-6,7-dipropyloxy-flavone (**23**), with two propyloxy groups on R6 and R7 and a methoxy group on R5. The EC_{50} of compound **23** was found to be 0.9 μM and the A_{max} is 10-fold higher than that of the control. This is more efficient than CSA, which shows an activity that is 7-fold higher than control. The presence of a methoxy group on the R5 position of compound **23** makes it slightly more toxic than compound **18** to KB and KB/MDR cells, but this cytotoxicity is not altered in the presence of P-gp 170

Table 2. Anti-P-gp Activity and Cytotoxicity of Alkylated Baicalein Compounds

	functional group					anti-P-gp activity[b]		cytotoxicity IC_{50}[e]	
compd	R5	R6	R7	R8	clogD[a]	EC_{50}[d]	A_{max}[c]	KB	KB/MDR
control							0.5 ± 0.1f		
CSA					2.9	1.2 ± 0.3	3.5 ± 0.3	0.6 ± 0.2	1.5 ± 0.7
VRM					4.5	14 ± 1.2	2.2 ± 0.1	19.6 ± 2.7	51.7 ± 4.7
1	OH	OH	OH	H	3.0	41 ± 5.1	1.7 ± 0.1	62.3 ± 3.7	87.1 ± 3.6
8	OH	OMe	OMe	H	3.5	4.6 ± 1.1	3.4 ± 0.3	>100	>100
6	OMe	OMe	OMe	H	2.9	5.5 ± 0.4	2.7 ± 0.2	85.9 ± 7.8	57.9 ± 5.9
19	OH	OCH2O		H	3.7	6.5 ± 1.3	1.2 ± 0.1	>100	>100
20	OMe	OCH2O		H	3.1	4.4 ± 2.1	1.5 ± 0.1	> 100	>100
32	OH	OEt	OH	H	3.6	2.3 ± 0.3	3.5 ± 0.3	24.6 ± 3.5	17.5 ± 5.6
38	OH	OEt	OMe	H	4.1	1.5 ± 0.3	2.3 ± 0.2	>100	>100
33	OH	OEt	OEt	H	4.6	1.8 ± 0.2	4.9 ± 0.2	>100	>100
44	OMe	OEt	OEt	H	3.9	1.1 ± 0.1	4.2 ± 1.1	81.7 ± 7.8	79.2 ± 5.8
17	OH	OPr	OH	H	4.1	2 ± 0.7	4.7 ± 0.1	58.9 ± 6.3	>100
21	OH	OPr	OMe	H	4.6	1.2 ± 0.4	4.6 ± 0.1	>100	>100
22	OMe	OPr	OMe	H	3.9	1.7 ± 0.1	4.6 ± 0.1	>100	>100
18	OH	OPr	OPr	H	5.6	1.4 ± 0.4	5.0 ± 0.2	>100	>100
23	OMe	OPr	OPr	H	5.0	0.9 ± 0.1	5.2 ± 0.1	86.4 ± 6.3	93.7 ± 2.2
45	OH	OC_4H_9	OC_4H_9	H	6.7	1.5 ± 0.3	3.2 ± 0.1	>100	>100
46	OMe	OC_4H_9	OC_4H_9	H	6.1	1.6 ± 0.2	4.4 ± 0.1	>100	>100
40	OH	OC_5H_{11}	OC_5H_{11}	H	7.8	1.8 ± 0.1	1.1 ± 0.1	>100	>100
41	OMe	OC_5H_{11}	OC_5H_{11}	H	7.1	1.5 ± 0.1	3.2 ± 0.1	75.4 ± 6.4	82.6 ± 8.4
42	OH	OC_6H_{13}	OC_6H_{13}	H	8.8	1 ± 0.1	1.0 ± 0.1	>100	>100
43	OMe	OC_6H_{13}	OC_6H_{13}	H	8.2	1.3 ± 0.2	1.1 ± 0.1	39.1 ± 8.5	44.8 ± 7.9
34	OH	OC_8H_{17}	OC_8H_{17}	H	10.9	7.4 ± 4.1	1.2 ± 0.1	>100	>100

[a] clogD was calculated using the following equation: $\text{clogD} = \text{clogP} - \log[1 + 10^{(\text{pH}-\text{p}K_a)}]$, where clogP is found using ChemDraw ULTRA, version 6.0.1, for 1-octanol/water system, the pH of the experiment was 7, and the pK_a was assumed to be 10 (values ranging from 8 to 11 made insignificant changes to the clogD value). [b] The anti-P-gp efflux activity is represented by intracellular vinblastine accumulation in 1 h with or without drug treatment; see Experimental Section for details. [c] Maximum vinblastine accumulation (pmoles) of flavones (<100 μM) treated cells in the 10^6 cells in 1 h. [d] EC_{50} calculated as the concentration (μM) that causes 50% of maximum vinblastine accumulation in the cells in 1 h. [e] Cytotoxicity IC_{50} calculated as the concentration (μM) required for 50% inhibition of cell growth, for each respective cell line, after 72 h of drug exposure. [f] Control cells were treated with vinblastine only. All values represent the mean ± SD of at least three identical experiments.

activity, which suggests that compound **23** is not a substrate of P-gp 170. Compounds with alkoxy substitutions of longer chain length lead to a decrease in A_{max}, without altering the EC_{50} substantially. No obvious correlation between a molecule's clog D value and anti-P-gp activity was observed.

Discussion and Conclusion

Other groups have explored the anti-P-gp activity of balcalein, which exhibits moderate instability in cell culture. In drug screens involving molecules isolated from Chinese herbal medicines, Thomas Efferth et al. found several compounds exhibiting anti-P-gp activity.[21] However, baicalein was not included in their list. With adjacent trihydroxyl groups on the A ring, baicalein is not a stable compound. It is easily oxidized and forms a green precipitate within a few hours under assay conditions. In our 1-h vinblastine accumulation experiment, we observed a moderate loss of baicalein. Since this molecule is so unstable, it is not surprising that it was not identified in the original 4−10 day anti-P-gp activity screening assay.

Our previous studies have shown that flavone has the highest anti-P-gp activity among all of the flavonoid subclasses, which include flavone, flavonol, isoflavone, flavanone, and glycosylated flavone. This conclusion is consistent with that observed by Gwenaelle Conseil et al.[22] who demonstrated that the 2,3-double bond on C ring and the hydroxyl group on the A ring are important affinity determinants for flavonoid binding to P-gp. In addition, Jose M. Perez-victoria et al.[23] showed that no matter how large a substitution is made, its effect is not as important as the 2,3-double bond in the C ring for binding affinity. The assay used in these studies, however, directly measures the binding affinity of compounds for the cytosolic nucleotide-binding domain of P-gp 170 protein and does not provide a measure of anti-P-gp 170 functional activity in living cells.

