Fatty Acid Synthase Inhibitors from Plants: Isolation, Structure Elucidation, and SAR Studies

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Fatty acid synthase (FAS) has been identified as a potential antifungal target. FAS prepared from *Saccharomyces cerevisiae* was employed for bioactivity-guided fractionation of *Chlorophora tinctoria, Paspalum conjugatum, Symphonia globulifera, Buchenavia parviflora,* and *Miconia pilgeriana*. Thirteen compounds (**1**−**13**), including three new natural products (**1**, **4**, **12**), were isolated and their structures identified by spectroscopic interpretation. They represented five chemotypes, namely, isoflavones, flavones, biflavonoids, hydrolyzable tannin-related derivatives, and triterpenoids. 3′-Formylgenistein (**1**) and ellagic acid 4-*O*-α-L-rhamnopyranoside (**9**) were the most potent compounds against FAS, with IC_{50} values of 2.3 and 7.5 μg/mL, respectively. Furthermore, 43 (**14**−**56**) analogues of the five chemotypes from our natural product repository and commercial sources were tested for their FAS inhibitory activity. Structure−activity relationships for some chemotypes were investigated. All these compounds were further evaluated for antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. Although there were several antifungal compounds in the set, correlation between the FAS inhibitory activity and antifungal activity could not be defined.

The enzyme fatty acid synthase (FAS) has emerged as a promising target for the development of novel therapeutic agents.[1] Lipid biosynthesis is essential for cell viability, and since differences exist in the FAS systems of different organisms, specificity for disease-causing microorganisms can be achieved. There are two major types of fatty acid synthase. The associated, or type I, systems exist in animals and fungi and comprise a single, multifunctional polypeptide.[2] The dissociated, or type II, systems exist in bacteria and plants and comprise a collection of discrete enzymes that each carry out an individual step in the cycles of chain elongation.[3] Triclosan and isoniazid are two commonly used antibacterial agents that target the fatty acid synthesis pathway, and cerulenin and thiolactomycin are examples of natural products that inhibit type II FAS. Cerulenin is not selective for type II and also inhibits mammalian type I FAS.[4]

We have recently employed FAS prepared from *Saccharomyces cerevisiae* in our search for potential prototype antifungal agents from plants.[5] A primary screening of 3784 plant samples produced 22 hits with IC_{50} values of 20−100 μg/mL in crude extracts. Five plants, *Chlorophora tinctoria* (L.) Gaud. (Moraceae), *Paspalum conjugatum* Berguis (Poaceae), *Symphonia globulifera* L.f. (Guttiferae), *Buchenavia parviflora* Spruce (Combretaceae), and *Miconia pilgeriana* (Melastomataceae), were selected for bioassay-guided fractionation. Among these, chemical studies have been reported on *C. tinctoria*[6−8] and *S. globulifera*.[9−11] In the current study, 13 compounds (**1**−**13**), including three new natural products (**1**, **4**, **12**), which belong to five chemotypes, isoflavones, flavones, biflavonoids, hydrolyzable tannin-related derivatives, and triterpenoids, were isolated from five plants. The active compounds (**1**, **6**−**9**, **13**) showed FAS inhibitory activity with IC_{50} values ranging from 2.3 to 30 μg/mL. In addition, 43 analogues of the five chemotypes from commercial sources and our natural product repository were screened for FAS activity. All the compounds were also tested in the cell-based antifungal assay against *Candida albicans* and *Cryptococcus neoformans*. The isolation and structure determination of FAS inhibitors from the five plants, as well as inhibition of FAS and antifungal activity for all compounds tested, are presented in this paper.

Results and Discussion

Detailed isolation procedures for compounds **1**−**13** from five plants, generally using normal-phase silica gel and reversed-phase silica gel as chromatographic materials, are elaborated in the Experimental Section.

Compounds **1**−**5** were obtained from the ethanol extract of the leaves and twigs of *C. tinctoria*. Three known compounds were identified by physical and spectral data to be orobol (**2**),[12] isowighteone (**3**),[13] and alpinum isoflavone (**5**).[14,15] Compound **1** displayed a protonated molecular ion peak at *m/z* 299.0559 $[M + H]^+$ in the high-resolution ESIMS, from which its molecular formula was determined to be $C_{16}H_{10}O_6$. The ^1H and ^{13}C NMR spectra of **1** showed a close resemblance to those of known isoflavones (**2**, **3**). A 5,7-dihydroxy isoflavone skeleton[12−15] in the molecule was suggested by the signals at δ_H 8.44 (1H, s), 6.24 and 6.41 (1H each, d, $J = 1.8$ Hz), which correlated with the signals at δ_C 154.9, 99.4 and 94.1, respectively, in the HMQC

