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Flavonoids from Eight Tropical Plant Species That Inhibit the Multidrug Resistance Transporter ABCG2

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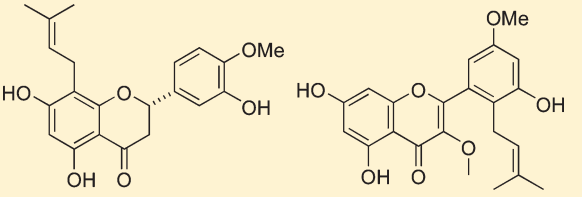
Supporting Information

ABSTRACT: Overexpression of ABCG2, a membrane-bound multidrug transporter, can make tumor cells resistant to treatment with conventional chemotherapeutic agents. A high-throughput screening effort with the NCI repository of natural product extracts revealed that eight tropical plant extracts significantly inhibited the function of ABCG2. This activity was tracked throughout the extract fractionation process to a series of ABCG2 inhibitory flavonoids (**1**−**13**). Their structures were identified by a combination of NMR, mass spectrometry, and circular dichroism studies, and this resulted in the elucidation of (2*S*)-5,7,3′-trihydroxy-4′-methoxy-8-(3″-methylbut-2″-enyl)-flavonone (**1**), (2*S*)-5,7,3′,5′-tetrahydroxy-8-[3″,8″-dimethylocta-2″(*E*),7″-dienyl]flavonone (**3**), and 5,7,3′-trihydroxy-3,5′-dimethoxy-2′-(3′-methylbut-2-enyl)flavone (**12**) as new compounds.



ABCG2 (also known as breast cancer resistance protein, BCRP) is a member of the ATP binding cassette (ABC) family of multidrug transporters.[1] It is a membrane-bound protein that can extrude cytotoxic agents from cells using the energy of ATP hydrolysis, and it has been implicated in the absorption, distribution, and excretion of a variety of drugs and cytotoxins. Overexpression of this cellular transporter is observed in a wide variety of human solid tumors, and an increase in ABCG2 has been associated with the development of resistance by tumor cells to chemotherapeutic agents such as topotecan and mitoxantrone.[2,3] ABCG2 can confer drug resistance independent of the well-known multidrug resistance protein P-glycoprotein 1 (Pgp-1), and it has been demonstrated that inhibition of ABCG2 function increases the effectiveness of topotecan treatment in cancer patients.[4] Therefore, new inhibitors of ABCG2 could be used to potentially enhance the efficacy and reduce the associated toxicity of several commonly administered anticancer drugs.

Flavonoids, a class of natural products ubiquitously present in higher plants, are reported to have an inhibitory effect on the activity of ABCG2.[5−8] Several in vitro studies performed using different classes of flavonoids in combination with cytotoxic drugs have confirmed their ability to inhibit ABCG2 and thereby enhance the anticancer activity of mitoxantrone and topotecan.[7,8] These studies have suggested some plausible structure−activity relationships for flavonoids as well.[3] Nearly 5000 flavonoids have been reported from terrestrial plants, and many of them occur in fruits, seeds, vegetables, spices, herbs, wines, and teas that are commonly consumed by humans.[9,10] Flavonoids are potent antioxidants and anti-inflammatory agents, and they have been reported to confer numerous health benefits.[9−11] It has been postulated that their ability to function as effective free-radical scavengers in a cellular environment contributes to the prevention of several diseases, including cancer and cardiovascular diseases. There are numerous reports about potential cancer chemopreventive properties of flavonoids, and some of these compounds have been investigated extensively in the past few years.[9,11] Based on these studies, there is considerable evidence to suggest that flavonoids may help prevent the occurrence of cancer in humans through a variety of different mechanisms.

