Flavonolignan and Flavone Inhibitors of a *Staphylococcus aureus* Multidrug Resistance Pump: Structure−Activity Relationships

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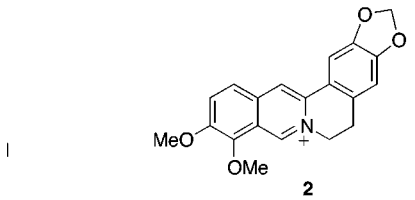
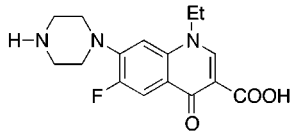
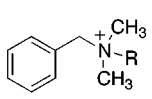
Although some progress has been reported on structure−activity relationships (SARs) for inhibitors of mammalian P-glycoprotein MDR efflux pumps, there is almost nothing in the literature regarding SARs for inhibitors of any bacterial efflux pump. Indeed, only a few of these have been described. Our discovery of a potent naturally occurring flavonolignan inhibitor of the NorA MDR pump of *Staphylococcus aureus* provided a structural foundation upon which SARs could be assessed via synthetic analogues. Several flavonolignans were prepared which proved to have greater potency than the natural isolate, 5′-methoxyhydnocarpin-D, while others showed decreased potency. Surprisingly, some simple alkylated flavones also were quite active MDR pump inhibitors. Variability of activity among the compounds tested was sufficient so that at least some SARs could be postulated and compared with those known for P-glycoprotein.

Introduction

Multidrug resistance (MDR) represents an increasing problem in the treatment of bacterial infections and cancer. It often appears after prolonged exposure of cells to a single drug and is often characterized by resistance to a series of structurally unrelated compounds.[1] One important resistance mechanism involves drug depletion in cells by membrane efflux proteins, for example P-glycoprotein (P-gp) in mammalian cells,[2] Bmr in *Bacillus subtilis*, and NorA in *Staphylococcus aureus*.[3] Although numerous small-molecule inhibitors of P-gp have been reported as well as some structure−activity relationships (SARs) postulated for these inhibitors, only a few inhibitors of bacterial MDR pumps are known. The alkaloid reserpine and some amide analogues were found to inhibit NorA in *S. aureus*.[4,5] Some tetracycline analogues can inhibit Tet protein efflux activity in *Escherichia coli*.[6] Diamide amines structurally reminiscent of those active against NorA[5] inhibited *Pseudomonas aeruginosa* efflux pumps.[7] We recently found two plant-derived compounds, the flavonolignan 5′-methoxyhydnocarpin-D[8] (**1**) and the porphyrin pheophorbide *a*,[9] to be potent inhibitors of the NorA MDR efflux pump in *S. aureus*. While exhibiting no activity themselves, **1** and pheophorbide *a* potentiated growth inhibitory activity of the natural antibacterial alkaloid berberine (**2**), the fluoroquinolone norfloxacin (**3**), and the antiseptic benzalkonium chloride (**4**) against resistant *S. aureus*. A number of hydnocarpin-type flavonolignans were synthesized[10] for structure determination purposes, and these were also found to exhibit a range of activity as potentiators of berberine activity. We report here the inhibition activities of those synthetics







(Figure 1) as well as those of an extended series of related compounds and simple flavones (Figure 2).

Chemistry

Synthetic flavonolignans (**1**, **5**, **6**, **8**−**12**, **14**) were prepared by reaction of a commercial catecholic flavone with a lignan (coniferyl alcohol or an analogue) via radical coupling.[10−12] Syntheses of **1**, **5**, and **6** were reported previously.[10] One of the couplings was initiated with horseradish peroxide (HRP) and the others with Ag_2CO_3. These methods typically gave different regioselectivities as described[10] for the preparation of **1** and **5**. The Ag_2CO_3 reactions yielded a mixture having the major product with the D ring in the "down" position (e.g. **1**) and the HRP reactions with that ring in the "up" position (e.g. **5**). Yields were not optimized in any of the reactions.

Syntheses of **7**, **8**, and **18** are exemplary (Scheme 1). A flavonolignan isolated from the plant *Onopordon corymbosum* was assigned structure **8** and given the name 5″-methoxyhydnocarpin.[13] There were no data presented which would distinguish **8** from its regioisomer **7**. Hydnocarpin itself had actually earlier been shown[14] to be the regioisomer **5** and not **6**, so the name

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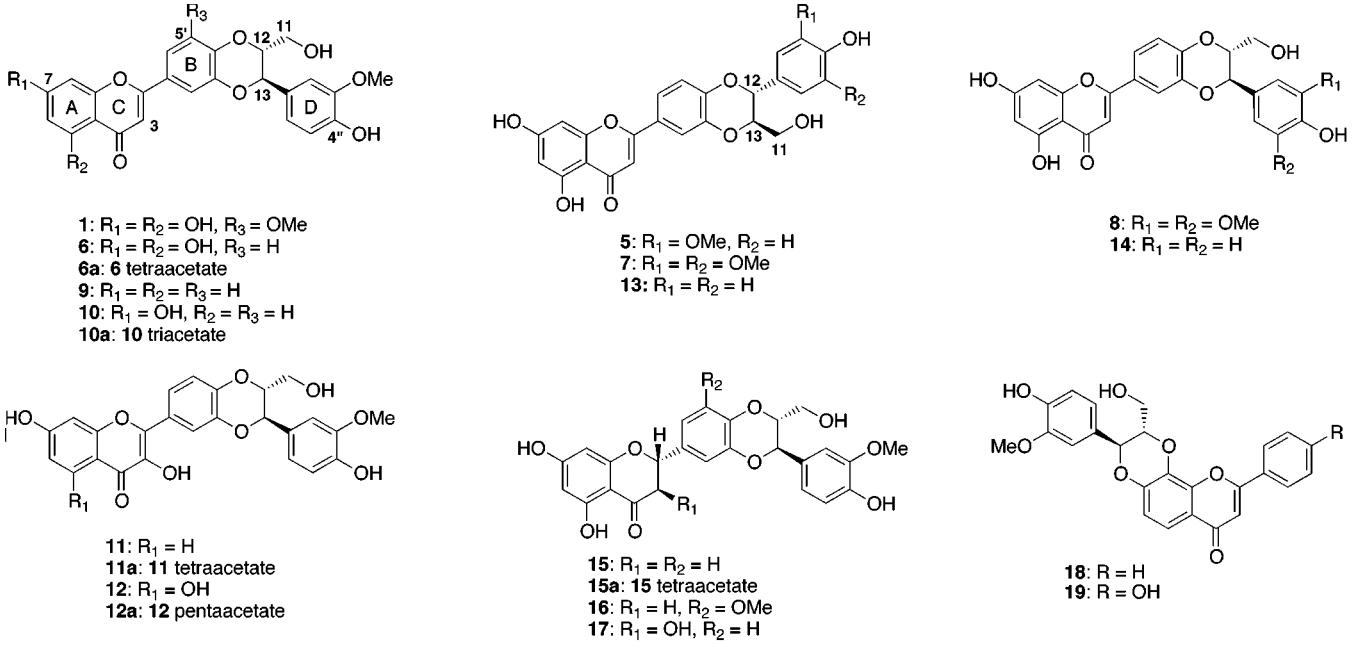


Figure 1. Flavonolignans tested as inhibitors of the *S. aureus* MDR efflux pump NorA.