Ahcene Boumendjel et al.[20] incorporated modified chrysin in their studies and achieved results similar to our own. Their experiments showed that the increase in hydrophobicity of chrysin by alkylation with methyl, isopropyl, benzyl, 3,3-dimethylallyl, or geranyl substituents correlated with an increase in affinity for in vitro binding to the P-gp cytosolic domain. Their anti-P-gp activity is therefore solely dependent on the number of isopropyl groups, irrespective of the A-ring position (6, 7, or 8). Our results indicate that the number of carbons permitted in the alkoxy group is limited to three. Alkoxy groups in all three positions 5, 6, or 7 make a contribution to anti-P-gp activity. Potency is dependent on the alkoxy(l) group and decreases in the following order: propyloxy > ethoxy > methoxy. In our studies, all flavones with alkoxy groups have the same or better anti-P-gp activity than that of cyclosporin A (measured by vinblastine accumulation) and much higher than that of the flavones with *C*-alkyl groups, which only accumulate about 20−30% of drug as compared to cyclosporin A. The impact on anti-P-gp activity caused by *n*-propyloxy substituents is greater than having *C*-isopropyl or *C*-dimethylallyl substituents on these compounds. Perhaps the high potency observed for their best compound, which has an *O*-dimethylallyl substitution pattern, results from the effect of oxygen alkylation and not from the dimethylallyl group itself. Moreover, the A_{max} of compound **23** is 167% of cyclosporin A and

236% of verapamil. One possible interpretation of these results is that the alkoxyflavone is more specific for P-gp 170 and has no other cellular effect, which is not the case of cyclosporin A or verapamil. In this manner, it can reach a higher level of apparent anti-P-gp activity in the KB/MDR cells.

Interestingly, all of the flavones with an alkoxy group of 4−8 carbons exhibited the same level of EC_{50} compared to compound **23**, indicating that maybe the binding affinity of this series of alkoxyflavones remain the same, since the long chain substituents did not decreased the anti-P-gp activity. One possibility is that the long carbon chain of the alkoxy group changes the orientation of the flavone (e.g., insertion into plasma membrane) relative to the P-gp 170 binding site, so that they still bind to P-gp but do not block pump activity.

The benzyl group substituent also showed a large impact on anti-P-gp activity both in the studies of Ahcene Boumendjel's[20] and ours. The benzyloxy group has a much stronger effect on the A_{max} than the benzyl group in both systems. On the other hand, both their and our benzyloxy groups are connected to the A-ring at positions 6 and 7. With the same benzyloxy group, the two acetoxy groups of our compound (5,6-diacetoxy-7-benzyloxyflavone, the A_{max} is 97% as compared to cyclosporin A) may have some supplementary effect compared to their compound (7-benzyloxy-5-hydroxyflavone, the A_{max} is 25% as compared to cyclosporin A). The addition of two benzyloxy groups on the A-ring caused the flavone (6, 7-dibenzyloxy-5-hydroxyflavone) to lose all of its anti-P-gp activity in our experiments, but (6-benzyl-7-benzyloxy-5-hydroxyflavone) showed an increase in both binding affinity and drug accumulation in their experiments. The only difference between these two molecules is the benzyl or benzyloxy group on position 6. It should also be noted that cytotoxicity increases greatly with benzyloxy substitution on the A-ring.

To achieve a more comprehensive overview of the structure−activity relationship of the flavones, we subcategorized them based on the position of their substituents. We first organized the same functional group on R6 and R7, to see the impact of R5 on anti-P-gp efflux activity. Compounds with the same substitutions on R6 and R7 were divided into four groups: compounds **3**, **4**, **7**, and **9** with two acetoxy groups on R5 and R7; compounds **8** and **6** with two methoxy groups; compounds **33** and **44** with two ethoxy groups; and compounds **18** and **23** with two propyloxy groups on the R6 and R7 position. The EC_{50} required to inhibit P-gp 170 activity of these four groups decrease in the following order: two acetoxys > two methoxys > two ethoxys > two propyloxys. In addition, the composition of the R5 substituents is not as dominant as the composition of the same two groups on R6 and R7. The anti-P-gp activity of flavones with two acetoxy groups on R6 and R7 is dependent on the functional group in the R5 position. The anti-P-gp activity of flavones with two methoxy or ethoxy groups on R6 and R7 did not vary with a change in the R5 functional group. Flavones with two propyloxy groups on R6 and R7 were the most potent inhibitors in this series of compounds, regardless of whether R5 is hydroxy or methoxy group. However, the methoxy group on the R5 position renders the flavones more toxic to cells as compared to the hydroxyl group.

In the case of a fixed functional group on the R5 and R7 position, the molecules can be divided into three groups, those with two hydroxy groups (compounds **1**, **2**, **15**, **17**, and **32**), two methoxy groups (compounds **5**, **6**, and **22**), and a hydroxy group on the R5 and methoxy group on the R7 (compounds **8**, **21**, and **38**). The anti-P-gp activity of the compounds with two hydroxyl groups was largely dependent on the functional group at the R6 position, from high (compounds **1** and **2**) to very low (compounds **15** and **32**) EC_{50}. The anti-P-gp efflux activity of compounds with two methoxy groups is also dependent on the functional group at the R6 position, showing both medium EC_{50} (compounds **5** and **6**) and very low (compound **22**) activity. The anti-P-gp 170 activity of compounds with a hydroxy group on R5 and a methoxy group on R7 do not show obvious dependence on the functional group at R6, since neither the methoxy ethoxy or propyloxy groups changed the EC_{50} value noticeably, however, all three were very good inhibitors. Their inhibitory effectiveness is shows the following decreasing order: propyloxy (**21**) > ethoxy (**38**) > methoxy (**8**).