In a previous study, a high-throughput screening campaign to identify natural product inhibitors of ABCG2 was conducted with the NCI natural products extract repository, and it was revealed that liphophilic extracts of eight tropical plants, *Anonna reticulata* L. (Annonaceae), *Artocarpus odoratissimus* Blanco (Moraceae), *Calycopteris floribunda* Lam. (Combretaceae), *Evodia confusa* Merr. (Rutaceae), *E. elleryana* F. Muell. (Rutaceae), *Macaranga bicolor* Muell. Arg. (Euphorbiaceae), *M. conifera* (Zoll.) Muell. Arg. (Euphorbiaceae), and *Tabernaemontana macrocarpa* Jack (Apocynaceae) showed significant ABCG2 inhibition. Bioassay-guided fractionation of these extracts yielded 13 flavonoids, and, in this paper, we report the isolation, structural characterization, and ABCG2-inhibitory activity of these compounds.

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RESULTS AND DISCUSSION

Organic solvent extracts of the eight plant species were fractionated by passing each individual extract through a diol solid-phase extraction column, followed by size-exclusion chromatography using Sephadex LH-20, while tracking the ABCG2-inhibitory activity via a cell-based fluorescence assay.[12] Further purification of the ABCG2-active fractions by HPLC on C_{18} afforded flavonoids **1**−**13** (Table 1). Characterization of these metabolites by NMR and mass spectrometry revealed that compounds **1**, **3**, and **12** are new, while compounds **2**, **4**−**11**, and **13** are known. The latter were identified by comparison of their spectroscopic data with previously published values.[13−23]

Table 1. ABCG2 Inhibitory Flavonoids Present in Eight Different Tropical Plant Extracts

species	compounds isolated (w/w % yield from extract)
Macaranga conifera	**1** (3.56), **2**[13] (5.03)
Macaranga bicolor	**3** (0.63), **4**[14] (4.23), **5**[15] (0.33), **6**[16] (0.90), **7**[17,18] (2.20)
Evodia confusa	**8**[19] (0.18)
Evodia elleryana	**9**[20] (0.42), **10**[21] (0.32)
Anonna reticulata	**11**[22] (1.06)
Calycopteris floribunda	**9**[20] (3.30)
Tabernaemontana macrocarpa	**12** (0.18)
Artocarpus odoratissimus	**13**[23] (10.00)

Analysis of the HRESIMS of compound **1** showed a $[M + H]^+$ ion at m/z 371.1506, consistent with a molecular formula of $C_{21}H_{22}O_6$. The ^1H and ^{13}C NMR data for compound **1** (Table 2) were indicative of a flavanone structure, while two 3H singlets at δ_H 1.63 and 1.59 were characteristic of two vinyl methyls of an isoprene unit (H_3-4″ and H_3-5″). This was supported by HMBC correlations from H_3-4″ to C-5″ (δ_C 26.1) and from H_3-5″ to C-4″ (δ_C 18.0), C-3″, and C-2″. H-1″ showed a COSY correlation with H-2″, and HMBC correlations with C-7 and C-8a, which established the connectivity of the isoprene moiety to the flavonoid skeleton at C-8. The singlet observed at δ_H 5.93 (H-6) had HMBC correlations with C-4a, C-5, and C-8 that further defined the A ring of a flavonone. Strong COSY correlations revealed two vicinal aromatic protons at δ_H 6.93 (H-6′) and 6.95 (H-5′), while HMBC correlations from H-5′ to C-4′, from H-6′ to C-2 and C-4′, and from an aromatic singlet δ_H 6.98 (H-2′) to