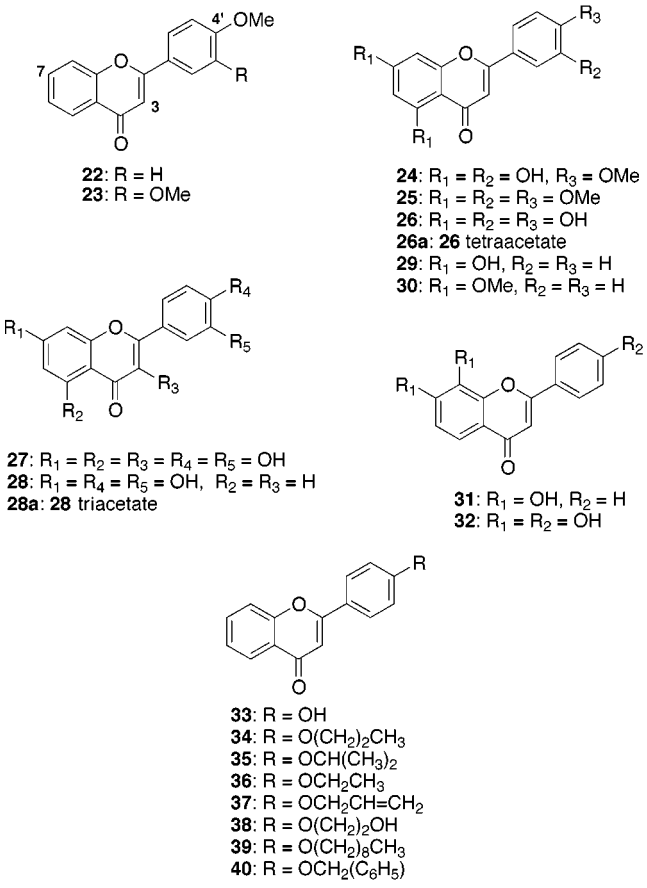


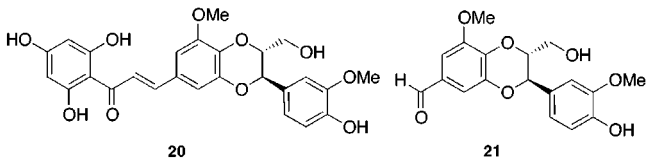
Figure 2. Flavones tested as inhibitors of the *S. aureus* MDR efflux pump NorA.

5″-methoxyhydnocarpin would not have been valid for **8**. Under our suggested nomenclature system,[10] **7** would be 5″-methoxyhydnocarpin and **8** would be 5″-methoxyhydnocarpin-D. Which structure represented the actual plant isolate[13] is not determinable without an authentic sample, which was unavailable. Our structures for **7** and **8** were verified by HMBC NMR.[10]

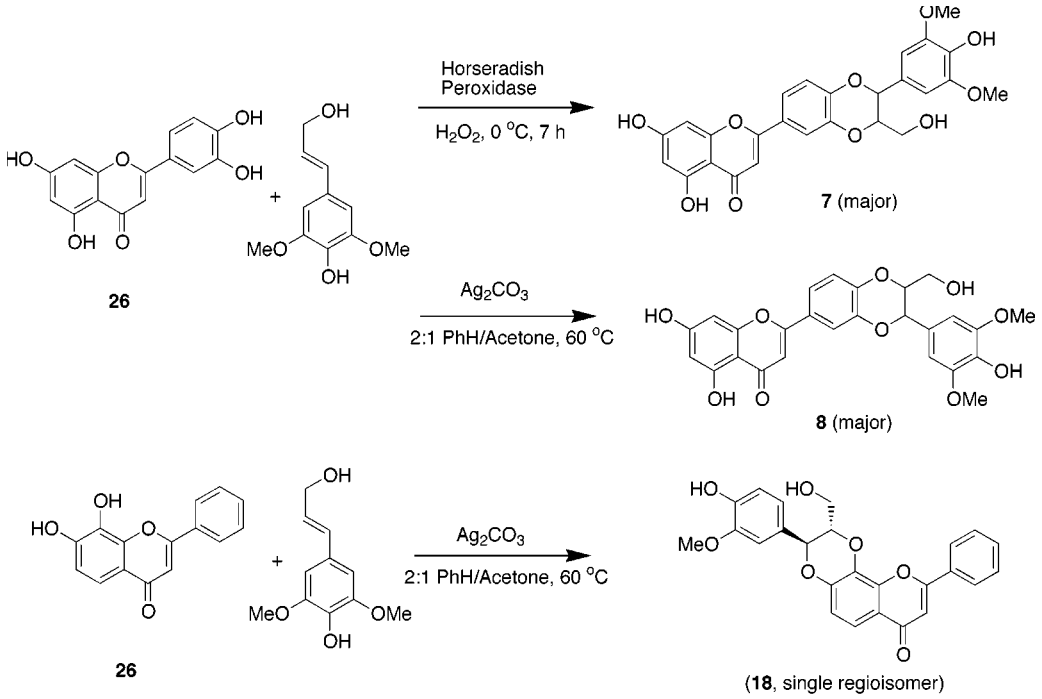
Flavonolignan **13** (sinaiticin) had been isolated from *Verbascum sinaiticum* and its structure confirmed by INEPT NMR.[15] In our hands, a regiopure sample of **13** could not be obtained via HRP initiation, but only a 3:2 **13**/**14** mixture. Regiopure **14** was obtained via Ag_2CO_3 initiation and the regiochemistry verified by HMBC NMR.[10] A synthesis of sinaiticin (**13**) was recently reported,[16] but no evidence was given which would distinguish the product from isomer **14** and a request for a sample was not answered.

The coupling of coniferyl alcohol to an A ring catecholic flavone proceeded with regiospecificity, unlike those exemplified by Scheme 1. The 5-OH analogues of **18** and **19** are known as scutellaprostin A and B, respectively, and were isolates from *Scutellaria prostrata*.[17] Both scutellaprostins were also reportedly synthesized as pure regioisomers via similar coupling reactions and Ag_2O.[17]

A few flavanolignans are also known as natural products. For example, (−)-silandrin (also known as 3-deoxyisosilybin) was isolated from *Silybum marianum* and was also synthesized.[18] We prepared the D ring "down" isomer, *rac*-silandrin-D (**15**) for testing, while the corresponding 5′-methoxy derivative **16** was available from previous synthetic work.[10] An extract of *Silibum marianum*, known as silymarin, is commerically available and was recrystallized from methanol to yield an approximately 50:50 mixture of silybin diastereomers **17** (the other diastereomer has the alternate trans stereochemistry of substituents on the benzodioxane ring). The chalcone **20** and its precursor **21**, intermediates in the synthesis[10] of **15** and **1**, were also available.



Scheme 1. Examples of Flavonolignan Syntheses



Some commercially available flavones were tested with varying results. Activity in a few, combined with the results on some ring A deoxy compounds, suggested the preparation and testing of 4′-hydroxyflavone (**33**) in comparison with some alkylated derivatives **22** and **34**−**40**, simply prepared from base-catalyzed reaction of **33** with several alkyl bromides.