Compounds with the same functional groups on R5 and R6 could also be divided into four categories. With a hydroxy group on R5 and a benzyloxy group on R6 (compounds **10** and **15**), the EC_{50} values decrease and are not affected by the substituent on R7, except for the molecule containing two benzyloxy groups (**16**), which lost both anti-P-gp 170 activity and cytotoxicity. The anti-P-gp 170 activity of compounds with a hydroxy group on R5 and an ethoxy group on R6 was not dependent on the functional group at R7 either (compounds **32**, **38**, and **33**). The anti-P-gp 170 efflux activity of compounds with a hydroxy group on R5 and a propyloxy group on R6 was also not dependent on the functional group at the R7 (compounds **17**, **21**, and **18**). Even the anti-P-gp activity of the most potent compounds, containing a methoxy group on R5 and a propyloxy group on R6 was not obviously dependent on the chemical function at R7 (compounds **22** and **23**). It appears that small substituent changes on position 7 do not have an impact on anti-P-gp activity.

The inhibition of P-gp 170 efflux activity itself should not cause cytotoxicity, and our data support this theory since there appears to be no correlation between P-gp inhibition and cytotoxicity in the compounds evaluated in this study. Therefore, the increased toxicity noted in some of the molecules is likely due to affinity of the compounds for additional biochemical targets with ATP-binding sites. Several flavonoids have been reported to be good inhibitors for a variety of ATP-binding proteins such as plasma membrane ATPases,[24,25] protein kinase A,[26] protein kinase C,[27] serine/threonine protein kinases,[28] tyrosine protein kinase,[29] and topoisomerase II.[30] Staurosporine produces high intrinsic cytotoxicity in human cells in addition to its anti-P-gp activity. On the basis of a structural analysis, R. B. Wang et al.[31] suggested that the isobenzopyrrolidone of staurosporine meets the binding requirement of the adenosine moiety of ATP and prevents ATP binding to the ATP-binding site. In this comparison, however, the A ring of galangin (3, 5, 7-trihydroxyflavone) does not

overlap well with the five-membered ring of adenosine, and that is the reason galangin does not exhibit cytotoxicity as a staurosporine analogue. A. D. Pietro et al.[32] indicated that among a total of 29 flavonoids examined, only three flavonols were found to bind to the ATP-binding site, and the hydroxyl group at position 3 of the flavonol is critical for binding. This requirement is similar to that observed for quercetin binding to the Hck tyrosine kinase, as demonstrated by cocrystallization[33] and for other ATPases by inhibition kinetics.[34] On the basis of these observations, most of our synthetic flavones are unlikely to be good candidates for ATP-binding site affinity because they lacking the hydroxyl group on R3, except the benzyloxy flavone which could potentially block ATP binding by fitting to the adenine site in the ATP-binding pocket like staurosporine.

When a benzyloxy group is connected to the A-ring of baicalein, its structure resembles L868276 (5,7-dihydroxy-8-[4-(3-hydroxy-1-methyl)piperidinyl]-flavone), which fits nicely to the adenine-binding pocket of CDK2,[19] and this may be a source of its increased cytotoxicity. Interestingly, the cytotoxicity is lost when we add another benzyloxy group to the A-ring of baicalein. This is similar to a pattern noted with staurosporine modification: the addition of one benzyl group causes a decrease in cytotoxicity (CGP41251),[35] and adding another totally abolishes the cytotoxic effect (CGP42700).[36] It is possible that the increased steric bulk added to the molecule by virtue of the second benzyl group renders the compounds incapable of entering the adenine-binding pocket of CDK2 due to steric hindrance. We are currently evaluating this hypothesis.

The nucleotide binding site of P-glycoprotein contains a region that interacts with hydrophobic steroid derivatives, such as RU486, called the steroid binding hydrophobic region (SBHR). This region is most likely located in close proximity to the ATP binding site since RU486 completely prevents or displaces the hydrophobic nucleotide derivative, 2(3)-methylanthraniloyl-ATP (MANT-ATP).[37] A tentative model for the interaction of flavonoids with P-glycoprotein and related multidrug transporters has been proposed by A. D. Pietro et al.[32] Galangin (5,7-dihydroxyflavonol), kaempferol (4′,5,7-trihydroxyflavone), kaempferide (3,5,7-trihydroxy-4′-methoxyflavone), and dehydrosilybin (3, 5, 7-trihydroxy-3′-monolignolflavone) appear to interact with the cytosolic nucleoside binding domain; the hydroxyl groups at positions 3 and 5, in addition to the ketone at position 4, are proposed to bind the ATP-binding site, whereas other parts of the molecules bind in the vicinal SBHR region. Prenylation of the A-ring would therefore increase the hydrophobic interactions with both the cytosolic steroid-interacting region and the drug-binding site. This would potentially produce a significant shift in flavonoid positioning, in such a way that overlap with the ATP binding site can no longer occur. Such a prenylflavonoid positioning appears to be efficient enough to directly inhibit P-gp 170 substrate binding and transport, while indirectly interfering with ATP hydrolysis and energy transduction. In our case, flavones without the 3-hydroxyl group may be acting in a similar fashion, since the alkoxyl group helps trihydroxyflavone (baicalein) to be a better P-gp modulator with less cytotoxicity and may be interacting with the steroid binding hydrophobic region of P-gp rather than the ATP-binding site. Benzyloxyflavone, on the other hand, may likely be overlapping the ATP-binding site, in order to decrease the efflux of P-gp 170 substrates such as vinblastine, and be exhibiting a high intrinsic cytotoxicity through interaction with the ATP-binding sites of other vital proteins.

In conclusion, acetylation, alkylation, or benzylation of hydroxyl groups on the A ring of baicalein can enhance interaction with the P-gp 170 protein and prevent its substrate efflux activity. The mode of interaction with these modified flavones appear be quite different, given the broad differences noted in EC_{50}, A_{max} and cytotoxicity. The alkoxyflavones may interact with P-gp at a site other than the ATP-binding site, whereas the other modified flavonoids likely mimic the adenosine moiety of ATP and block the ATP-binding site. This suggests that the alkoxyflavones may have a lower propensity to interact with the ATP-binding site of other proteins, as observed by their lower cytotoxicity. In summary, the alkoxyflavones appear to be quite promising modulators of P-gp 170 function and warrant further exploration.

Experimental Section

General Chemistry Methods. All solvents and reagents were obtained from commercial suppliers and were used without further purification. Unless otherwise specified, reactions were performed under a nitrogen atmosphere with exclusion of moisture. All reaction mixtures were magnetically stirred and monitored by TLC using Si250F precoated plates from J. T. Baker (0.25 mm). Flash column chromatography was performed on 32−63 D 60 Å silica gel from ICN SiliTech (ICN Biomedicals GmbH). Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-400, Bruker AM-500, or GE QE-plus 300 spectrometer. Chemical shifts are reported using chloroform-*d* (δ 7.24 ppm) or DMSO-d_6 (2.50 ppm). All coupling constants are described in Hz. Mass spectra were conducted at the Mass Spectrometry Laboratory of the University of Illinois.