Table 2. ^1H and ^{13}C NMR Data (500 MHz, CD_3OD) for Compounds 1, 3, and 12

	compound **1**			compound **3**			compound **12**		
position	δ_C	δ_H (J in Hz)	HMBC[a]	δ_C	δ_H (J in Hz)	HMBC[a]	δ_C	δ_H (J in Hz)	HMBC[a]
2	80.3	5.30 1H, dd (12.5, 2.5)		80.6	5.25 1H, dd (12.5, 3.0)		158.3		
3	44.2	3.03 1H, dd (17.0, 12.5)	2, 1′	44.4	3.05 1H, dd (17.0, 12.5)	2	139.7		
		2.73 1H, dd (17.0, 2.5)			2.69 1H, dd (17.0, 3.0)				
4	198.1			197.9			180.1		
4a	103.5			103.3			106.0		
5	166.2			162.7			163.2		
6	96.6	5.93 1H, s	4a, 5, 8	95.7	5.94 1H, s	4a, 5, 7, 8	99.9	6.19 1H, s	4a, 5, 7, 8
7	163.3			166.1			166.0		
8	109.2			109.9			94.9	6.38 1H, s	4a, 6, 8a
8a	161.6			162.6			158.5		
1′	133.6			132.2			122.0		
2′	114.7	6.98 1H, d (1.3)	1′	119.4	6.79 1H, s	2, 1′, 3′	129.6		
3′	147.9			147.0			148.8		
4′	149.4			114.8	6.91 1H, s	3′, 5′, 6′	110.2	7.54 1H, s	3′, 6′
5′	112.7	6.95 1H, d (8.5)	4′	146.7			148.7		
6′	119.0	6.93 1H, dd (8.5, 1.3)	2, 4′	116.4	6.79 1H, s	2, 1′, 3′	124.1	7.54 1H, s	2, 1′, 4′, 5′
1″	22.6	3.17 1H, dd (7.6, 14.4)	7, 8a	22.0	3.21 2H, d (7.3)	7, 8, 8a, 2″, 3″	29.0	3.36 2H, d (7.0)	1′, 2′, 3′, 2″
		3.20 1H, dd (7.6, 14.4)	7, 8a						
2″	124.1	5.15 1H, t (7.5)		124.2	5.19 1H, br t (7.0)		123.5	5.35 1H, br t (7.0)	1″, 4″, 5″
3″	131.8			135.4			133.9		
4″	18.0	1.63 3H s	5″	16.3	1.75 3H, s	2″, 3″	18.9	1.75 3H, s	3″, 5″
5″	26.1	1.59 3H s	2″, 3″, 4″	41.0	1.95 2H, t (7.2)	2″, 3″, 6″	26.1	1.77 3H, s	4″, 3″
6″				27.9	2.06 2H, dt (6.8, 7.4)	5″, 8″			
7″				125.6	5.06 1H, br t (7.4)				
8″				132.1					
9″				17.9	1.56 3H, s	8″, 10″			
10″				26.0	1.62 3H, s	8″, 9″			
3-OCH_3							60.7	3.77 3H, s	3
4′-OCH_3	56.6	3.87 3H s	4′						
5′-OCH_3							56.8	3.93 3H, s	5′

[a] HMBC correlations are from the proton(s) stated to the indicated carbons.

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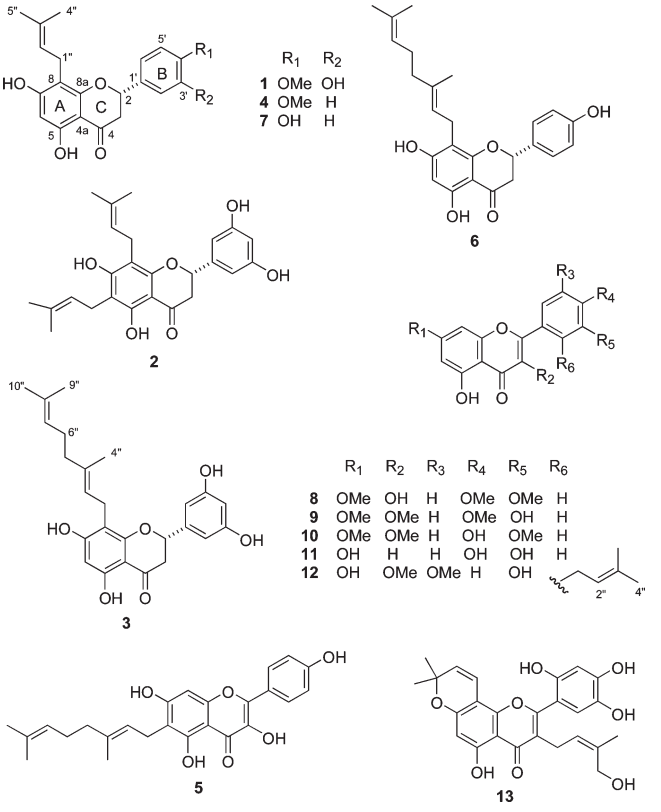
C-1′ were consistent with a 1,3,4-trisubstituted benzene (ring B) attached to C-2 (δ_C 80.3). The H-2 oxymethine (δ_H 5.30) showed COSY correlations with both H-3a (δ_H 3.03) and H-3b (δ_H 2.73), while the C-4 resonance (δ_C 198.1) was assigned as a conjugated ketone to complete the flavanone C ring. A 3H singlet at δ_H 3.87 (δ_C 56.6) established the presence of a methoxy group, and its HMBC correlation with C-4′ confirmed it was substituted on this carbon. Both the ^{1}H and ^{13}C NMR chemical shifts assigned for rings A, B, and C of **1** were in good agreement with the values reported for related compounds.[6−9] The absolute stereochemistry at C-2 was assigned by the analysis of the CD spectrum. Compound **1** showed a negative Cotton effect resulting from a $\pi-\pi^*$ transition at 290 nm, and this was highly indicative of a 2*S* configuration.[24] The structure of **1** was thus assigned as (2*S*)-5,7,3′-trihydroxy-4′-methoxy-8-(3″-methylbut-2″-enyl)flavanone.