Biological Assays

Cell Culturing and Susceptibility Testing. Growth of bacteria and susceptibility measurements were performed according to the National Center for Clinical Laboratory Standards recommendations. *S. aureus* RN4222[8] were cultured in Mueller−Hinton (MH) broth overnight with aeration at 37 °C. Cells were then inoculated into fresh MH medium at a 1:10 dilution and were allowed to grow for 1 h. This suspension was diluted 1:2000 into MH broth and 0.05 mL was dispensed per well of microtiter plates. For measurements of direct antimicrobial activity, test substances were serially diluted 2-fold in the wells. The final volume of a well was 0.2 mL, and the cell concentration was 10^5/mL. MIC was defined as a concentration of an antimicrobial that completely prevented cell growth during an 18-h incubation at 37 °C. Growth was assayed with a microtiter plate reader (Biorad) by absorption at 600 nm. Tests for MDR inhibitory activity were done similarly, but with berberine present at a subinhibitory concentration (30 μg/mL, 1/8 MIC) throughout. The test substance was then serially diluted 2-fold, and MIC for test substances was then defined as their minimal concentration that completely inhibited cell growth in the presence of 30 μg/mL berberine. MIC values (Tables 1 and 2) could vary within a factor of 2.

Results and Discussion

Bioassay results are summarized for flavonolignans (Table 1) and flavones (Table 2). None of compounds showed independent activity (with no berberine present) against *S. aureus*, except flavonolignan **18** and flavone **34**, which were only very weakly active (MIC = 125 μg/mL). Many compounds were, however, synergistic (some very potently so) with a subinhibitory concentration of berberine (**2**). A number of the tested flavonolignans (**6**, **6a**, **9**, **10**, **10a**, **18**, **19**) were as potent as or more potent (<2 μg/mL) than the natural product **1**. Flavonolignans with and without free phenolic groups at the 5 and 7 positions were comparably active (**6**, **9**, **10**, **18**, **19**), although the most potent, **9**, completely lacks A ring OH groups. The potency of peracetate derivatives (**6a**, **10a**, **15a**) was approximately the same as that of their parent compounds. These peracetates could be inherently active or, perhaps less likely, deacetylated by *S. aureus* and hence considered "prodrugs." The presence of a 3-hydroxy group resulted in markedly decreased activity (**11**)

Table 1. Minimum Inhibitory Concentration of Flavonolignans That Inhibit Growth of *S. aureus* in the Presence of Subinhibitory (30 μg/mL) Berberine, **2**

compd	MIC (μg/mL)	compd	MIC (μg/mL)	compd	MIC (μg/mL)
1	1−2	**10**	0.8	**16**	1.9
5	3.1	**10a**	1.6	**17**	12.5
6	0.1	**11**	163	**18**	1.9
6a	0.1	**12**, **12a**	inactive	**19**	0.6
7	7.8	**14**	inactive	**20**, **21**	inactive
8	4−8	**15**	3.1		
9	0.08	**15a**	1.6		

Table 2. Minimum Inhibitory Concentration of Flavones That Inhibit Growth of *S. aureus* in the Presence of Subinhibitory (30 μg/mL) Berberine, **2**

compd	MIC (μg/mL)	compd	MIC (μg/mL)	compd	MIC (μg/mL)
22	6.3	**30**	25	**37**	6.3
23	6.3	**31**−**33**	inactive	**38**	25
24	15	**34**	0.4	**39**, **40**	inactive
25−**28a**	inactive	**35**	1.9		
29	25	**36**	1.6		

or no activity (**12**, **12a**) in flavone-derived systems. In the case of the one 3-hydroxyflavanone we tested, silybin (**17**), potency was, however, still fairly high.

In contrast to the case of A ring substitution, some changes on the D ring played an important role. The majority of flavonolignans had 3-methoxy-4-hydroxy substitution and were quite active, while 3,5-dimethoxy-4-hydroxy D ring substituted compounds (**7**, **8**) were slightly less active. On the other hand, **14**, with only 4-hydroxy substitution on ring D was, perhaps remarkably, completely inactive. The regiochemistry of the D ring benzodioxane ring fusion in the flavonolignans was important in one case where we were able to compare isomers (**5** vs **6**), but not in another case (**7** vs **8**). The two scutellaprostin analogues **18** and **19** were both quite active, so in this system H vs OH in ring B is not critical. Two synthetic intermediates, chalcone **20** and its precursor **21**, were inactive.

Most of the commercially available simple flavones (Figure 2 and Table 2), especially those with free phenolic groups or as peracetates, had little or no activity (**26**, **26a**, **27**, **28**, **28a**, **29**, **32**). Luteolin (**26**), which was the flavone most often used for preparation of flavonolignans (Scheme 1), was not active, nor was coniferyl alcohol, often used as the coupling partner (Scheme 1) with the catecholic flavones. Flavones devoid of B ring substitution (chrysin (**29**) and its dimethyl ether **30**) as well as two devoid of substitution on the A ring (**22** and **23**) had moderate activity. Diosmetin (**24**), which is the 4′ methyl ether of the inactive luteolin, was surprisingly active (15 μg/mL). The relatively high activity of **22**−**24** suggested that 4′-alkylation might be of importance and led to the synthesis of various ethers of 4′-hydroxyflavone (**33**). While **33** was inactive, derivatives with small lipophilic alkylated side chains (**34**−**38**) were quite active. As lipophilicity was increased, however, activity was completely lost (**39**, **40**). The difference between **33** and **34** is particularly striking. The importance of maintaining lipophilicity at the 4′ carbon was also demonstrated by the decreased activity for **38** as compared to **34**. Because of the potency of **34**−**36** and their simplified structures as compared to the flavonolignans, they were also tested against a mutant *S. aureus* strain lacking NorA. They did not potentiate the activity of norfloxacin against the mutant, as was similarly the case for the flavonolignan **1**, thus indicating that the activities for **34**−**36** against the wild-type *S. aureus* were indeed due to NorA inhibition.

It is of interest to compare these *S. aureus* NorA inhibitors with some inhibitors of the mammalian MDR P-gp efflux proteins. Any inhibitor of a microbial MDR pump developed for therapeutic use should not affect P-gp, which plays a role in xenobiotic efflux of normal tissues. SARs of existing inhibitors will help in designing therapeutic compounds that specifically inhibit microbial MDR pumps but not P-gp. A comparison of SARs of these two different proteins, NorA belonging to the major facilitator family of transporters and P-gp being a member of the ABC family, might shed light on some general principles of drug binding and recognition by multidrug pumps. An extremely wide variety of structurally unrelated P-gp inhibitors is known,[19] but as has been pointed out,[20] P-gp nevertheless "displays a significant sensitivity to structural changes within a particular series of compounds". This seems to be true for *S. aureus* NorA inhibitors as well since the data (Tables 1 and 2) show very clear SARs among fairly closely related compounds, even though the other natural inhibitor we discovered[9] was the structurally completely unrelated porphyrin pheophorbide *a*. A cyanobacterium porphinoid was also reported to be a P-gp inhibitor.[21] It is possible that these effects in P-gp stem from the fact that P-gp has both ATP-binding and steroid-interacting sites,[22,23] or one large binding site with a number of distinct subpockets.[24] These sites or subpockets in P-gp and NorA could interact differentially with structurally very diverse inhibitors. A previously known naturally occurring inhibitor of NorA was the indole alkaloid reserpine, while an active nitroindole, a biarylurea, and other inhibitors were found by chemical library screening.[5] One study[25] has genetically compared a bacterial-resistance protein (LmrA in *Lactobacillus lactis*) with the P-gp gene. In one case where some diaryl peptide bacterial efflux pump inhibitors were tested for P-gp inhibitory activity, they proved inactive.[7]