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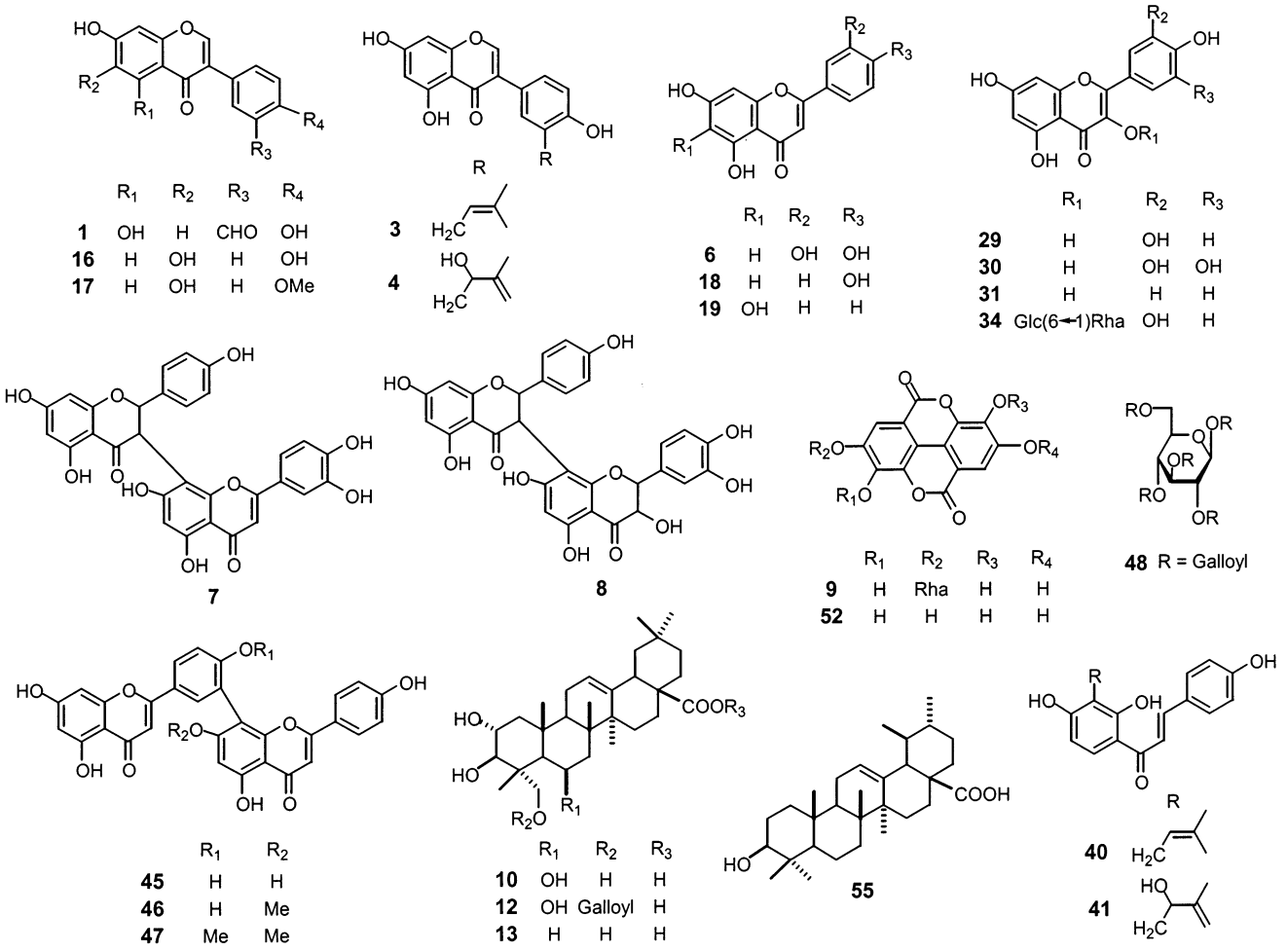
Chart 1



spectrum. Three additional signals at δ_H 7.88 (1H, d, J = 2.0 Hz), 7.71 (1H, dd, J = 8.6, 2.0 Hz), and 7.08 (1H, d, J = 8.6 Hz) indicated a 3,4-substituted B-ring. The presence of an aldehyde signal (δ_H, 10.31/δ_C, 191.4) in the molecule distinguished **1** from most naturally occurring isoflavones. This aldehyde group and the remaining hydroxy group could be located on the B-ring. The placements of the aldehyde group at C-3′ and the hydroxy group at C-4′ were indicated by the chemical shifts of the proton signals of the B-ring, since the 3′-substituted aldehyde group would induce deshielding of H-2′. Confirmation was made by the HMBC spectrum, which showed key correlations between H-2′ and the aldehyde carbon, between the aldehyde proton and C-2′, and between both H-2′ and H-6′ and C-3. This was further supported by the NOESY experiment in which a cross-peak between the aldehyde proton and H-2′ was observed. Other key HMBC and NOESY correlations are summarized in Figure 1. Therefore, the structure of **1** was established as 5-(5,7-dihydroxy-4-oxo-4*H*-chromen-3-yl)-2-hydroxybenzaldehyde, which has been given the trivial name 3′-formylgenistein (**1**). Isoflavonoids containing an aldehyde group are very rare in nature. To our knowledge, this is only the third report following the isolation of 5′-formylpratensein from *Erythrina sacleuxii*[16] and erysubin C from *Erythrina suberosa* var. *glabrescens*.[17]