Compound **3** displayed a $[M + H]^+$ ion at *m*/*z* 425.1963 by HRESIMS, which established a molecular formula of $C_{25}H_{28}O_6$. It also had ^{1}H and ^{13}C NMR data that were consistent with a substituted flavonone structure (Table 2). The A ring displayed only one aromatic singlet at δ_H 5.94 (H-6) that showed HMBC correlations with four quaternary carbons assigned as C-4a, C-5, C-7, and C-8. A doublet at δ_H 3.14 (H-1″) had HMBC correlations with C-8 and two oxygen-substituted aromatic carbons at δ_C 166.12 (C-7) and 162.6 (C-8a). These observations and the respective carbon chemical shift values were characteristic of a pentasubstituted flavanone ring A subunit with the C-1″ methylene attached at C-8. This side chain was extended to be a bis-isoprene unit partly on the basis of HMBC correlations from H-1″ to C-2″ and C-3″ and from H_3-4″ to C-2″ and C-3″. The upfield chemical shift of C-4″ (δ_C 16.3) was indicative of *E* geometry for the C-2″, C-3″ double bond. The side chain was further elaborated by HMBC correlations observed from H-5″ to C-3″ and from H-6″ to C-5″ and C-8″. Finally, ^{1}H−^{1}H COSY coupling data and HMBC correlations from both H_3-10″ and H_3-9″ to C-8″ confirmed the attachment of a second isoprene unit to C-5″, which fully defined the geranyl side chain in compound **3**.

Ring B was assigned as a 1,3,5-trisubstituted benzene ring on the basis of a lack of ^{1}H−^{1}H coupling observed for the three ring-associated aromatic protons and HMBC correlations from H-6′ to C-1′, C-2, and C-5′, from H-4′ to C-3′, C-5′, and C-6′, and from H-2′ to C-2, C-1′, and C-3′. The chemical shifts for C-3′ (δ_C 147.0) and C-5′ (δ_C 146.7) were consistent with the substitution of hydroxy groups at these positions. While ring B is symmetric in its substitution pattern, the nonequivalence in chemical shift of these two carbons as well as C-2′ (δ_C 119.4) and C-6′ (δ_C 116.4) indicates there is restricted rotation of this ring. Similar NMR data have been reported for other flavonoids with the same substituted B ring.[25,26] The connectivity of ring B to ring C was established by the HMBC correlations from H-6′ and H-2′ to the oxymethine C-2 (δ_C 80.6). Vicinal coupling between H-2 and both H-3 methylene protons in conjunction with ^{13}C chemical shift comparisons[27] defined the flavanone ring C in **3**, which completed the planar structural assignment. Observation of a negative Cotton effect at 295 nm in the CD spectrum allowed assignment of *S* absolute stereochemistry for C-2, and thus compound **3** was defined as (2*S*)-5,7,3′,5′-tetrahydroxy-8-[3″,8″-dimethylocta-2″(*E*),7″-dienyl]flavanone.