There have been a few SAR studies on P-gp inhibitors which can be compared with the present results. Most closely related to the present work were studies on some natural flavonoids,[26] some synthetically modified flavonoid derivatives,[27] and some halogenated chalcones.[28] These papers also provide an entrance into extensive literature in this area. Among the natural flavonoids, kaempferide (3,5,7-trihydroxy-4′-methoxyflavone) was found to be most active, while kaempferol (3,5,7,4′-tetrahydroxyflavone) and chrysin (**29**) were less so.[26] The reverse occurred with NorA inhibition (Table 2), with chrysin being the most active followed by kaempferide (100 μg/mL). The latter two flavones have 3-OH substituents, which were suggested[26] to be valuable for P-gp inhibition, while we found such substitution to be detrimental to NorA inhibition (**11**, **12**). Active MDR modulators of human leukemia cells were reported[29] to display two or more lipophilic ring systems separated by a linker containing a basic nitrogen. As a result of studies on some verapamil analogues, it was concluded that besides overall lipophilicity, weak polar interactions provided by overlapping of aromatic rings were valuable for binding MDR-reverting agents to P-gp.[30] The importance of a basic nitrogen atom for best P-gp-mediated efflux was also affirmed by some studies on active tetrahydroisoquinolines, but here high molar refractivity values were considered to be more important than lipophilicity.[31] Some natural flavones were modified by attaching substituted *N*-benzylpiperazinyl side chains to the 5- and 7-OH groups.[27] Several of these were quite potent, but it was also found that activity depended not only on these substituents but also on the type of flavonoid moiety and the nature of substituents on other parts of the flavonoid. Acylation of the 7-OH was very important since derivatives with a free 7-OH were devoid of activity, as was the parent diosmetin (**24**). In the case of NorA inhibition, however, presence of a 7-OH did not negate activity, although the most potent compounds in both the flavonolignan and flavone series lacked any OH groups in ring A. Diosmetin itself was also quite active against NorA. Replacement of the flavone ring by a flavanone did not markedly alter MDR-

modulating efficiency,[27] and this was true in our flavonolignan series as well (**15**−**17**). The one chalcone we tested, **20**, was inactive against NorA, while several were found[28] to have high-affinity binding to P-gp. The most active in that case was 2′,4′,6′-trihydroxy-4-iodochalcone. The work in general showed an importance for lipophilicity at C-4, with a 4-OMe group conferring higher activity than a 4-OH group and increasing activity in the sequence 4-Cl/4-Br/4-I. This is partially mirrored by our results (Table 2) with simple flavones, although here presence of a free 4′-OH (which corresponds to the 4-OH in chalcones) completely negates MDR inhibition activity. In a synthesis of a complex natural product, ningalin B (which contains four phenolic groups), a synthetic tetramethoxy intermediate proved to have activity against P-gp although ningalin B did not.[32] An intermediate bearing a carboxylic acid was inactive, but the corresponding methyl ester was active. The presence/absence of acidic functions (either phenolic or as a carboxylic acid) thus seems to sometimes have marked influence on the potency of MDR inhibitors in both the bacterial NorA and mammalian P-gp systems. In summary, we have found both overall structural similarities between some inhibitors of these two efflux pump proteins but also differences in effects related to changes of functional groups. The flavonolignan **1** was also tested as an inhibitor against the Gram-negative bacteria *E. coli* and *P. aeruginosa* but was ineffective.

Conclusions

Some potent potentiators of antimicrobial activity, which apparently act by inhibition of the *S. aureus* MDR efflux pump protein NorA, have been found. The range of activites discovered have provided SARs which, in comparison with some SARs known for P-gp inhibitors, provide information which could be useful for understanding inhibitor/NorA interactions. Further systematic studies of bacterial MDR efflux pump inhibitors should prove valuable in helping to understand and combat antibiotic resistance.[33]

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Inova spectrometer at 400 and 100 MHz, respectively, using $CDCl_3$, acetone-d_6, methanol-d_4, or DMSO-d_6 as the solvent and internal reference. Melting points were determined on a Laboratory Device's Mel-Temp and are uncorrected. All solvents were distilled prior to use. THF was freshly distilled from benzophenone-ketyl and benzene was freshly distilled from CaH_2. ACS acetone was stored over 4-Å molecular sieves. All nonaqueous reactions were performed in dry glassware under an argon atmosphere. All starting materials were used as received. Acetylations were performed using standard acetic anhydride/pyridine conditions. All flavonoids except quercetin dihydrate were purchased from Indofine Chemical Co. and all other reagents purchased from Aldrich Chemical Co. All column chromatography separations (CC) were performed with normal-phase silica gel (Scientific Adsorbents Inc.; 32−63-μm particle size, 60-Å pore size). Coniferyl, sinapyl, and *p*-coumaryl alcohols were prepared by a DIBAL reduction (THF, 0 °C, 12 h, >75% yield) of the corresponding aldehyde.

Flavonolignans. 5″-Methoxyhydnocarpin (7). To a 100-mL three-neck round-bottom flask were added 0.300 g (1.05 mmol) of luteolin and 0.220 g (1.05 mmol) of sinapyl alcohol. Next, 20 mL of acetone and 5 mL of a 0.2 M citric acid/phosphate buffer were added to the reaction flask and the flask cooled to 0 °C. Two drops of 30% H_2O_2 and 1 mL of a horseradish peroxidase solution (1.5 mg HRP (1100 U/mg)/3 mL water) were added. The HRP solution (1 mL) was added every 15 min thereafter, and the reaction then allowed to stir at 0 °C for 7 h. The reaction solution was allowed to warm to room temperature, washed with brine, and extracted with EtOAc. The EtOAc was dried with anhydrous $MgSO_4$, filtered, and removed by rotary evaporation to yield a brown-orange solid. The solid was subjected to CC using 92:8 $CHCl_3$/MeOH to yield 0.192 g (37%) of a yellow-orange solid, 3:2 ratio of **7**/**8** (by ^{1}H NMR) with minor impurities present. This solid was recrystallized from 9:1 MeOH/H_2O to give 0.025 g of regiopure **7**: yellow crystals, mp 260 °C dec; ^{1}H NMR (75 °C, DMSO-d_6) δ 3.46 (m), 3.63 (dd, J = 12.6, 2.8 Hz), 4.29 (m), 4.77 (t, CH_2−O*H*), 5.03 (d, J = 7.8 Hz), 6.22 (d, J = 2.0 Hz), 6.51 (d, J = 2.0 Hz), 6.76 (s), 6.79 (s), 7.09 (d, J = 8.4 Hz), 7.58 (d, J = 8.6, 2.0 Hz), 7.63 (d, J = 2.0 Hz); ^{13}C NMR (25 °C, DMSO-d_6) δ 56.1, 60.1, 76.7, 77.9, 94.1, 98.9, 103.8, 103.9, 105.4, 114.8, 117.6, 119.9, 123.7, 126.0, 136.1, 143.7, 147.1, 148.0, 157.3, 161.4, 162.9, 164.3, 181.8. Anal. ($C_{26}H_{22}O_{10}\cdot 3H_2O$) C, H.