The second new compound (**4**) was assigned as a derivative of isowighteone (**3**)[13] on the basis of comparison of their ^{1}H and ^{13}C NMR spectra. The high-resolution ESIMS indicated that **4** has one more hydroxy group than **3**. Besides the isoflavone skeleton, additional signals at δ_C

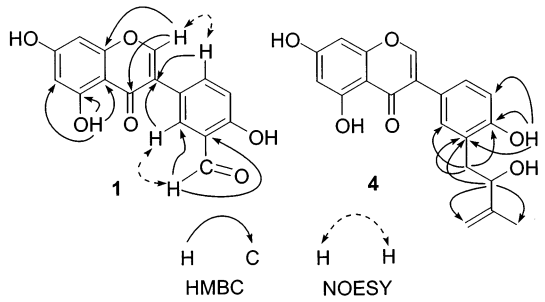


Figure 1. Key HMBC and NOESY correlations of **1** and **4**.

18.3 (CH_3), 38.9 (CH_2), 77.1 (CH−O), 110.7 (=CH_2), and 148.5 (=C<) and signals at δ_H 1.82 (3H, s), 2.92 (2H, m), 4.30 (br dd, J = 10, 9 Hz), and 4.83/4.67 (1H each, s) suggested the presence of a 2-hydroxy-3-methyl-3-butenyl structural moiety as a side chain.[18,19] With the aid of the HMBC experiment, the side chain proved to be attached to C-3′ of the B-ring (Figure 1). It was noted that the 2″-hydroxy group on the side chain formed an intramolecular hydrogen bond with HO-4′, as indicated by the presence of the relatively sharp signal at δ 9.15 in the ^{1}H NMR spectrum, which showed correlations with C-4′, C-3′, and C-5′ in the HMBC spectrum (Figure 1). The HMQC and HMBC spectra of **4** allowed a complete assignment of its ^{1}H and ^{13}C NMR signals (Table 1). Thus, the structure of **4** was assigned as 5,7,4′-trihydroxy-3′-(2-hydroxy-3-methyl-3-butenyl)isoflavone. Compound **4** had a specific rotation of ca. 1°, but showed no absorptions in its CD spectrum. This is indicative of an almost fully racemized stereo-

Table 1. NMR Data for Compounds **1** and **4** (ppm)

	1 (DMSO-d_6)		**4** (acetone-d_6)	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
2	154.9	8.44 s	154.3	8.15 s
3	121.6		124.1	
4	180.2		181.7	
5	162.3		164.0	
6	99.4	6.24 d (1.8)	100.0	6.29 d (1.9)
7	164.8		165.0	
8	94.1	6.41 d (1.8)	94.5	6.42 d (1.9)
9	158.0		159.1	
10	104.8		106.2	
1′	122.3[a]		123.1	
2′	129.7	7.88 d (2.0)	132.9	7.37 d (2.0)
3′	122.5[a]		126.8	
4′	160.9		157.1	
5′	117.6	7.08 d (8.6)	116.8	6.89 d (8.3)
6′	137.2	7.71 dd (8.6, 2.0)	131.2	7.35 dd (8.3, 2.0)
CHO	191.4	10.31 s		
HO-4		12.82 s		13.04 s
HO-4′				9.15
1″			38.9	2.92 m
2″			77.1	4.30 br dd (9.5, 10.3)
3″			148.5	
4″			110.7	4.79 s, 4.99 s
5″			18.3	1.82 s

[a] Signals may be interchangeable.

center.[20] This is the first report of the isolation of isoflavones from *C. tinctoria*, although flavones,[6] a flavonol,[6,8] a benzophenone,[6] dihydroflavanols,[6,8] flavanones,[6] and xanthones[6,7] have been reported. Among the five isolated isoflavones, only **1** showed inhibitory activity against FAS with an IC_{50} of 2.3 μg/mL.

Compound **6** was the only active compound isolated from the ethanol extract of the roots and leaves of *Paspalum conjugatum* and was identified by spectral data as luteolin.[21,22] This flavone showed an IC_{50} of 9 μg/mL against FAS.

Compounds **7** and **8** were isolated from the ethanol extract of the leaves of *Symphonia globulifera* and identified by comparison with literature values as morelloflavone (**7**)[23,24] and GB-2 (**8**).[23,25] This is the first isolation of biflavonoids from *S. globulifera*, although the isolation of xanthones and benzophenones[9−11] was reported. The two biflavonoids, **7** and **8**, were active against FAS with IC_{50} values of 30 and 23 μg/mL, respectively.