6-Acetoxy-5,7-dihydroxyflavone (2). Baicalein **1** (54 mg, 0.2 mmol) was dissolved in acetic anhydride (1 mL) and pyridine (1 mL), and the solution was stirred at room temperature for 2 h. The reaction mixture was poured into ice−water (10 mL), and the precipitate was collected by filtration and purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2/MeOH (20:1) to yield compound **2** (35 mg, 56%) as a yellow powder. mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ 2.28 (s, 3H), 6.67 (s, 1H), 7.01 (s, 1H), 7.57 (m, 3H), 8.79 (m, 2H), 11.28 (s, 1H), 12.97 (s, 1H,); MS (EI) *m*/*z* 312 $[M]^+$, 270 (base).

6,7-Diacetoxy-5-hydroxyflavone (3) and 5,6,7-Triacetoxyflavone (4). Baicalein **1** (216 mg, 0.8 mmol) was dissolved in acetic anhydride (40 mL) and pyridine (12 mL), and the solution was stirred at room temperature for 48 h. The reaction mixture was poured into ice−water (100 mL), and the precipitate was collected by filtration and purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2/MeOH (40:1) to give compounds **3** (72 mg, 25%) and **4** (220 mg, 69%) as a pale yellow powder, respectively. (**3**): mp 204−206 °C; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 6H), 6.74 (s, 1H), 6.98 (s, 1H), 7.55 (m, 3H), 7.89 (m, 2H), 12.95 (s, 1H); MS (EI) *m*/*z* 354 $[M]^+$, 312, 270 (base). (**4**): mp 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 2.36, 2.37, 2.46 (each s, 9H), 6.67 (s, 1H), 7.52 (s, 1H), 7.54 (m, 3H), 7.87 (m, 2H); MS (EI) *m*/*z* 396 $[M]^+$, 354, 312, 270 (base).

6-Acetoxy-5,7-dimethoxyflavone (5). To a stirred solution of **2** (25 mg, 0.08 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added trimethylsilyldiazomethane (TM-

$SCHN_2$, 2 M in hexanes, 0.4 mL, 0.8 mmol). The reaction mixture was stirred at room temperature for 12 h and then evaporated. Flash chromatography of the residue, eluting with *n*-hexane/EtOAc (1:1), gave compound **5** (5 mg, 18%) as a pale yellow powder. mp 208−210 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.96 (s, 6H), 6.70 (s, 1H), 6.88 (s, 1H), 7.54 (m, 3H), 7.88 (m, 2H); MS (EI) *m*/*z* 340 $[M]^+$, 298, 280 (base).

5,6,7-Trimethoxyflavone (6). To a stirred solution of **1** (54 mg, 0.2 mmol) in a mixture of MeOH (6 mL) and THF (12 mL) was added $TMSCHN_2$ (2 M in hexanes, 1.2 mL, 2.4 mmol). The reaction mixture was stirred at room temperature for 36 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (15:1), gave compound **6** (30 mg, 48%) as a pale yellow powder. mp 164−165 °C; ^{1}H NMR ($CDCl_3$) δ 3.90, 3.97, 3.99 (s, 9H), 6.67 (s, 1H), 6.81 (s, 1H), 7.50 (m, 3H,), 7.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 56.71, 61.96, 62.60, 96.67, 108.84, 126.39, 129.38, 131.68, 132.03, 154.98, 158.20; MS (EI) *m*/*z* 312 $[M]^+$, 297 (base). HRMS for $C_{18}H_{16}O_5$ $[M]^+$: calculated, 312.0998; found, 312.0995.

6,7-Diacetoxy-5-methoxyflavone (7). To a stirred solution of **3** (25 mg, 0.07 mmol) in a mixture of MeOH (2 mL) and THF (4 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.21 mL, 0.42 mmol). The reaction mixture was stirred at room temperature for 12 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (30:1), gave compound **7** (6 mg, 23%) as a pale yellow powder. mp 240−242 °C; ^{1}H NMR ($CDCl_3$) δ 2.35, 2.45 (s, 6H), 3.95 (s, 3H), 6.61 (s, 1H), 6.96 (s, 1H), 7.52 (m, 3H), 7.85 (m, 2H); MS (EI) *m*/*z* 368 $[M]^+$, 326, 284 (base).

5-Hydroxy-6,7-dimethoxyflavone (8). To a stirred solution of **1** (54 mg, 0.2 mmol) in a mixture of MeOH (6 mL) and THF (12 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.6 mL, 1.2 mmol). The reaction mixture was stirred at room temperature for 8 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (40:1 to 20:1), gave compound **8** (8 mg, 13%) as a pale yellow powder. mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 3.94, 3.99 (s, 6H), 6.59 (s, 1H), 6.70 (s, 1H), 7.55 (m, 3H), 7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 56.78, 61.31, 91.04, 106.07, 126.68, 129.53, 131.73, 132.27, 153.46, 159.31, 164.37, 183.16; MS (EI) *m*/*z* 298 ($[M]^+$, base), 283. HRMS for $C_{17}H_{14}O_5$ $[M]^+$: calculated, 298.0841; found, 298.0844.

6,7-Diacetoxy-5-(benzyloxy)flavone (9) and 7-Acetoxy-6-(benzyloxy)-5-hydroxyflavone (10). A mixture of **3** (21 mg, 0.06 mmol), benzyl bromide (0.03 mL), and anhydrous K_2CO_3 (26 mg) in acetone (15 mL) was refluxed for 8 h with stirring. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. Flash chromatography of the residue, eluting with *n*-hexane/EtOAc (3:2), afforded compounds **9** (18 mg, 68%) and **10** (8 mg, 33%) as a pale yellow powder, respectively. (**9**): mp 175−177 °C; ^{1}H NMR ($CDCl_3$) δ 2.32, 2.47 (s, 6H), 5.22 (s, 2H), 6.61 (s, 1H), 7.02 (s, 1H), 7.42 (m, 5H), 7.52 (m, 3H), 7.84 (m, 2H); MS (EI) *m*/*z* 444 $[M]^+$, 402, 360, 269 (base). (**10**): mp 184−185 °C; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H), 5.22 (s, 2H), 6.64 (s, 1H), 6.70 (s, 1H), 7.42 (m, 5H), 7.55 (m, 3H), 7.88 (m, 2H), 13.00 (s, 1H); MS (EI) *m*/*z* 402 $[M]^+$, 360, 269 (base).