5″-Methoxyhydnocarpin-D (8). To a 100-mL three-neck round-bottom flask were added 0.160 g (0.559 mmol) of luteolin, 0.118 g (0.559 mmol) of sinapyl alcohol, 40 mL benzene and 20 mL of acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.154 g (0.559 mmol) of Ag_2CO_3 was added and the reaction solution stirred vigorously for 10 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a pale yellow powder. The powder was subjected to CC using 4:1 CH_2Cl_2/$(CH_3)_2CO$ to yield 0.052 g of pure **8** (19%, pale yellow powder, 6:1 **8**/**7**). The purified material was recrystallized from 1:1 H_2O/MeOH to give 0.033 g of regiopure **8**: pale yellow white crystals, mp 213−214 °C; ^{1}H NMR (DMSO-d_6) δ 3.38 (dd, J = 12.6, 4.4 Hz), 3.57 (dd, J = 12.6, 2.8 Hz), 3.78 (s, OMe), 4.33 (m), 4.95 (d, J = 8.0 Hz), 6.19 (d, J = 2.0 Hz), 6.50 (d, J = 2.0 Hz), 6.77 (s), 6.87 (s), 7.12 (d, J = 8.4 Hz), 7.63 (dd, J = 8.4, 2.0 Hz), 7.67 (d, J = 2.0 Hz) 12.91 (s); ^{13}C NMR (DMSO-d_6) δ 56.1, 60.0, 76.2, 78.5, 94.1, 98.9, 103.8, 103.9, 105.4, 115.1, 117.4, 120.1, 123.4, 126.1, 136.1, 144.0, 146.9, 148.0, 157.3, 161.4, 162.9, 164.3, 181.8. Anal. ($C_{26}H_{22}O_{10}\cdot 2.5H_2O$), C, H.

5,7-Deoxyhydnocarpin-D (9). The title compound was synthesized in the same manner as **8** using 0.291 g (1.15 mmol) of 3,4-dihydroxyflavone, 0.207 g (1.15 mmol) of coniferyl alcohol, 25 mL of benzene, 12.5 mL of acetone, and 0.317 g (1.15 mmol) of Ag_2CO_3 to yield a dark yellow solid. This solid was subjected to CC using 93:7 $CHCl_3$/MeOH to yield 0.129 g of a 7:1 down/up mixture of regioisomers and minor impurities. A portion (0.016 g) of the purified product **9** was subjected to acetylation to give 0.014 g of acetylated product that was further purified by CC (1:1 hexanes/EtOAc) to give 12 fractions of pure **9a**, in which 3 fractions (0.006 g) contained a 15:1 down/up mixture (by ^{1}H NMR) of regioisomers: white microcrystalline solid, mp 172−174 °C. Peracetate: ^{1}H NMR ($CDCl_3$) δ 2.09 (s), 2.35 (s), 3.89 (s, OMe), 4.04 (dd, J = 12.4, 4.4 Hz), 4.36 (m), 4.42 (dd, J = 12.4, 3.2 Hz), 5.01 (d, J = 8.0 Hz), 6.76 (s), 7.00 (d, J = 2.0 Hz), 7.02 (m), 7.12 (J = 8.0 Hz), 7.12 (d, J = 8.0 Hz), 7.42 (ddd, J = 8.2, 6.8, 0.8 Hz), 7.52 (d, J = 2.0 Hz), 7.54 (d, J = 2.0 Hz), 7.54 (dd, J = 8.4, 1.6 Hz), 7.61 (d, J = 2.0 Hz), 7.63 (dd, J = 8.0, 2.0 Hz), 7.70 (ddd, J = 8.6, 7.0, 1.6 Hz), 8.23 (dd, J = 8.4, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.7, 56.0, 62.5, 75.9, 76.4, 106.7, 111.0, 115.5, 117.8, 118.0, 119.8, 120.4, 123.4, 123.9, 125.2, 125.4, 125.7, 133.7, 134.0, 140.7, 143.7, 145.9, 151.7, 156.2, 163.0, 168.7, 170.4, 178.3. Anal. ($C_{25}H_{20}O_7\cdot 0.5H_2O$) C, H.

5-Deoxyhydnocarpin-D (10). To a 250-mL three-neck round-bottom flask were added 0.400 g (1.48 mmol) of 7,3′,4′-trihydroxyflavone, 0.267 g (1.48 mmol) of coniferyl alcohol, 70 mL of benzene, and 35 mL of acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.408 g (1.48 mmol) of Ag_2CO_3 was added and the reaction solution stirred vigorously for 36 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield a yellow powder. The

yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.172 g of a pale yellow powder that contained a 6:1 down/up (by ^1H NMR) mixture of regioisomers and dehydrodiconiferyl alcohol. VLC (vacuum liquid chromatography) using C-18 silica gel (4:1 H_2O/MeOH to 100% MeOH) yielded 0.073 g of pure **10** (11%, pale yellow powder, 6:1 down/up regioisomers). A portion (0.035 g) of the purified flavonolignan was acetylated (0.039 g, 89%) and recrystallized from 1:1 H_2O/MeOH to yield 0.025 g (64% from acetylated product) of regiopure **10a**. Peracetate: white microcrystalline sold, mp 212 °C; ^1H NMR ($CDCl_3$) δ 2.09 (s), 2.34 (s), 2.37(s), 3.88 (s, OMe), 4.05 (dd, J = 12.3, 4.0 Hz), 4.35 (m), 4.42 (dd, J = 12.3, 3.2 Hz), 6.75 (s), 7.01 (m, 2H), 7.11 (d, J = 8.8 Hz), 7.12 (d, J = 8.0 Hz), 7.16 (dd, J = 8.0, 2.2 Hz), 7.37 (d, J = 2.2 Hz), 7.50 (dd, J = 8.4, 2.2 Hz), 7.57 (d, J = 2.2 Hz), 8.24 (d, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 21.2, 56.0, 62.4, 75.9, 76.3, 106.6, 110.9, 111.0, 115.4, 117.9, 119.3, 119.8, 120.4, 121.6, 123.3, 125.0, 127.1, 134.0, 140.7, 143.7, 146.1, 151.7, 154.5, 156.6, 163.2, 168.5, 168.7, 170.3, 177.6. Anal. ($C_{31}H_{26}O_{11}$) C, H.