Compounds **9**−**12** were isolated from the ethanol extract of the roots of *Buchenavia parviflora*. Three known compounds were identified by spectral data as ellagic acid 4-α-L-rhamnopyranoside (**9**),[26] terminolic acid (**10**),[27,28] and chebuloside II (**11**).[29] Since the NMR data of **10** in the literature are inaccurate and incomplete,[27,28] the carbon signals and important proton signals of this compound were assigned by a combination of 1D and 2D NMR (DEPT, COSY, HMQC, and HMBC) experiments and are listed in Table 2. Compound **12** is new, and its structure was established as follows. The molecular formula was determined to be $C_{37}H_{52}O_{10}$ from a combination of its high-resolution ESIMS and ^{13}C NMR spectral data. The ^{13}C NMR spectrum of **12** was similar to that of **10** with the exception of a set of additional aromatic carbon signals at δ 167.1 (C), 147.6 (C × 2), 140.9 (C), 121.3 (C), and 109.9 (CH × 2). These signals were attributed to a galloyl structural moiety, which was further supported by the signal at δ_H 7.84 integrating for two protons in the ^1H NMR spectrum.[30] Thus, **12** is a galloyl derivative of **10**. In the ^1H NMR spectrum of **12**, two significant downfield proton signals at δ 4.91 (1H, d, J = 11 Hz) and 4.77 (1H, d, J = 11 Hz), assignable to H-23 from the HMQC spectrum,

Table 2. NMR Data for Compounds **10** and **12** in Pyridine-d_5 (ppm)[a]

	10		**12**	
	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	50.0		49.9	
2	69.0	4.42 br d (9.4)	68.4	4.38 br d (9.4)
3	78.2	4.25 d (9.4)	78.0	4.03 d (9.4)
4	44.5		43.8	
5	48.7		49.5[b]	
6	67.5	5.10 br s	67.5	4.88 br s
7	41.0		41.1	
8	39.3		39.1	
9	48.7		48.9[b]	
10	38.2		38.0	
11	23.6[b]	5.55 br s	23.5[c]	5.52 br s
12	122.8		122.5	
13	144.2		144.1	
14	42.7		42.6	
15	28.2		27.8	
16	24.0[b]		23.8[c]	
17	46.6		46.6	
18	42.0	3.32 br d (11)	42.0	3.28 br d (11)
19	46.4		46.3	
20	30.9		30.8	
21	34.2		34.1	
22	33.2		33.0	
23	66.1	4.42 d (10)	66.8	4.91 d (11)
		4.06 d (10)		4.77 d (11)
24	15.9	1.74 s	15.4	1.72 s
25	18.9	1.77 s	18.8	1.71 s
26	18.6	1.62 s	18.6	1.54 s
27	26.2	1.21 s	26.0	1.20 s
28	180.2		180.1	
29	33.2	0.91 s	33.1	0.93 s
30	23.7	0.99 s	23.6	0.99 s
1′			121.3	
2′,6′			109.9	7.84 s
3′,5′			147.6	
4′			140.9	
7′			167.1	

[a] Assignments were made on the basis of 1D and 2D NMR (COSY, HMQC, and HMBC) spectra. [b,c] Signals may be interchangeable.

indicated that the C-23 hydroxy group was galloylated. It was also noted that the 23-galloyl substitution resulted in significant shielding of H-3 ($\Delta\delta$, −0.22 ppm) and H-6 ($\Delta\delta$, −0.22 ppm) when compared with **10** (Table 2). This was further supported by the HMBC spectrum, which showed correlations between the two H-23 protons and the carbonyl carbon of the galloyl moiety. Therefore, compound **12** is 23-galloylterminolic acid. We assumed that its configuration would be the same as that of terminolic acid. While the three triterpenoids (**10**−**12**) were inactive in the FAS assay, the ellagic acid derivative **9** exhibited an IC_{50} value of 7.5 μg/mL.

Compound **13** was isolated from the ethanol extract of the roots of *Miconia pilgeriana* and identified by spectral data as arjunolic acid.[31,32] It showed moderate activity against FAS (IC_{50}, 27.5 μg/mL).

The above results indicate that representatives of five chemotypes of plant metabolites, isoflavones, flavones, biflavonoids, hydrolyzable tannin-related derivatives, and triterpenoids, possess inhibitory activity against FAS. In an attempt to search for more active compounds and obtain some structure−activity relationship information, 43 analogues of the five chemotypes from commercial sources and our natural product repository were selected to test the FAS inhibitory activity. These included four isoflavones (**14**−**17**), 11 flavones (**18**−**28**), six flavonols (**29**−**34**), two flavanones (**35**, **36**), one dihydroflavonol (**37**), two auranols (**38**, **39**), two chalcones (**40**, **41**), two dihydrochalcones (**42**,

Table 3. Fatty Acid Synthase (FAS) Inhibitory Activity and Antifungal Activity for Compounds **1**−**56** (IC_{50}, μg/mL)[a]