HRESIMS analysis of compound **12** provided a $[M + H]^+$ ion at *m*/*z* 399.1456 consistent with a molecular formula of $C_{22}H_{22}O_7$. The ^{1}H NMR spectrum contained a singlet at δ_H 6.38 (H-8), which had HMBC correlations with C-6, C-8a, and C-4a. A second aromatic singlet at δ_H 6.19 (H-6) showed HMBC correlations with C-5, C-7, C-8, and C-4a that helped define ring A. An overlapped two-proton singlet at δ_H 7.54 (H-4′/6′) had HMBC correlations with C-2, C-3′, C-4′, C-5′, and C-6′ that were consistent with the substitution pattern assigned for ring B. The chemical shifts of C-3′ (δ_C 148.8) and C-5′ (δ_C 148.7) revealed oxygenation at these carbons, and an OMe group appearing at δ_H 3.93 showed a HMBC correlation with C-5′, which affirmed its attachment at this position. HMBC correlations from a methylene resonance at δ_H 3.36 (H-1″) to C-2′, C-3′, C-2″, and C-3″ helped establish the attachment of an isoprene substituent at C-2′. The isoprenoid subunit was further defined by HMBC correlations from both H_3-4″ (δ_H 1.77) and H_3-5″ (δ_H 1.75) to C-3″ and C-2″. An OMe group that resonated at δ_H 3.76 was correlated by HMBC to C-3 (δ_C 139.7), which secured the C ring of a flavone. The structure of compound **12** could then be assigned as 5,7,3′-trihydroxy-3,5′-dimethoxy-2′-(3″-methylbut-2-enyl)flavone.



A cell-based high-throughput assay developed in our laboratory[12] was used to assess the ABCG2 inhibitory activity of flavanoids **1**−**13** (Table 3). This assay measured cellular accumulation of the fluorescent chlorophyll derivative pheophorbide a (PhA), which is a substrate specific for ABCG2. When the ABCG2 transporter is fully functional, PhA is actively pumped out of cells, and there is little cellular associated fluorescence. However, ABCG2 inhibition results in the accumulation of PhA within the cells and increased cellular fluorescence. The ABCG2 inhibitory activity of test compounds was measured in terms of cell-associated fluorescence intensity (670 nm) relative to the positive control compound fumitremorogin C (FTC).[12] DMSO solutions of compounds **1**−**13** were tested for their ability to

Table 3. ABCG2 Inhibitory Activity of Compounds 1−13

compound	ABCG2 inhibition IC_{50} (μM)
1	6.6
2	4.1
3	15.5
4	22.6
5	5.7
6	12.3
7	3.1
8	na[a]
9	5.7
10	17.2
11	8.9
12	8.4
13	6.5
FTC[b]	0.8

[a] Not tested. [b] Fumitremorogin C.

inhibit ABCG2, and the results are summarized in Table 3. All of the test materials were active against ABCG2, and IC_{50} potencies ranged from 3.1 to 22.6 μM. These results are in line with previous reports of ABCG2 inhibitory activity for flavonoids. While there has been some speculation about possible structure−activity relationships regarding the substituents and the substitution patterns for various classes of flavonoids,[3] the present ABCG2 test results did not support any clear trends in this regard. Comprehensive testing of a much larger set of related compounds will be required to define what structural parameters are associated with potency, efficacy, and specificity with regard to ABCG2. The fact that prenylated flavonoids with ABCG2 inhibitory properties occur in a wide diversity of plant sources is intriguing and raises important questions about the physiological role and function of these compounds in the host plant.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using a Na lamp at 25 °C. Infrared spectra were obtained with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. Ultraviolet−visible experiments were measured on a Varian Cary 50 Bio UV spectrophotometer. CD spectra were obtained from a JASCO J720 spectropolarimeter. ^{1}H, ^{13}C, gHSQC, gHMBC, and ^{1}H−^{1}H COSY NMR spectra were obtained on a Varian Inova 500 instrument operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using residual solvent resonances for internal reference. ^{1}H chemical shifts were recorded relative to δ 7.24 ($CDCl_3$), whereas the ^{13}C shifts were referenced to δ 77.23 ($CDCl_3$). High-resolution mass spectra were recorded on an Agilent Q-TOF 6520 mass spectrometer. Low-resolution mass spectra were recorded on an Agilent Series 1100 LC-MS. HPLC was performed with a Varian Prostar multisolvent delivery system connected to a Varian Prostar photodiode array detector using a (5 μm) Phenomenex C_{18} column (250 mm × 10 mm).