5-Deoxy-3-hydroxyhydocarpin-D (11). The title compound was synthesized in the same manner as **10** using 0.200 g (0.699 mmol) of fisetin, 0.126 g (0.699 mmol) of coniferyl alcohol, 50 mL of benzene, 25 mL of acetone and 0.193 g (0.699 mmol) of Ag_2CO_3 for 36 h to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.172 g of a pale yellow powder that contained a 14:1 down/up (by ^1H NMR) mixture of regioisomers and dehydrodiconiferyl alcohol. VLC using C-18 silica gel (4:1 H_2O/MeOH to 100% MeOH) yielded 0.086 of pure **12**: 27%, pale yellow powder; ^1H NMR (acetone-d_6) δ 3.55 (dd, J = 12.3, 4.0 Hz), 3.79 (dd, J = 12.3, 2.5 Hz), 3.89 (s, OMe), 4.24 (m), 5.06 (d, J = 8.4 Hz), 6.90 (d, J = 8.1 Hz), 6.99 (dd, J = 8.7, 2.1 Hz), 7.01 (dd, J = 8.1, 1.8 Hz), 7.08 (d, J = 9.0 Hz), 7.09, (d, J = 2.1 Hz), 7.18 (d, J = 1.8 Hz), 7.86 (m, 2H), 8.02 (d, J = 8.7 Hz). Peracetate **11a**: white microcrystalline solid, mp 199−200 °C; ^1H NMR ($CDCl_3$) δ 2.09 (s), 2.34 (s), 2.37 (s), 2.38 (s), 3.88 (s, OMe), 4.03 (dd, J = 12.3, 4.0 Hz), 4.34 (m), 4.42 (dd, J = 12.3, 3.2 Hz), 5.01 (d, 8.0 Hz), 7.00 (dd, J = 8.4, 1.6 Hz), 7.01 (d, J = 1.6 Hz), 7.11 (d, J = 8.4 Hz), 7.12 (d, J = 8.4 Hz), 7.17 (dd, J = 8.8, 2.4 Hz), 7.39 (d, J = 2.4 Hz), 7.51 (J = 8.4, 2.4 Hz), 7.57 (d, J = 2.4 Hz), 8.25 (d, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.6, 20.7, 21.2, 56.0, 62.5, 75.9, 76.3, 110.9, 111.0, 117.5, 119.4, 119.8, 121.3, 122.4, 123.2, 123.3, 127.4, 133.4, 134.0, 140.6, 143.5, 145.7, 151.7, 154.7, 155.7, 155.9, 168.0, 168.4, 168.7, 170.4, 171.5. Anal. ($C_{33}H_{28}O_{13}$) C, H.

3-Hydroxyhydnocarpin-D (12). The title compound was synthesized in the same manner as **10** using 0.600 g (1.77 mmol) of quercetin dihydrate, 0.320 g (1.77 mmol) of coniferyl alcohol, 90 mL of benzene and 45 mL of acetone, and 0.489 g (1.77 mmol) of Ag_2CO_3 to yield a yellow powder. The yellow powder was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.341 g of a yellow powder that contained >30:1 down/up ratio of regioisomers (by ^1H NMR) plus minor impurities. In the more concentrated CC fractions, the product crystallized out to give regiopure samples of pure **12** (0.055 g). The remaining fractions were subjected to VLC using C-18 silica gel (3:2 H_2O/MeOH to 1:4 H_2O/MeOH) to give 0.142 g of a regiopure sample of pure **12**: 23% combined, yellow powder, mp 279−280 °C; ^1H NMR (acetone-d_6) δ 3.54 (dd, J = 12.4, 4.0 Hz), 3.78 (dd, J = 12.4, 2.4 Hz), 3.89 (s, OMe), 4.25 (m), 5.06 (d, J = 8.4 Hz), 6.27 (d, J = 2.0 Hz), 6.59 (d, J = 2.0 Hz), 6.90 (d, J = 8.0 Hz), 7.01 (dd, J = 8.4, 2.0 Hz), 7.10 (d, J = 8.4 Hz), 7.17 (d, J = 2.0 Hz), 7.85 (dd, J = 8.4, 2.0 Hz), 7.86 (d, J = 2.0 Hz), 12.14 (s); ^{13}C NMR (acetone-d_6) δ 56.3, 61.7, 77.2, 80.0, 94.6, 99.2, 104.2, 111.9, 115.8, 117.2, 117.7, 121.7, 122.2, 124.9, 128.9, 137.1, 144.8, 146.1, 146.5, 148.1, 148.5, 157.8, 162.3, 165.1, 172.8, 176.6. Peracetate **13a**: pale yellow microcrystals, mp 176−178 °C; ^1H NMR ($CDCl_3$) δ 2.09 (s), 2.34 (s), 2.35 (s), 2.35 (s), 2.44 (s), 3.88 (s, OMe), 4.02 (dd, J = 12.0, 4.0 Hz), 4.34 (m), 4.41 (dd, J = 12.0, 3.2 Hz), 5.00 (d, J = 8.0 Hz), 6.99 (dd, J = 8.4, 2.0 Hz), 7.01 (d, J = 2.0 Hz), 7.09 (d, J = 8.4 Hz), 7.12 (d, J = 8.4 Hz), 7.32 (d, J = 2.0 Hz), 7.47 (dd, J = 8.4, 2.0 Hz), 7.53 (d, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 20.9, 21.3, 21.4, 56.2, 62.7, 76.1, 76.5, 109.1, 111.2, 113.9, 117.6, 117.7, 120.0, 122.6, 123.0, 123.5, 133.7, 134.2, 140.9, 143.7, 146.0, 150.5, 151.9, 154.3, 154.9, 157.0, 168.0, 168.1, 168.9, 169.5, 170.3, 170.6. Anal. ($C_{35}H_{30}O_{15}$) C, H.

Sinaiticin-D (14). The title compound was synthesized in the same manner as **8** using 0.200 g (0.699 mmol) of luteolin, 0.105 g (0.699 mmol) of *p*-coumaryl alcohol, 25 mL of benzene, 12.5 mL of acetone, and 0.193 g (0.699 mmol) of Ag_2CO_3 to yield a brown-orange solid. The solid was subjected to CC using 91:9 $CHCl_3$/MeOH to yield 0.146 g (48%) of a 1:2 mixture of **14**/**13** and minor impurities. The solid was recrystallized from MeOH to yield 0.038 g of regiopure **14**: 13%, yellow crystals, mp 269 °C; ^1H NMR (DMSO-d_6) δ 3.34 (dd, J = 12.0, 4.0 Hz), 3.58 (dd, J = 12.0, 2.4 Hz), 4.26 (m), 4.98 (d, J = 8.0 Hz), 6.19 (d, J = 1.8 Hz), 6.50 (d, J = 1.8 Hz), 6.81 (d, J = 8.8 Hz), 6.87 (s), 7.11 (d, J = 8.8 Hz), 7.29 (d, J = 8.8 Hz), 7.62 (d, J = 8.8, 2.0 Hz), 7.65 (d, J = 2.0 Hz), 12.90 (s); ^{13}C NMR (DMSO-d_6) δ 60.0, 75.7, 78.6, 94.1, 98.9, 103.7, 103.9, 115.0, 115.3, 117.4, 120.1, 123.5, 126.5, 129.2, 144.0, 146.9, 157.3, 157.9,161.4, 162.9, 164.5, 181.8. Anal. ($C_{24}H_{18}O_8$) C, H.