6,7-(Diphenylmethylenedioxy)-5-methoxyflavone (13). A mixture of **1** (27 mg, 0.1 mmol) and dichlorodiphenylmethane (0.02 mL, 0.1 mmol) was stirred under nitrogen at 170 °C for 1 h. The reaction mixture was cooled to 30 °C and then dissolved in a minimum amount of CH_2Cl_2. The crude product was purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2 to yield compound **11** (35 mg, 81%). To a stirred solution of **11** (14 mg, 0.03 mmol) in a mixture of MeOH (2 mL) and THF (4 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.1 mL, 0.2 mmol). The reaction mixture was stirred at room temperature for 24 h and then evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/MeOH (40:1), gave compound **13** (13 mg, 90%) as a pale yellow powder. mp 238−240 °C; ^{1}H NMR ($CDCl_3$) δ 4.24 (s, 3H), 6.67 (s, 1H), 6.81 (s, 1H), 7.42 (m, 6H), 7.50 (m, 3H), 7.61 (m, 4H), 7.85 (m, 2H); MS (EI) *m*/*z* 448 $[M]^+$, 402, 371, 266, 167 (base).

5,6-Diacetoxy-7-(benzyloxy)flavone (14). A mixture of **4** (34 mg, 0.086 mmol), benzyl bromide (0.05 mL), KI (3.5 mg), and anhydrous K_2CO_3 (30 mg) in acetone (15 mL) was refluxed for 24 h with stirring. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. Flash chromatography of the residue, eluting with *n*-hexane/EtOAc (3:2), afforded compound **14** (20 mg, 52%) as a white solid. mp 174−175 °C; ^{1}H NMR ($CDCl_3$) δ 2.27, 2.48 (s, 6H), 5.14 (s, 2H), 6.53 (s, 1H), 6.97 (s, 1H), 7.38 (m, 5H), 7.47 (m, 3H), 7.76 (m, 2H); MS (EI) *m*/*z* 444 $[M]^+$, 402, 360, 269 (base).

6-(Benzyloxy)-5,7-dihydroxyflavone (15) and 6,7-(Dibenzyloxy)-5-hydroxy-flavone (16). A mixture of **1** (54 mg, 0.2 mmol), benzyl bromide (0.12 mL), and anhydrous K_2CO_3 (83 mg) in acetone (15 mL) was refluxed for 8 h with stirring. The reaction mixture was filtered, and the solvent was evaporated under reduced pressure. Flash chromatography of the residue, eluting with CH_2Cl_2/MeOH (100:1 to 50:1), afforded compound **15** (30 mg, 42%) as a yellow powder and compound **16** (24 mg, 27%) as a pale yellow powder. (**15**): mp 195−197 °C; ^{1}H NMR ($CDCl_3$) δ 5.27 (s, 2H), 6.67 (s, 2H), 7.44 (m, 5H), 7.52 (m, 3H), 7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 71.78, 92.25, 105.87, 106.69, 126.69, 127.99, 129.02, 129.28, 129.48, 130.39, 131.86, 132.18, 135.77, 146.35, 150.93, 152.22, 164.57, 183.09; MS(EI) *m*/*z* 360 $[M]^+$, 269 (base). HRMS for $C_{22}H_{16}O_5$ $[M]^+$: calculated, 360.0998; found, 360.0996. (**16**): mp 191−193 °C; ^{1}H NMR ($CDCl_3$) δ 5.17, 5.19 (s, 4H), 6.59 (s, 1H), 6.69 (s, 1H), 7.30−7.55 (m, 13H), 7.87 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 71.36, 75.25, 92.38, 106.07, 126.68, 127.68, 128.36, 128.60, 128.68, 129.05, 120.10, 129.49, 131.76, 132.22, 136.16, 137.89, 153.64, 153.94, 158.59, 164.38, 183.16; MS (EI) *m*/*z* 450 $[M]^+$, 359, 269, 91 (base). HRMS for $C_{29}H_{22}O_5$ $[M]^+$: calculated, 450.1467; found, 450.1465.

5,7-Dihydroxy-6-propyloxyflavone (17) and 5-Hydroxy-6,7-dipropyloxyflavone (18). A mixture of **1** (54 mg, 0.2 mmol), *n*-propyl iodide (0.06 mL), and anhydrous K_2CO_3 (110 mg) in acetone (20 mL) was refluxed with stirring for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with water (30 mL), and extracted with CH_2Cl_2 (3 × 30 mL). The extract was washed with water and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2/MeOH (70:1 to 50:1) to yield compounds **17** (7 mg, 11%) and **18** (44 mg, 62%) as a yellow powder, respectively. (**17**): mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3H, J = 7.5 Hz), 1.95 (sextet, 2H, J = 7.5 Hz), 4.13 (t, 2H, J = 7.5 Hz), 6.62 (s, 1H), 6.69 (s, 1H), 7.54 (m, 3H), 7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 12.94, 24.83, 73.58, 93.63, 107.99, 108.54, 128.79, 131.62, 132.22, 134.04, 134.29, 148.21, 153.22, 154.79, 165.56, 185.24; MS (EI) *m*/*z* 312 $[M]^+$, 297, 283, 270 (base). HRMS for $C_{18}H_{16}O_5$ $[M]^+$: calculated, 312.0998; found, 312.0993. (**18**): mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ 1.07, 1.11 (t, 6H, J = 7.5 Hz), 1.82, 1.92 (sextet, 4H, J = 7.5 Hz), 4.02, 4.05 (t, 4H, J = 7.5 Hz), 6.54 (s, 1H), 6.66 (s, 1H), 7.53 (m, 3H), 7.88 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.86, 10.92, 22.78, 23.81, 30.09, 71.06, 75.43, 91.67, 106.02, 106.54, 126.64, 129.47, 131.87, 132.13, 132.47, 153.63, 153.71, 159.22, 164.19, 183.13; MS (EI) *m*/*z* 354 ($[M]^+$, base), 325, 311, 283, 270. HRMS for $C_{21}H_{22}O_5$ $[M]^+$: calculated, 354.1467; found, 354.1464.