	FAS	Ca[b]	Cn[c]		FAS	Ca[b]	Cn[c]
1	2.3	*d*	*d*	**30**	15	*d*	*d*
3	*d*	5	7.5	**31**	50	*d*	*d*
6	9	*d*	*d*	**34**	*d*	*d*	30
7	30	*d*	*d*	**40**	*d*	2	45
8	23	*d*	*d*	**41**	46	20	50
9	7.5	*d*	*d*	**45**	7	*d*	*d*
13	27.5	*d*	20	**46**	25	*d*	*d*
16	50	*d*	*d*	**47**	49	*d*	*d*
17	50	*d*	*d*	**48**	2.8	*d*	*d*
18	37.5	*d*	20	**52**	20	*d*	5.5
19	25	6	*d*	**55**	38	*d*	*d*
29	12	*d*	*d*	cerulenin[e]	0.15	0.40	0.15

[a] Compounds **2**, **4**, **5**, **10**−**12**, **14**, **15**, **20**−**28**, **32**, **33**, **35**−**39**, **42**−**44**, **49**−**51**, **53**, **54**, and **56** were not active at 50 μg/mL. [b] *Candida albicans*. [c] *Cryptococcus neoformans*. [d] Not active at 50 μg/mL. [e] Positive control substance.

43), one flavan-3-ol (**44**), three biflavonoids (**45**−**47**), seven tannin-related derivatives (**48**−**54**), and two triterpenoids (**55**, **56**) (refer to the names of these compounds in the Experimental Section). The FAS inhibitory activities of compounds **1**−**56** are listed in Table 3, and their structure−activity relationships are discussed below (structures of inactive compounds are available in the Supporting Information section).

For the isoflavone chemotype (**1**−**5**, **14**−**17**), only compound **1**, with an aldehyde substituent at C-3′, showed significant activity against FAS. Compounds **16** and **17** showed marginal activity. Thus, the aldehyde group appears to play an important role in mediating the enzyme inhibition. It is envisioned that the aldehyde group can be readily reacting with nucleophilic sites on the enzyme due to its strong electrophilic properties.

Among the flavones (**6**, **18**−**28**), the basic skeleton required for the activity seems to be 5,7-dihydroxyflavone. Compounds with an additional hydroxy group, e.g., **18** and **19**, exhibited weak activity (IC_{50} = 37.5 and 25 μg/mL, respectively). Luteolin (**6**), with an additional hydroxy group at C-3′, was the most potent representative of this chemotype (IC_{50}, 9 μg/mL). Compounds **20**−**28**, which lack a free 5-hydroxy group, a free 7-hydroxy group, or both, showed no activity.

Like the flavone chemotype, the flavonol **33**, which lacks a 5-hydroxy group, did not show activity. Presumably because of the presence of a large, polar sugar moiety attached to the hydroxy group of C-3, compound **34** (rutin) was not active. It is interesting to compare the activity of compounds **29**−**32** of the flavonol chemotype, whose structural differences lie in the number of hydroxy groups on the B-ring. From zero to trihydroxy substitution, the activity increases and then decreases (IC_{50} values of >50 to 50, 12, and 15 μg/mL, corresponding to **32**, **31**, **29**, and **30**, respectively). The dihydroxy-substituted compound **29** (quercetin) was the most active flavonol, exhibiting activity comparable to the flavone-type luteolin (**6**), which lacks only a 3-hydroxy group. The slight decrease of activity from **6** to **29** could be due to the interference of the hydrogen bond between the 5-hydroxy group and the 4-carbonyl group after introduction of a 3-hydroxy group.

Flavanones (**35**, **36**) and a dihydroflavonol (**37**) did not show any activity in the FAS assay. Auranols (**38**, **39**), chalcones (**40**, **41**), dihydrochalcones (**42**, **43**), and a flavan-3-ol (**44**), which are in various respects structurally different from the above-discussed chemotypes, were also inactive, except for **41**, which exhibited marginal activity (IC_{50}, 46 μg/mL).

It was concluded that 5,7,3′,4′-tetrahydroxyflavone (**6**) (luteolin) is the optimal structural skeleton and substituent pattern required for FAS inhibitory activity among the flavonoids investigated. This assertion can be applied to interpretation of the activity of the biflavonoids investigated. Compound **45**, composed of two apigenin (**18**) units with a (I-3′→II-8) linkage, was the most active (IC_{50}, 7 μg/mL) of the five biflavonoids (**7**, **8**, **45**−**47**) tested. This compound can also be regarded as an analogue of **6**, in which the 3′-hydroxy group is replaced by another apigenin unit. Decreased activity was seen in **45**−**47** through blocking the 4′- and 7″-hydroxy groups. Morelloflavone (**7**), with a weak activity (IC_{50}, 30 μg/mL), also possesses the same structural moiety of **6**, but from the activity of GB-2 (**8**) (IC_{50}, 23 μg/mL), it was inferred that the upper flavanone unit may also play a role in the inhibition of the enzyme. Of course, the stereochemistry and conformations of such compounds must be critical for the activity.