***rac*-Silandrin-D (15).** The title compound was synthesized in the same manner as **10** using 0.400 g (1.39 mmol) of eriodictyol, 0.250 g (1.39 mmol) of coniferyl alcohol, 60 mL of benzene, 30 mL of acetone, and 0.383 g (1.39 mmol) of Ag_2CO_3 to yield a brown-white solid. This solid was subjected to CC 95:5 $CHCl_3$/MeOH to yield 0.262 g of a diastereomeric mixture of near pure **15** with only the down regioisomer detectible by ^1H NMR. This sample was further purfied using CC (7:3 hexanes/EtOAc) to yield 0.208 g of a regiopure diastereomeric mixture of **15**: 24%, white microcrystalline solid; key ^1H NMR resonances (acetone-d_6) δ 2.78 (m), 3.18 (dd, J = 17.0, 12.8 Hz), 3.52 (dd, J = 12.4, 4.0 Hz), 3.75 (dd, J = 12.4, 2.5 Hz), 3.87 (s, OMe), 4.14 (m), 5.00 (d, J = 8.0 Hz), 5.48 (m), 5,96 (m), 6.84−7.14 (m), 12.17 (s), 12.18 (s) (59:41 mixture of diastereomers by ^1H NMR). Key peracetate ^1H NMR resonances ($CDCl_3$): δ 2.07 (s), 2.08 (s), 2.30 (s), 2.31 (s), 2.33 (s), 2.39 (s), 2.39 (s), 2.76 (br d, J = 16.8 Hz), 3.05 (br d, J = 16.8 Hz), 3.86 (s, OMe), 4.00 (dd, J = 12.0, 4.0 Hz), 4.26 (m), 4.37 (dd, J = 12.0, 2.5 Hz), 4.96 (d, J = 8.0 Hz), 5.42 (br d, J = 13.2 Hz), 6.54 (m), 6.79 (m), 6.95−7.10 (m). Anal. ($C_{33}H_{30}O_{13}$) C, H.

5-Deoxyscutellaprostin-A (18). To a 250-mL three-neck round-bottom flask were added 0.400 g (0.157 mmol) of 7,8-dihydroxyflavone, 0.284 g (0.157 mmol) of coniferyl alcohol, 130 mL of benzene, and 65 mL of acetone. The reaction vessel was placed in a 60 °C oil bath and let stir for 10 min. Next, 0.758 g (2.75 mmol) of Ag_2CO_3 was added and the reaction vigorously stirred for 24 h. The reaction was then allowed to cool, filtered through a Buchner funnel, and the solvent removed by rotary evaporation to yield an orange solid. The solid was subjected to CC using 95:5 $CHCl_3$/MeOH to yield 0.270 g of near pure sample of **18**. This sample was subjected to VLC using C-18 silica gel (1:1 H_2O/MeOH to 3:7 H_2O/MeOH) to yield 0.142 g of pure **18**: 21%, yellow microcrystals, mp 233−234 °C; ^1H NMR (35 °C, DMSO-d_6) δ 3.48 (br d, J = 12.4 Hz), 3.74 (br d, J = 12.4 Hz), 3.80 (s, OMe), 4.40 (m), 5.08 (t, CH_2−OH), 5.14 (d, J = 7.6 Hz), 6.83 (d, J = 8.0 Hz), 6.92 (dd, J = 8.0, 2.0 Hz), 7.01 (s), 7.07 (d, J = 2.0 Hz), 7.09 (d, J = 8.8 Hz), 7.54 (d, J = 8.8 Hz), 7.61 (m), 8.13 (m), 9.17 (s); ^{13}C NMR (35 °C, DMSO-d_6) δ 55.7, 60.0, 76.3. 78.0, 106.6, 111.9, 114.8, 115.4, 115.9, 117.9, 120.6, 126.2, 126.7, 129.1, 131.2, 131.7, 132.2, 145.8, 147.2, 147.6, 161.8, 176.4. Anal. ($C_{25}H_{20}O_7$) C, H.

5-Deoxyscutellaprostin-B (19). The title compound was synthesized in the same manner as **18** using 0.350 g (1.30 mmol) of 7,8,4′-trihydroxyflavone, 0.233 g (1.30 mmol) of coniferyl alcohol, 110 mL of benzene, and 55 mL of acetone and 0.625 g (2.28 mmol) of Ag_2CO_3 to yield an orange oil. The oil was subjected to column chromatography (CC) using 95:5 $CHCl_3$/MeOH to yield 0.145 g of a regiopure sample of **19** and dehydrodiconiferyl alcohol. VLC using C-18 silica gel (1:1 H_2O/MeOH to 1:4 H_2O/MeOH) yielded 0.050 g of pure **19**: 12%, white powder, mp 254 °C; ^1H NMR (DMSO-d_6) δ 3.45 (br d, J = 12.8 Hz), 3.71 (br d, J = 12.8 Hz), 3.79 (s, OMe), 4.39 (m), 5.10 (t, CH_2−OH), 5.11 (d, J = 7.6 Hz), 6.82 (d, J = 8.0 Hz),

6.83 (s), 6.91 (d, J = 8.0, 2.0 Hz), 6.94 (d, J = 8.8 Hz), 7.06 (d, J = 8.8 Hz), 7.07 (d, J = 2.0 Hz), 7.50 (d, A of A_2B_2), 8.13 (d, B of A_2B_2), 9.22 (bs), 10.32 (bs); ^{13}C NMR (DMSO-d_6) δ 55.7, 60.0, 76.4, 78.1, 104.4, 111.8, 114.5, 115.4, 115.8, 115.9, 117.9, 120.6, 121.6, 126.8, 128.2, 132.2, 145.7, 147.2, 147.5, 147.7, 160.9, 162.3, 176.3. Anal. ($C_{25}H_{20}O_8$) HRFAB$^+$.

Flavones. 4′-Benzyloxyflavone (40). To a 100-mL three-neck round-bottom flask were added 0.075 g (0.315 mmol) of **33**, 25 mL of dry acetone, 0.060 g (0.351 mmol) of benzyl bromide, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3. The reaction solution was heated at reflux for 9 h, cooled to room temperature, poured into a solution of brine, the acetone removed in vacuo, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed in vacuo to yield a white powder. The sample was recrystallized from MeOH to yield 0.086 g of pure **40**: 84%, white powder, mp 192 °C; ^{1}H NMR ($CDCl_3$) δ 5.17 (s), 7.10 (A of A_2B_2), 7.42 (m, 6H), 7.55 (d, J = 8.4, 0.8 Hz), 7.70 (ddd, J = 8.4, 7.0, 2.0 Hz) 7.90 (B of A_2B_2), 8.24 (J = 8.0, 2.0 Hz); ^{13}C NMR ($CDCl_3$) δ 70.2, 106.2, 115.3, 117.9, 123.9, 124.3, 125.1, 125.7, 127.5, 128.0, 128.3, 128.7, 133.6, 136.2, 156.2, 161.5, 163.3, 178.4. Anal. ($C_{22}H_{16}O_3$) C, H.

4′-Nonoxyflavone (39). The title compound was synthesized in the same manner as **40** using 0.075 g (0.315 mmol) of **33**, 25 mL of dry acetone, 0.065 g (0.315 mmol) of 1-bromononane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 12 h to yield an off-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.048 g of pure **39**: 45%, white powder, mp 102 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t), 1.34 (m, 10 H), 1.49 (m, 2H), 1.83 (m, 2H), 4.04 (t), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J = 8.4, 7.0, 1.2 Hz), 7.56 (dd, J = 8.4, 1.2 Hz), 7.69 (ddd, J = 8.4, 7.0, 1.2 Hz), 7.88 (B of A_2B_2), 8.23 (dd, J = 8.0, 1.2); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 26.0, 29.1, 29.2, 29.3, 29.5, 31.8, 68.3, 106.1, 114.9, 117.9, 123.7, 123.9, 125.0, 125.6, 127.9, 133.5, 156.2, 162.0, 163.5, 178.4. Anal. ($C_{24}H_{28}O_3$) C, H.