5-Hydroxy-6,7-(methylenedioxy)flavone (19). A mixture of **1** (81 mg, 0.3 mmol) and cesium carbonate (244 mg, 0.75 mmol) in DMF (5 mL) was stirred at room temperature for 30 min. Bromochloromethane (0.05 mL, 0.75 mmol) was added to the DMF solution, and the mixture was stirred at 50 °C for 8 h then diluted with CH_2Cl_2. The dichloromethane solution was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2/MeOH (100:1 to 50:1) to give compound **19** (29 mg, 33%) as a pale yellow powder. mp 213-215 °C; ^{1}H NMR ($CDCl_3$) δ 6.12 (s, 2H), 6.61 (s, 1H), 6.70 (s, 1H), 7.55 (m, 3H), 7.87 (m, 2H); MS (EI) *m*/*z* 282 ($[M]^+$, base), 149.

5-Methoxy-6,7-(methylenedioxy)flavone (20). To a stirred solution of **19** (17 mg, 0.06 mmol) in a mixture of MeOH (3 mL) and THF (6 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.3 mL, 0.6 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (30:1), afforded

compound **20** (12 mg, 68%) as a white solid. mp 202−204 °C; ^{1}H NMR ($CDCl_3$) δ 4.14 (s, 3H), 6.08 (s, 2H), 6.71 (s, 1H), 6.75 (s, 1H), 7.51 (m, 3H), 7.86 (m, 2H); MS (EI) *m*/*z* 296 $[M]^+$, 268 (base), 250.

5-Hydroxy-7-methoxy-6-propyloxyflavone (21) and 5,7-Dimethoxy-6-propyloxy-flavone (22). To a stirred solution of **17** (19 mg, 0.06 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.2 mL, 0.4 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/MeOH (30:1), gave compound **21** (4 mg, 20%) as a yellow powder and compound **22** (9.2 mg, 45%) as a pale yellow powder. (**21**): mp 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3H, J = 7.5 Hz), 1.95 (sextet, 2H, J = 7.5 Hz), 3.93 (s, 3H), 4.09 (t, 2H, J = 7.5 Hz), 6.57 (s, 1H), 6.69 (s, 1H), 7.55 (m, 3H), 7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.91, 22.73, 61.23, 71.13, 91.77, 106.02, 106.54, 126.66, 129.49, 131.81, 132.19, 133.30, 153.55, 153.73, 159.00, 164.28, 183.11; MS (EI) *m*/*z* 326 ($[M]^+$, base), 283, 269. HRMS for $C_{19}H_{18}O_5$ $[M]^+$: calculated, 326.1154; found, 326.1149. (**22**): mp 135−136 °C; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.5 Hz), 1.96 (sextet, 2H, J = 7.5 Hz), 3.92, 4.01 (s, 6H), 4.09 (t, 2H, J = 7.5 Hz), 6.73 (s, 1H), 6.82 (s, 1H), 7.52 (m, 3H), 7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.93, 22.71, 58.92, 61.86, 62.63, 71.10, 97.28, 108.62, 113.62, 115.71, 121.02, 126.43, 129.38, 130.02, 131.72, 132.00, 141.00, 153.05, 155.02, 157.98, 161.66, 177.67; MS (EI) *m*/*z* 340 $[M]^+$, 325 (base), 283. HRMS for $C_{20}H_{20}O_5$ $[M]^+$: calculated, 340.1311; found, 340.1316.

5-Methoxy-6,7-dipropyloxyflavone (23). To a stirred solution of **18** (28 mg, 0.08 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.32 mL, 0.64 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (15:1), gave compound **23** (23 mg, 78%) as a pale yellow powder. mp 109−110 °C; ^{1}H NMR ($CDCl_3$) δ 1.08, 1.12 (t, 6H, J = 7.5 Hz), 1.82, 1.95 (sextet, 4H, J = 7.5 Hz), 3.99 (s, 3H), 4.00, 4.06 (t, 4H, J = 7.5 Hz), 6.72 (s, 1H), 6.81 (s, 1H), 7.52 (m, 3H), 7.89 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 10.95, 22.76, 23.89, 62.46, 71.00, 76.28, 97.19, 108.75, 113.16, 126.38, 129.36, 131.61, 132.11, 140.26, 153.17, 154.90, 158.13, 161.47, 177.69.; MS (EI) *m*/*z* 368 $[M]^+$, 325 (base), 283. HRMS for $C_{22}H_{24}O_5$ $[M]^+$: calculated, 368.1624; found, 368.1630.

6,7-Diacetoxy-8-bromo-5-hydroxyflavone (24). A mixture of **4** (84 mg, 0.21 mmol) and *N*-bromosuccinimide (NBS, 56 mg, 0.32 mmol) in THF (8 mL) and concd H_2SO_4 (10 μL) was stirred at room temperature for 48 h. The reaction mixture was extracted with EtOAc, washed with 10% aqueous $NaHSO_4$ solution and water, dried over $MgSO_4$, and then concentrated in vacuo. The residue was recrystallized from MeOH to give compound **24** (50 mg, 55%) as a yellow powder. mp 244−246 °C; ^{1}H NMR ($CDCl_3$) δ 2.38, 2.44 (each s, 6H), 6.82 (s, 1H), 7.59 (m, 3H), 8.01 (m, 2H); MS (EI) *m*/*z* 434 $[M + 2]^+$, 432 $[M]^+$, 390, 348 (base), 270, 269.

8-Bromo-5,6,7-trihydroxyflavone (25). A mixture of **1** (35 mg, 0.13 mmol) and NBS (33 mg, 0.19 mmol) in THF (4 mL) and concd H_2SO_4 (5 μL) was stirred at room temperature for 12 h. The reaction mixture was extracted with EtOAc, washed with 10% aqueous $NaHSO_4$ solution and water, dried over $MgSO_4$, and then concentrated in vacuo. The residue was recrystallized from MeOH to give compound **25** (26 mg, 57%) as a yellow powder. mp 263−265 °C; ^{1}H NMR (DMSO-d_6) δ 7.07 (s, 1H), 7.59 (m, 3H), 8.12 (m, 2H), 9.58, 10.92, 12.76 (s, 3H); MS (EI) *m*/*z* 350 $[M + 2]^+$, 348 ($[M]^+$, base), 270.

