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Platensimycin and Platencin Congeners from *Streptomyces platensis*

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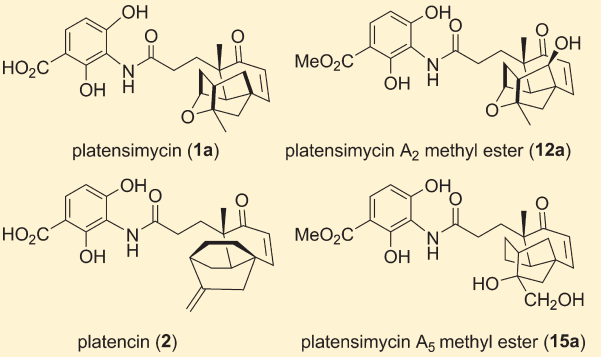
Supporting Information

ABSTRACT: Platensimycin (**1a**) and platencin (**2**) are inhibitors of FabF and FabF/H bacterial fatty acid synthase. The discovery of natural congeners is an approach that can render a better understanding of the structure−function relationships of complex natural products. The isolation and structure elucidation of nine new congeners (**11**−**20**) of platensimycin and platencin are described from a fermentation broth of *Streptomyces platensis*. These hydroxylated congeners are likely derived by cytochrome P450 oxidation of the terpenoid units post-cyclization. Polar groups in the terpenoid portion of the molecule produce negative interactions with the hydrophobic pocket of FabF, resulting in poor activities. However, the discovery of these compounds serves an important purpose, not only to understand structure−function relationships, which cannot be easily accessed by chemical modification, but also to provide access to compounds that could be used for structural identification/confirmation of the oxidative trace metabolites produced in vivo during animal experiments.



Bacterial resistance to antibiotics continues to grow at an alarming rate. Over 60% of hospital strains of *Staphylococcus aureus* are resistant to methicillin, and this leads to a significant and unacceptable loss of lives in the United States.[1] Historically, natural products have been the main sources of antibiotics.[2] Platensimycin (**1a**) and platencin (**2**) are two novel antibiotics that were reported recently from soil bacterial strains of *Streptomyces platensis*. These natural products were discovered by employing a novel antisense differential sensitivity screening strategy.[3−8] Compounds of the platensimycin family inhibit bacterial fatty acid synthesis. Platensimycin (**1a**) was shown to be a selective inhibitor of fatty acid acyl carrier protein synthase II (FabF),[3] and platencin (**2**) is a balanced dual inhibitor of both fatty acid acyl carrier protein synthases II (FabF) and III (FabH).[6] More specifically these compounds bind to the malonyl CoA binding site and uniquely inhibit an acyl−enzyme intermediate. Both of these compounds showed potent in vitro activities against cell-free and whole-cell assay systems. Unfortunately the potent in vitro activities did not translate well in an in vivo mouse model when dosed conventionally (p.o., s.c., and i.p.). The poor in vivo activity exerted by these compounds was attributed to their rapid clearance, leading to low systemic exposure. To circumvent their rapid clearance, these compounds were infused continuously to allow for adequate exposure, leading to robust efficacy. While continuous infusion can be used as a dosing paradigm in hospital for serious infections, it is generally less desirable for most patients. Therefore, attenuation of the clearance along with improvement of potency and half-life are much needed attributes for these compounds to render them as successful antibiotics. Many approaches exist that will allow the achievement of these objectives and the associated study of structure−activity relationships. These approaches include chemical modifications,[9,10] total syntheses,[11−18] discovery of new congeners,[19−25] synthetic biology,[26,27] and biotransformation and inactivation of pathway-specific repressors.[28] Simple chemical modification led to compounds without any improvement of activity. Total synthetic approaches have not led to compounds with improved activity with the exception of lipophilic substitutions in the tetracyclic enone portion of the molecule, such as 7-phenyl- and 11-methyl-7-phenylplatensimycin.[16] Biosynthetic studies suggest that the C-17 enone acid portion of these compounds is biosynthesized by the non-mevalonate terpenoid pathway, and the 3-amino-2,4-dihydroxybenzoic acid unit is biosynthesized via the TCA cycle.[29,30]

A comprehensive search for minor congeners of platensimycin (**1a**) and platencin (**2**) by both biological and chemical screening methods has led to the isolation of a series of compounds with modifications on the polycyclic enone acid and the aromatic units of both lead molecules. The compounds have been named systematically based on the specific modifications at each unit of the molecules. Any modifications in the terpenoid portion of the molecule have been named in the A series, and modifications in the anilide portion of the molecule refer to the B series. Compounds with a complete replacement of the aromatic unit or a change in the carbon length of the enone acid were named in a