4′-*n*-Propoxyflavone (34). The title compound was synthesized in the same manner as **40** using 0.074 g (0.311 mmol) of **33**, 25 mL of dry acetone, 0.076 g (0.622 mmol) of 1-bromopropane, and 0.215 g (1.56 mmol) of anhydrous K_2CO_3 heating at reflux for 12 h to yield a white powder. The white powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.071 g of pure **34**: 82%, white powder, mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 1.08 (t), 1.86 (m), 4.01 (t), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J = 8.4, 7.0, 0.8 Hz), 7.56 (dd, J = 8.0, 1.2 Hz), 7.69 (ddd, J = 8.4, 7.0, 1.2 Hz), 7.88 (B of A_2B_2), 8.23 (dd, J = 8.0, 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ 10.5, 22.4, 69.8, 106.1, 114.9, 117.9, 123.7, 123.9, 125.0, 125.6, 128.0, 133.5, 156.2, 162.0, 163.5, 178.4. Anal. ($C_{18}H_{16}O_3$) C, H.

4′-Isopropoxyflavone (35). The title compound was synthesized in the same manner as **40** using 0.075 g (0.315 mmol) of **33**, 25 mL of dry acetone, 0.078 g (0.630 mmol) of 2-bromopropane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 12 h to give an off-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.041 g of pure **35**: 47%, white powder, mp 102 °C; ^{1}H NMR ($CDCl_3$) δ 1.39 (s), 1.40 (s), 4.67 (m), 6.75 (s), 7.00 (A of A_2B_2), 7.41 (ddd, J = 8.4, 7.0, 0.8 Hz), 7.55 (dd, J = 8.4, 1.2 Hz), 7.69 (ddd, J = 8.4, 7.0, 1.2 Hz), 7.87 (B of A_2B_2), 8.23 (dd, J = 8.0, 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ 21.9, 70.2, 106.0, 115.9, 117.9, 132.5, 123.9, 125.0, 125.6, 128.0, 133.5, 156.2, 160.9, 163.5, 178.4. Anal. ($C_{18}H_{16}O_3$) C, H.

4′-Ethoxyflavone (36). To a 100-mL three-neck round-bottom flask were added 0.078 g (0.327 mmol) of **33**, 25 mL dry acetone, 0.237 g (1.31 mmol) of bromoethane, and 0.226 g (1.63 mmol) of anhydrous K_2CO_3. The reaction flask was placed in a 37 °C oil bath and stirred for 17 h. The reaction was then allowed to cool to room temperature, poured into a solution of brine, the acetone removed in vacuo, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed in vacuo to yield a white powder of near pure **36**. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.076 g of pure **36**: 87%, white amorphous solid, mp 122−123 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (t), 4.13 (q), 6.76 (s), 7.02 (A of A_2B_2), 7.42 (ddd, J = 8.0, 7.6, 1.2 Hz), 7.56 (dd, J = 7.6, 1.2 Hz), 7.70 (ddd, J = 8.6, 7.0, 1.4 Hz), 7.89 (B of A_2B_2), 8.24 (dd, J = 8.0, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.7, 63.8, 106.2, 114.9, 123.9, 124.0, 125.0, 125.7, 128.0, 133.5, 156.2, 161.8, 163.5, 178.4. Anal. ($C_{17}H_{14}O_3$) C, H.

4′-Allyloxyflavone (37). The title compound was synthesized in the same manner as **40** using 0.075 g (0.315 mmol) of **33**, 25 mL of dry acetone, 0.076 g (0.630 mmol) of allyl bromide, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3 heating at reflux for 18 h to yield a brown-white powder. The powder was subjected to CC using 7:3 hexanes/EtOAc to yield 0.065 g of near pure **37**. This sample was recrystallized from hot 99:1 hexanes/EtOAc to yield 0.050 g of pure **37**: 57%, white fibrous crystals, mp 108−109 °C; ^{1}H NMR ($CDCl_3$) δ 4.64 (dd, J = 5.2, 1.6 Hz), 4.65 (dd, J = 3.2, 1.6 Hz), 5.35 (dd, J = 10.2, 1.6 Hz), 5.46 (dd, J = 17.2, 1.6 Hz), 6.08 (dddd, J = 17.2, 10.2, 5.2, 3.2 Hz), 6.76 (s), 7.05 (A of A_2B_2), 7.42 (ddd, J = 8.4, 7.0, 0.8 Hz), 7.56 (dd, J = 8.4, 0.8 Hz), 7.70, (ddd, J = 8.4, 7.0, 2.0 Hz), 7.89 (B of A_2B_2), 8.24 (dd, J = 7.6, 1.6 Hz); ^{13}C NMR ($CDCl_3$) δ 69.0, 106.3, 115.2, 118.0, 118.3, 124.0, 124.2, 125.1, 125.7, 128.0, 132.5, 133.6, 156.2, 161.4, 163.3, 178.4. Anal. ($C_{18}H_{14}O_3$) C, H.

4′-(2″-Hydroxyethyl)flavone (38). To a 100-mL three-neck round-bottom flask were added 0.075 g (0.315 mmol) of **33**, 25 mL of dry acetone, 0.075 g (0.315 mmol) of (2-bromoethoxy)-*tert*-butyldimethylsilane, and 0.218 g (1.58 mmol) of anhydrous K_2CO_3. The reaction solution was heated at reflux for a total of 9 h with 2 separate additions of 1 equiv of (2-bromoethoxy)-*tert*-butyldimethylsilane added at 2 and 5 h. The reaction solution was then cooled to room temperature, poured into a solution of brine, the acetone removed in vacuo, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed in vacuo to yield a white powder. The sample was subjected to CC using 1:1 hexanes/EtOAc to yield 0.030 g of the pure ether (24%, white microcrystalline powder). The ether and 15 mL of THF were added to a 50-mL three-neck round-bottom flask and the reaction solution cooled to 0 °C by an ice bath. After stirring 10 min, 0.2 mL of tetrabutylammonium fluoride (TBAF, 1.0 M in THF) was added and the reaction stirred for 30 min at 0 °C. The contents of the flask were then poured into water, THF removed in vacuo, and the organics extracted with EtOAc. The EtOAc was dried with $MgSO_4$, filtered, and removed in vacuo to yield a white powder. The powder was subjected to CC using 3:7 hexanes/EtOAc to yield 0.018 g of pure **38**: 86%, white microcrystalline powder, mp 142−143 °C; ^{1}H NMR (methanol-d_4) δ 3.93 (m, 2H), 4.15 (m, 2H), 6.84 (s), 7.15 (*A* of A_2B_2), 7.50 (ddd, J = 8.4, 7.2, 0.8 Hz), 7.72 (dd, J = 8.4, 0.8 Hz), 7.82 (ddd, J = 8.4, 7.0, Hz), 8.02 (*B* of A_2B_2), 8.14 (dd, J = 8.4, 1.6 Hz); ^{13}C NMR (methanol-d_4) δ 61.7, 71.1, 106.3, 116.4, 119.5, 124.7, 125.0, 126.3, 126.8, 129.6, 135.7, 157.9, 163.9, 166.2, 180.7. Anal. ($C_{17}H_{14}O_4$), C, H.

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