6-Ethoxy-5,7-dihydroxyflavone (32) and 6,7-Diethoxy-5-hydroxyflavone (33). A mixture of **1** (81 mg, 0.3 mmol), ethyl iodide (0.07 mL), and anhydrous K_2CO_3 (166 mg) in acetone (25 mL) was refluxed with stirring for 18 h. The reaction mixture was concentrated under reduced pressure, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The extract was washed with water and dried over $MgSO_4$ and the solvent evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel and eluted with CH_2Cl_2/MeOH (50:1) to give compounds **32** (30 mg, 34%) and **33** (12 mg, 12%) as a yellow powder, respectively. (**32**): mp 190−192 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (t, 3H, J = 6.9 Hz), 4.23 (q, 2H, J = 6.9 Hz), 6.60 (s, 1H), 6.68 (s, 1H), 7.53 (m, 3H), 7.89 (m, 2H), 12.50 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.04, 64.57, 90.44, 104.87, 105.42, 125.67, 128.50, 129.05, 130.91, 131.17, 145.08, 150.12, 151.56, 163.46, 182.10; MS (EI) *m*/*z* 298 ($[M]^+$, base), 283, 270, 269, 254. HRMS for $C_{17}H_{14}O_5$ $[M - H]^+$: calculated, 297.0763; found, 297.0762. (**33**): mp 132−133 °C; ^{1}H NMR ($CDCl_3$) δ 1.41, 1.53 (t, 6H, J = 7.2 Hz), 4.13, 4.19 (q, 4H, J = 7.2 Hz), 6.55 (s, 1H), 6.67 (s, 1H), 7.54 (m, 3H), 7.89 (m, 2H,), 12.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.01, 15.94, 65.18, 69.30, 91.65, 106.03, 106.55, 126.66, 129.50, 131.83, 132.08, 132.19, 153.69, 153.79, 159.08, 164.24, 183.13; MS (EI) *m*/*z* 326 $[M]^+$, 311, 297 (base), 269. HRMS for $C_{19}H_{18}O_5$ $[M]^+$: calculated, 326.1154; found, 326.1155.

5-Hydroxy-6,7-(dioctyloxy)flavone (34). A mixture of **1** (81 mg, 0.3 mmol), 1-iodooctane (0.16 mL), and anhydrous K_2CO_3 (166 mg) in acetone (25 mL) was refluxed with stirring for 30 h. The reaction mixture was concentrated under reduced pressure, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). The extract was washed with water and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel and eluted with CH_2Cl_2/MeOH (100:1 to 50:1) to give compound **34** (122 mg, 82%) as a pale yellow powder. mp 85−86 °C; ^{1}H NMR ($CDCl_3$) δ 0.89, 0.90 (t, 6H, J = 6.9 Hz), 1.31−1.52 (m, 20H), 1.79, 1.89 (t, 4H, J = 6.9 Hz), 4.03, 4.07 (t, 4H, J = 6.9 Hz), 6.53 (s, 1H), 6.64 (s, 1H), 7.51 (m, 3H), 7.86 (m, 2H), 12.45 (s, 1H); MS (EI) *m*/*z* 494 $[M]^+$, 382, 270 (base).

6-Ethoxy-5-hydroxy-7-methoxyflavone (38). To a stirred solution of **32** (10 mg, 0.034 mmol) in a mixture of MeOH (3 mL) and THF (6 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.02 mL, 0.04 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/MeOH (50:1), afforded compound **38** (7.4 mg, 70%) as a pale yellow powder. mp 133−134 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (t, 3H, J = 6.9 Hz), 3.93 (s, 3H), 4.21 (q, 2H, J = 6.9 Hz), 6.57 (s, 1H), 6.69 (s, 1H), 7.56 (m, 3H), 7.90 (m, 2H), 12.68 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 15.01, 61.19, 65.25, 91.72, 106.04, 106.58, 126.66, 129.49, 131.81, 132.19, 133.29, 153.55, 153.72, 158.75, 164.30, 183.11; MS (EI) *m*/*z* 312 ($[M]^+$, base), 283. HRMS for $C_{18}H_{16}O_5$ $[M]^+$: calculated, 312.0998; found, 312.0997.

5-Hydroxy-6,7-(dipentyloxy)flavone (40). A mixture of **1** (51 mg, 0.19 mmol), 1-iodopentane (0.076 mL), and anhydrous K_2CO_3 (110 mg) in acetone (20 mL) was refluxed with stirring for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with water (40 mL), and extracted with CH_2Cl_2 (40 mL × 3). The extract was washed with water and dried over $MgSO_4$ and the solvent evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel and eluted with CH_2Cl_2/MeOH (60:1) to give compound **40** (68 mg, 87%) as a pale yellow powder. mp 111−112 °C; ^{1}H NMR ($CDCl_3$) δ 0.94, 0.97 (t, 6H, J = 6.6 Hz), 1.39−1.52 (m, 8H), 1.81, 1.91 (quintet, 4H, J = 6.6 Hz), 4.05, 4.09 (t, 4H, J = 6.6 Hz), 6.56 (s, 1H), 6.68 (s, 1H), 7.54 (m, 3H), 7.90 (m, 2H); MS (EI) *m*/*z* 410 $[M]^+$.

5-Methoxy-6,7-(dipentyloxy)flavone (41). To a stirred solution of **40** (33 mg, 0.08 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.32 mL, 0.64 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (20:1), gave compound **41** (32.6 mg, 98%) as a white solid. mp 107−108 °C; ^{1}H NMR ($CDCl_3$) δ 0.95, 0.97 (t, 6H, J = 6.6 Hz), 1.39−1.54 (m, 8H), 1.81, 1.93 (quintet, 4H, J = 6.6 Hz), 3.99 (s, 3H), 4.03, 4.10 (t, 4H, J = 6.6 Hz), 6.78 (s, 1H), 6.81 (s, 1H), 7.53 (m, 3H), 7.90 (m, 2H); MS (EI) *m*/*z* 424 $[M]^+$.

6,7-(Dihexyloxy)-5-hydroxyflavone (42). A mixture of **1** (54 mg, 0.2 mmol), 1-bromohexane (0.084 mL), and anhydrous K_2CO_3 (110 mg) in acetone (20 mL) was refluxed with stirring for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with water (40 mL) and extracted with CH_2Cl_2 (40 mL × 3). The extract was washed with water and dried

over $MgSO_4$ and the solvent evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel and eluted with CH_2Cl_2/MeOH (60:1) to give compound **42** (71.4 mg, 82%) as a pale yellow powder. mp 96−97 °C; ^{1}H NMR ($CDCl_3$) δ 0.92, 0.93 (t, 6H, J = 6.6 Hz), 1.32−1.41 (m, 8H), 1.48−1.53 (m, 4H), 1.80, 1.90 (quintet, 4H, J = 6.6 Hz), 4.05, 4.09 (t, 4H, J = 6.6 Hz), 6.56 (s, 1H), 6.68 (s, 1H), 7.54 (m, 3H), 7.90 (m, 2H); MS (EI) m/z 438 $[M]^+$.