Among the eight hydrolyzable tannin-related derivatives (**9**, **48**−**54**) studied, compound **48** (1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose) exhibited significant activity against FAS (IC_{50}, 2.8 μg/mL), while the other three glucose-based hydrolyzable tannins (**49**−**51**) were inactive. Compound **9**, which is composed of ellagic acid (**52**, IC_{50}, 20 μg/mL) and rhamnose, showed good activity (IC_{50}, 7.5 μg/mL). The greater activity of **9** could be due to its polarity and optimal binding sites with the enzyme. Blocking the hydroxy groups on the ellagic acid skeleton in **53** and **54** resulted in a loss of activity.

Among the triterpenoids (**10**−**13**, **55**, **56**), only compounds **13** and **55** showed moderate or marginal activity. The limited number of compounds tested precludes conclusions regarding structure−activity relationships being made.

To establish the correlation of FAS inhibitory effects and antifungal activity, compounds **1**−**56** were evaluated for their antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, two major opportunistic pathogens associated with life-threatening disseminated candidiasis[33,34] and cryptococcosis,[35,36] respectively, in AIDS patients. The results (Table 3) indicated that compounds (**1**, **6**, **9**, **29**, **30**, **45**, **48**) with relatively higher FAS inhibitory activity (IC_{50} < 20 μg/mL) did not exhibit antifungal activity against the above two pathogens. Only compounds **19** and **52** with moderate FAS inhibitory activity showed clear antifungal activity. On the contrary, compounds **3** and **40**, which showed antifungal activity, did not inhibit the FAS enzyme. Therefore, it has been concluded that FAS inhibition is not directly correlated to antifungal activity, at least for the chemotypes examined in this study. Nevertheless, it may be possible that antifungal leads can be discovered on the basis of FAS inhibition from further classes of natural products or synthetic candidates.

In our study, we experienced loss of biological activity with some crude extracts or column fractions during the bioassay-guided isolation procedure. It was also noted that the FAS inhibitory activities of the compounds isolated were not as potent as anticipated from the crude extracts with potent inhibitory activity (IC_{50} < 20 μg/mL). It is concluded that the observed activity for the crude extracts or column fractions was, to some extent, due to the contribution of the nonselective inhibitory effects of unknown tannins or other factors that might have been present.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas-Hoover capillary melting point ap-

paratus and are uncorrected. Optical rotations were determined on an AutoPol IV automatic polarimeter. UV spectra were measured on a Hewlett-Packard 8453 spectrometer. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz), DRX-400 (400 MHz), or DRX-500 (500 MHz) NMR spectrometer. Chemical shifts are expressed relative to the internal standard TMS or the deuterated solvent. COSY, HMQC, HMBC (*J*, 10 Hz), and NOESY (mixing time, 800 ms) NMR spectra were performed with standard pulse programs. ESI-FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Column chromatography was run using silica gel (40 μm, J. T. Baker), reversed-phase silica gel (RP-18, 40 μm, J. T. Baker), or Sephadex LH-20 (Supelco). TLC was performed on silica gel sheets (Alugram Sil G/UV$_{254}$, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F$_{254S}$, Merck, Germany). Compounds purchased from Indofine Chemical Company, Inc. (Somerville, NJ) included **14** (genistein), **15** (biochanin), **16** (6,7,4′-trihydroxyisoflavone), **17** (formonetin), **18** (apigenin), **19** (baicalein), **20** (baicalein trimethyl ether), **21** (baicalin), **22** (6-chloro-7-methylflavone), **23** (6,8-dibromoflavone), **24** (4′-chloro-6,8-dibromoflavone), **25** (4′-chloro-6-methylflavone), **26** (6-bromo-4′-chloroflavone), **27** (gardenin), **28** (8-carboxy-3-methylflavone), **29** (quercetin), **30** (myricetin), **31** (kaempferol), **32** (galangin), **33** (fisetin), **34** (rutin), **35** (flavanone), **36** (5,7, 4′-trihydroxyflavanone), and **37** (3,5,7,2′,4′-pentahydroxydihydroflavonol). Compounds from our natural product repository were isolated from plant sources: **38** (amaranol A),[37] **39** (amaranol B),[37] **40** (isobacachalcone),[19] **41** [4,2′,4′-trihydroxy-3′-(2-hydroxy-3-methylbut-3-enyl) chalcone],[19] **42** (4,2′,6′-trihydroxy-4′-methoxydihydrochalcone),[38] **43** (2′,6′-dihydroxy-4′-methoxydihydrochalcone),[38] **45** (amentoflavone),[39] **46** (7″-*O*-methylamentoflavone),[39] **47** (4′,7″-*O*-methylamentoflavone),[39] **48** (1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose),[39] **49** (chebulanin),[39] **50** (chebulagic acid),[39] **51** (chebulinic acid),[39] **52** (ellagic acid),[40] **53** (3,4,3′-tri-*O*-methylellagic acid 4′-*O*-glucoside),[40] and **54** (nassoside).[40] Compounds **44** (epicatechin), **55** (ursolic acid), and **56** (betulinic acid) were purchased from Aldrich (Milwaukee, WI).