Plant Material. Specimens of *Anonna reticulata* L. (Annonaceae) were collected in Belize at 89°04′ W and 17°06′ N on October 13, 1994, and identified by Rosita Arvigo of the Institute of Economic Botany, New York Botanical Garden (voucher number OCJT2026). *Artocarpus odoratissimus* Blanco (Moraceae) was collected in the Similajan forest in Sarawak, Malaysia, at longitude 113°03′ E and latitude 3°20′ N on September 5, 1987. The sample was identified by D. D. Soerjarto of the University of Illinois at Chicago (voucher number Q6601979). *Calycopteris floribunda* Lam. (Combretaceae) was collected in the Chittagong district in Bangladesh at longitude 91°05′ and latitude 22°34′, on April 13, 1994, and identified by Ahmed M. Huq (voucher number OFCZ11). Samples of *Evodia confusa* Merr. (Rutaceae) were collected in Borneo north of Safoda Camp in Telupid on September 8, 1994, and identified by W. Meijer (voucher number OFCZ1145). *Evodia elleryana* F. Muell (Rutaceae) was collected in Madang Province in Papua New Guinea at longitude 145°58′ and latitude −5°18′ on January 27, 1989. The sample was identified by W. Takeuchi (voucher number Q6606980). *Macaranga bicolor* Muell. Arg. (Euphorbiaceae) was collected in Palawan, Philippines, at longitude 118°02′ and latitude 9°51′ on April 14, 1989. The sample was identified by D. D. Soerjarto of the University of Illinois at Chicago (voucher number Q6608058). *Macaranga conifera* (Zoll.) Muell. Arg. (Euphorbiaceae) was collected in the Sandakan district, Saba, Borneo, near Kebun China on September 22, 1987, and identified by W. Meijer (voucher number Q6605205). *Tabernaemontana macrocarpa* Jack (Apocynaceae) was collected in Sarawak, Malaysia, at longitude 113°03′ E and latitude 3°20′ N on September 12, 1987. The sample was identified by M. M. J. Van Balgooy (voucher number Q6602165).

Extraction and Isolation. The standardized NCI extraction protocol for terrestrial plant samples has recently been described in detail by McCloud.[28] In brief, dried, ground plant material was sequentially extracted with MeOH−CH_2Cl_2 (1:1) followed by 100% MeOH. These two solutions were combined and evaporated under reduced pressure to give the crude organic solvent extract. An aliquot of each active plant extract was individually loaded onto prepacked diol SPE cartridges (100 mg of extract per 500 mg cartridge) and fractionated by elution with the following solvent combinations: (A) hexane−CH_2Cl_2 (9:1), (B) CH_2Cl_2−EtOAc (20:1), (C) EtOAc (100%), (D) EtOAc−MeOH (5:1), (E) MeOH (100%). Chromatography fractions that inhibited ABCG2 were further separated by size-exclusion chromatography on Sephadex LH-20 eluted with hexane−CH_2Cl_2−MeOH (2:5:1). Compounds **1** and **2** were obtained in pure form directly from the LH-20 separation of fraction C from *Macaranga conifera*. LH-20 separation of fraction A from *M. bicolor* provided compound **4** directly, while similar processing of the B fraction gave compounds **6** and **7**. Final purification of compounds **3** and **5** was accomplished by HPLC on C_{18} eluted with CH_3CN−water (6:4) followed by 100% CH_3CN. The B fraction from *Evodia confusa* was fractionated on LH-20 followed by C_{18} HPLC eluted with 100% H_2O (20 min), CH_3CN−H_2O (1:1, 15 min), and 100% CH_3CN (5 min) to give compound **8**. The B fraction from *E. elleryana* was chromatographed in an identical manner to that described for *E. confusa* to provide compounds **9** and **10**. The B fraction from *Calycopteris floribunda* was treated identically to give compound **9**. The C fraction from *Tabernaemontana macrocarpa* was passed through LH-20 eluted with CH_2Cl_2−MeOH (1:1) and then C_{18} HPLC sequentially eluted with H_2O−CH_3CN (9:1), followed by 100% CH_3CN, to give compound **12**. Fraction D from *Artocarpus odoratissimus* was separated by LH-20 eluted with CH_2Cl_2−MeOH (1:1) and then C_{18} HPLC eluted with H_2O−CH_3CN (55:45) to give compound **13**.