6,7-(Dihexyloxy)-5-methoxyflavone (43). To a stirred solution of **42** (47 mg, 0.1 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.4 mL, 0.8 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (15:1), gave compound **43** (41 mg, 91%) as a white solid. mp 93−95 °C; ^{1}H NMR ($CDCl_3$) δ 0.92, 0.94 (t, 6H, J = 6.6 Hz), 1.30−1.41 (m, 8H), 1.51−1.55 (m, 4H), 1.80, 1.92 (quintet, 4H, J = 6.6 Hz), 3.99 (s, 3H), 4.03, 4.10 (t, 4H, J = 6.6 Hz), 6.76 (s, 1H), 6.81 (s, 1H), 7.52 (m, 3H), 7.90 (m, 2H); MS (EI) m/z 452 $[M]^+$.

6,7-Diethoxy-5-methoxyflavone (44). To a stirred solution of **33** (33 mg, 0.1 mmol) in a mixture of MeOH (4 mL) and THF (8 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.4 mL, 0.8 mmol). The reaction mixture was stirred at room temperature for 24 h and evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (10:1 to 5:1), afforded compound **44** (33.8 mg, 99%) as a white solid. mp 126−128 °C; ^{1}H NMR ($CDCl_3$) δ 1.42, 1.55 (t, 6H, J = 6.9 Hz), 4.00 (s, 3H), 4.13, 4.19 (q, 4H, J = 6.9 Hz), 6.77 (s, 1H), 6.81 (s, 1H), 7.52 (m, 3H), 7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.94, 16.05, 62.39, 65.13, 70.22, 97.15, 108.78, 113.18, 126.37, 129.36, 131.61, 132.10, 139.93, 153.27, 154.92, 157.94, 161.46, 177.67; MS (EI) m/z 340 $[M]^+$. HRMS for $C_{20}H_{20}O_5$ $[M]^+$: calculated, 340.1311; found, 340.1315.

6,7-Dibutoxy-5-hydroxyflavone (45). A mixture of **1** (81 mg, 0.3 mmol), 1-iodobutane (0.1 mL), and anhydrous K_2CO_3 (165 mg) in acetone (20 mL) was refluxed with stirring for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with water (50 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The extract was washed with water and dried over $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by flash chromatography on a column of silica gel eluted with CH_2Cl_2/MeOH (60:1) to give compound **45** (86 mg, 75%) as a yellow powder. mp 116−117 °C; ^{1}H NMR ($CDCl_3$) δ 0.99, 1.02 (t, 6H, J = 6.6 Hz), 1.53−1.58 (m, 4H), 1.79, 1.89 (quintet, 4H, J = 6.6 Hz), 4.06, 4.10 (t, 4H, J = 6.6 Hz), 6.56 (s, 1H), 6.68 (s, 1H), 7.54 (m, 3H), 7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.17, 14.31, 19.55, 19.62, 31.40, 32.61, 69.26, 73.51, 91.65, 106.00, 106.52, 126.63, 129.47, 131.86, 132.13, 132.49, 153.63, 153.71, 159.25, 164.17, 183.11; MS (EI) m/z 382 $[M]^+$. HRMS for $C_{23}H_{26}O_5$ $[M]^+$: calculated, 382.1780; found, 382.1780.

6,7-Dibutoxy-5-methoxyflavone (46). To a stirred solution of **45** (49 mg, 0.128 mmol) in a mixture of MeOH (5 mL) and THF (10 mL) was added $TMSCHN_2$ (2 M in hexanes, 0.5 mL, 1 mmol). The reaction mixture was stirred at room temperature for 24 h and then evaporated. Flash chromatography of the residue, eluting with CH_2Cl_2/acetone (15:1), gave compound **46** (46 mg, 91%) as a white solid. mp 103−105 °C; ^{1}H NMR ($CDCl_3$) δ 0.99, 1.03 (t, 6H, J = 6.6 Hz), 1.55−1.59 (m, 4H), 1.78, 1.91 (quintet, 4H, J = 6.6 Hz), 3.99 (s, 3H), 4.04, 4.11 (t, 4H, J = 6.6 Hz), 6.81 (s, 1H), 6.82 (s, 1H), 7.53 (m, 3H), 7.90 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.18, 14.26, 19.56, 19.63, 31.37, 32.69, 62.44, 69.19, 74.33, 97.17, 108.78, 113.18, 126.36, 129.34, 129.63, 130.21, 131.58, 132.13, 140.25, 153.18, 154.89, 158.11, 161.41, 177.68; MS (EI) m/z 396 $[M]^+$. HRMS for $C_{24}H_{28}O_5$ $[M]^+$: calculated, 396.1937; found, 396.1939.

Vinblastine Uptake by the KB/MDR Cells. KB/MDR cells,[38] which overexpress human P-gp 170 protein, were seeded into 24-well tissue culture plates in RPMI 1640 medium plus 10% fetal bovine serum for 24 h without the selecting agent doxorubicin (37 nM). The cells were then treated with [^{3}H]vinblastine plus synthetic flavones in HBSS for 60 min. The cells were washed twice with ice-cold PBS, harvested with 1 N NaOH, and neutralized with 1 N HCl. The cell aliquots were transferred to scintillation vials and counted with a β-counter (Beckman, model LS 5000) after adding 10 mL scintillation fluids (SafeScint, American Bioanalytical Co., Natick, MA). Data presented are the mean of three independent experiments.

Growth Inhibitory Assay. Approximately 10^4 KB or KB/MDR cells were seeded into 24-well tissue culture plates in RPMI 1640 medium plus 10% fetal bovine serum for 24 h, after which the cells were treated with various concentrations of synthetic flavones in culture medium for 3 days. The cells were then fixed and stained with methylene blue in 50% MeOH, washed thoroughly with tap water, and dissolved with 0.5 mL of 0.5% sarcosyl.[39] The amount of cellular protein, which is proportional to the cell number, is estimated by the absorption ($OD_{595\ nm}$). The growth inhibitory assay, which is presented as an IC_{50} value, represents the concentration of compound required to inhibit 50% of cell growth. The cell doubling time of KB and KB/MDR cells is about 20 to 24 h. The data presented are the mean of three independent experiments.

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Supporting Information Available: HPLC analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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