Fatty Acid Synthase Assay. The enzyme fatty acid synthase (FAS) was prepared from the yeast *Saccharomyces cerevisiae*. The cell lysate was prepared using a glass bead mill in 125 mM phosphate buffer (pH 6.6) containing 1 mM EDTA, 1 mM DTT, 0.7 μg/mL pepstatin, 0.2 μg/mL aprotinin, and 0.2 μg/mL leupeptin. Lysate was centrifuged at 30,000*g* for 30 min at 4 °C. To the supernatant was added ammonium sulfate (50% solubility), and the mixture was stirred at 4 °C for 30 min. The protein pellet was obtained by centrifuging at 12,000*g* for 20 min at 4 °C and resuspended in the same buffer. This semipurified enzyme was dialyzed overnight and stored at 4 °C for up to two weeks. The activity was determined and the enzyme preparation was diluted to give an activity of 250 mOD/min^{-1} mL^{-1} prior to assay.

To screen for inhibitors of FAS activity, the assay was performed on 96-well plates in a total volume of 100 μL. The samples to be tested were diluted with the enzyme and incubated at room temperature for 30 min. An aliquot (50 μL) of the diluted samples was mixed with 50 μL of assay mixture containing 1 mM each of malonyl CoA and NADPH, 40 μM acetyl CoA, and 2 mM DTT in phosphate buffer (250 mM). The plate was read immediately in the kinetic mode on a microplate reader, at 340 nm for 10 min, and kinetic plots were obtained. Rate of reaction of the initial 2 min was measured as mOD/min. IC$_{50}$ values was estimated from graphically plotted dose−response curves. Cerulenin was used as a positive control. A pure compound with an inhibition of <50% at the concentration of 50 μg/mL, i.e., IC$_{50}$ >50 μg/mL, was considered to be inactive.

Plant Materials. The plant parts of *Chlorophora tinctoria* (April 1993; Voucher IBE 10519), *Paspalum conjugatum* (October 1995; Voucher IBE 11423), *Symphonia globulifera* (July 1990; Voucher IBE 9713), *Buchenavia parviflora* (October 1997; Voucher IBE 12113), and *Miconia pilgeriana* (October 1997; Voucher IBE 12134) were collected by Mr. Manuel Rimachi in Maynas, Loreto, Peru, and identified by Mr. M. Rimachi and Prof. Sidney McDaniel. Voucher specimens of these plants are deposited at the Herbarium of Mississippi State University.

General Extraction Procedure. The dried plant materials of each plant were ground to a coarse powder and percolated with 95% EtOH four times. Removal of the solvent under a vacuum at 45 °C yielded a EtOH extract for bioassay and fractionation in each case.

Isolation of Compounds 1−5 from *C. tinctoria*. The EtOH extract (28.5 g, IC$_{50}$, 70 μg/mL) from 800 g of the leaves and twigs was dissolved in MeOH−H$_2$O (9:1, 300 mL) and defatted with hexane (300 mL × 3). The aqueous MeOH layer was further extracted with CHCl$_3$ (350 mL × 3). The CHCl$_3$ extract (17.4 g, IC$_{50}$, 90 μg/mL) was fractionated on a silica gel column (507 g) using CHCl$_3$−acetone mixtures (0 → 100%), resulting in 30 combined fractions. Fraction 6 (300 mg), eluted with 2% acetone−CHCl$_3$ (100 mL), was crystallized from acetone−CHCl$_3$ to give **5** (alpinum isoflavone)[14,15] (179 mg). Fraction 11 (710 mg), eluted with 4% acetone−CHCl$_3$ (180 mL), was further chromatographed on a silica gel column (25 g) to yield **1** (2.5 mg). Fraction 16 (398 mg), eluted with 6% acetone−CHCl$_3$ (80 mL), was further chromatographed on a silica gel column (25 g) to give **3** (isowighteone)[13] (43 mg). Fraction 20 (1.56 g), eluted with 20% acetone−CHCl$_3$ (500 mL), was further separated on a Chromatotron using a 1 mm thick silica gel plate with mixtures of CHCl$_3$−acetone (0 → 100%) and MeOH as solvents. The fractions containing **4**, eluted with 10% acetone−CHCl$_3$ (150 mL), were further purified by reversed-phase TLC (MeOH−H$_2$O, 7:3) to give **4** (22 mg; R_f = 0.52). Fraction 22 (890 mg), eluted with 20% acetone−CHCl$_3$ (600 mL), was chromatographed on a reversed-phase silica gel column (50% MeOH−H$_2$O) to give **2** (orobol)[12] (61 mg).

3′-Formylgenistein (1): white amorphous powder, mp 273−275 °C, UV (MeOH) λ_{max} (log ϵ) 212 (4.11), 260 (4.21) nm; NMR data, see Table 1; ESIMS *m/z* 299.0559 {calcd for [M(C$_{16}$H$_{10}$O$_6$) + H]$^+$, 299.0556}.