(2*S*)-5,7,3′-Trihydroxy-4′-methoxy-8-(3″-methylbut-2″-enyl)flavanone (1): colorless, amorphous solid; $[\alpha]^{25}_D$ −20.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (ε) 210 (1510), 225 (3700), 235 (1469), 245 (3700), 275 (3700), 310 (1488), 320 (1223) nm; IR (thin film) ν_{max} 3391 (br), 2928, 1634, 1515, 1442, 1385, 1272, 1174, 1131, 1078, 1027, 807, 763, 737 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; HRESIMS *m*/*z* $[M + H]^+$ 371.1506 ($C_{21}H_{23}O_6$ requires 371.1489).

(2*S*)-5,7,3′,5′-Tetrahydroxy-8-(3″,8″-dimethylocta-2″,7″-dienyl)flavanone (3): colorless, amorphous solid; $[\alpha]^{25}_D$ −7.2 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (ε) 244 (16 920), 260 (16 920), 270 (7598), 295 (16 920), 305 (6851) nm; IR (thin film) ν_{max} 3367 (br), 2924, 1634, 1455, 1284, 1159, 1131, 1087, 815 cm^{-1}; ^{1}H and ^{13}C

NMR data, see Table 2; HRESIMS m/z 425.1963 $[M + H]^+$ ($C_{26}H_{29}O_6$ requires 425.1958).

5,7,3′-Trihdroxy-3,5′-dimethoxy-2′-(3′-methylbut-2-enyl)-flavone (12): colorless, amorphous solid; $[\alpha]^{25}_D$ −22.8 (c 0.14, MeOH); UV (MeOH) λ_{max} (ε) 208 (16 920), 254, 360 (16 920), 270 (7598), 295 (16 920), 305 (6851) nm; IR (thin film) ν_{max} 3139 (br), 2853, 1650, 1597, 1581, 1494, 1441, 1378, 1352, 1172, 1162, 1070, 838, 806 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESIMS m/z 399.1456 $[M + H]^+$ ($C_{22}H_{23}O_7$ requires 399.1438).

Bioactivity Determination. ABCG2 inhibition of the chromatographic fractions and pure compounds was determined using a fluorescence accumulation assay previously developed in our laboratory.[12] This assay was performed with NCI-H460 human lung non-small-cell carcinoma cells maintained in RPMI 1640 supplemented with penicillin, streptomycin, 10% FBS, and 20 nM mitoxantrone. Inclusion of mitoxantrone results in selection of cells that overexpress ABCG2.[29] The cells and the media were prepared according to the previously published protocol and transferred to 384-well assay plates.[12] PhA was added to the cells and then immediately followed by the addition of the test compounds, the DMSO vehicle control, or the FTC positive control. After incubation (37 °C, 2−20 h) the medium was removed, and the plates were washed with PBS in order to reduce background fluorescence. Fluorescence was read using a fluorescence plate reader with the emission set at 670 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. HRESIMS, ^1H NMR, ^{13}C NMR, gHSQC, and gHMBC spectra for (2*S*)-5,7,3′-trihydroxy-4′-methoxy-8-(3″-methylbut-2″-enyl)flavanone (**1**), (2*S*)-5,7,3′,5′-tetrahydroxy-8-[3″,8″-dimethylocta-2″(*E*),7″-dienyl]flavanone (**3**), and 5,7,3′-trihdroxy-3,5′-dimethoxy-2′-(3′-methylbut-2-enyl)flavone (**12**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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