5,7,4′-Trihydroxy-3′-(2-hydroxy-3-methyl-3-butenyl)-isoflavone (4): light yellow powder, $[\alpha]_\lambda^{25}$ 1.4° (λ = 586 nm) and 0.86° (λ = 546 nm) (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.63), 262 (4.64) nm; CD (*c* 9.2 × 10^{-5} M, MeOH): no absorption in the 200−400 nm region; IR (KBr) ν_{max} 3400 (br), 1652 (C=O), 1618, 1577, 1503, 1447, 1364, 1261 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 355.1135 {calcd for [M(C$_{20}$H$_{18}$O$_6$) + H]$^+$, 355.1182}.

Isolation of Compound 6 from *P. conjugatum*. The EtOH extract (2.7 g, IC$_{50}$ < 20 μg/mL) of 100 g of the roots and leaves was dissolved in MeOH−H$_2$O (9:1, 50 mL) and defatted with hexane (50 mL × 4). The aqueous MeOH layer was further extracted with CHCl$_3$ (50 mL × 3). The remaining aqueous MeOH layer was brought to 50% aqueous MeOH and then extracted with EtOAc (50 mL × 2). The EtOAc extract (264 mg, IC$_{50}$ < 20 μg/mL) was chromatographed on a silica gel column using 15% CHCl$_3$−MeOH (150 mL) to afford **6** (luteolin)[21,22] (102 mg).

Isolation of Compounds 7 and 8 from *S. globulifera*. The EtOH extract (52 g, IC$_{50}$ < 20 μg/mL) of 750 g of the leaves was dissolved in MeOH−H$_2$O (9:1, 540 mL) and defatted with hexane (450 mL × 4). The aqueous MeOH layer was further extracted with CHCl$_3$ (350 mL × 3). The CHCl$_3$ extract (13.5 g, IC$_{50}$ < 20 μg/mL) was fractionated on a silica gel column (400 g) using CHCl$_3$−acetone (0 → 100%), resulting in 16 combined fractions. The most active fraction, 12 (329 mg) (IC$_{50}$ < 20 μg/mL), eluted with 30% acetone−CHCl$_3$ (95 mL), was further chromatographed on a silica gel column using 10% MeOH−CHCl$_3$ to afford **7** (morelloflavone)[23,24] (75 mg) and **8** (GB-2)[23,25] (17 mg).

Isolation of Compounds 9−12 from *B. parviflora*. The EtOH extract (42 g, IC$_{50}$, 55 μg/mL) was obtained from 450 g of the roots, of which 5 g was subjected to column chromatog-

raphy on reversed-phase silica gel C_{18} using an aqueous methanol system (0% → 100% MeOH in water) to give 16 pooled fractions (A−Q). Compounds **9** (ellagic acid 4-α-L-rhamnopyranoside)[26] (60 mg), **11** (chebuloside II)[29] (15 mg), and **10** (terminolic acid)[27,28] (33 mg) were obtained from fractions I, M, and P, respectively, by crystallization from MeOH. Fraction N was chromatographed on silica gel eluting with CH_2Cl_2−MeOH (9:1) to yield compound **12** (6 mg).

23-Galloylterminolic acid (12): white powder, $[\alpha]_D^{25}$ −7.4° (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ϵ) 222 (4.66), 278 (4.45) nm; IR (KBr) ν_{max} 3421 (br), 2944, 1697, 1614, 1463, 1347, 1228, 1034 cm^{-1}; NMR data in pyridine-d_5, see Table 2; ^1H NMR (MeOH-d_4, 300 MHz) δ 7.05 (2H, s, Gal H-2,6), 5.29 (1H, br s, H-12), 4.25 (1H, d, J = 11 Hz, H-23a), 4.23 (1H, br s, H-6), 4.15 (1H, d, J = 11 Hz, H-23b), 3.77 (1H, ddd, J = 9.8, 9.8, 4.6 Hz, H-2), 3.38 (1H, d, J = 9.6 Hz, H-3), 2.85 (1H, dd, J = 12.0, 2.3 Hz, H-18), 1.41, 1.19, 1.08, 1.06, 0.94, 0.91 (3H each, H-24, -25, -27, -29, -30); ESIMS *m*/*z* 679.3437 {calcd for $[M(C_{37}H_{52}O_{10}) + Na]^+$, 679.3452}.

Isolation of Compound 13 from *M. pilgeriana*. The EtOH extract (34.7 g, IC_{50}, 50 μg/mL) was obtained from 749 g of the roots. A portion of the EtOH extract (100 mg) was chromatographed on reversed-phase silica gel (6 g) using aqueous MeOH (0 → 100%, 50 mL) to give **13** (arjunolic acid)[31,32] (12 mg).

Antifungal Assay. The procedures used for *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90113 have been described.[19]

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Supporting Information Available: Structures of compounds **2**, **5**, **11**, **14**, **15**, **20**−**28**, **32**, **33**, **35**−**39**, **42**−**44**, **49**−**51**, **53**, **54**, and **56**, which were not active in any of the bioassays used. This information is available free on the Internet at http://pubs.acs.org.

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