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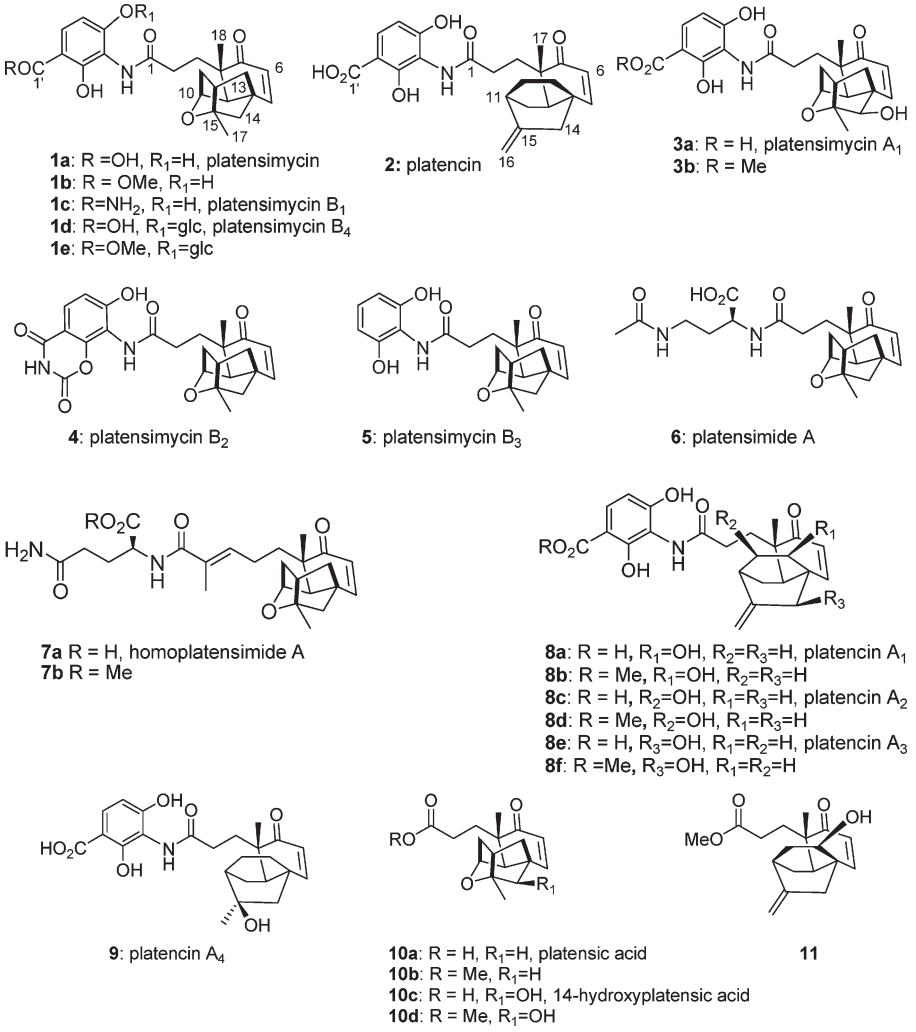
Chart 1



different manner. A number of these compounds have been reported as congeners from *Streptomyces platensis*, including platensimycins B_1−B_4 (**1c**−**e**, **4**, **5**),[21,24] platensimycin A_1 (**3a**),[23] platensimide A (**6**),[19] homoplatensimide A (**7a**),[20] platencins A_1−A_4 (**8a**−**f**, **9**),[22,25] platensic acid and the methyl ester (**10a** and **10b**),[19] 14-hydroxyplatensic acid and the methyl ester (**10c** and **10d**),[23] and 13-hydroxyplatencinic acid methyl ester (**11**).[22] Recently, Shen and his associates described the isolation of the natural hydroxylated platencins A_2−A_{10} by genetic knock-down of a PTN resistance gene.[28] This article presents a comprehensive report on the discovery of all the compounds of these series including the isolation, structure elucidation, and biological activities of nine new compounds (**12**−**20**). The overall discovery of 32 congeners has provided significant structural diversity that allows for a better understanding of the structure−activity relationships, which would not have been possible by chemical modifications or by total synthesis alone without the commitment of considerable resources.

RESULTS AND DISCUSSIONS

Overall Compound Isolation. The platensimycin-producing strain *S. platensis* MA7327 was subjected to medium- and time-course optimization followed by fermentation in a 70 L stirred tank on CLA production medium (see Experimental Section). The production of platensimycin (**1a**) was monitored by HPLC quantification, which indicated the maximal platensimycin titer of 52 mg/L at eight days (Figure 1), and harvested at day nine without any change of the titer at the harvest. The broth was acidified (pH 2.7), extracted with MeOH, and chromatographed on Amberchrome. The column was eluted with a step gradient of 40−100% aqueous MeOH (Scheme S1, Supporting Information). Reversed-phase HPLC of the fractions eluting with 60−70% aqueous MeOH afforded 14-hydroxyplatensic acid (**10c**) (1 mg, 0.1 mg/L, Table S1, Supporting Information) and platensimide A (**6**) (2.4 mg, 0.22 mg/L). The fraction eluting with 70−80% aqueous MeOH from the Amberchrome column was extracted with CH_2Cl_2 at pH 9 and pH 2 and chromatographed by silica gel and reversed-phase HPLC to yield homoplatensimide A (**7a**) (0.4 mg, 0.01 mg/L: the mg/L value represents better relative titer of each compound compared to absolute amount due to use of only a portion of material for purification of many compounds), the methyl ester of homoplatensimide A (**7b**) (1.6 mg, 0.04 mg/L), and platensimycin A_1 (**3a**) (4 mg, 0.1 mg/L). The Amberchrome fractions eluting with

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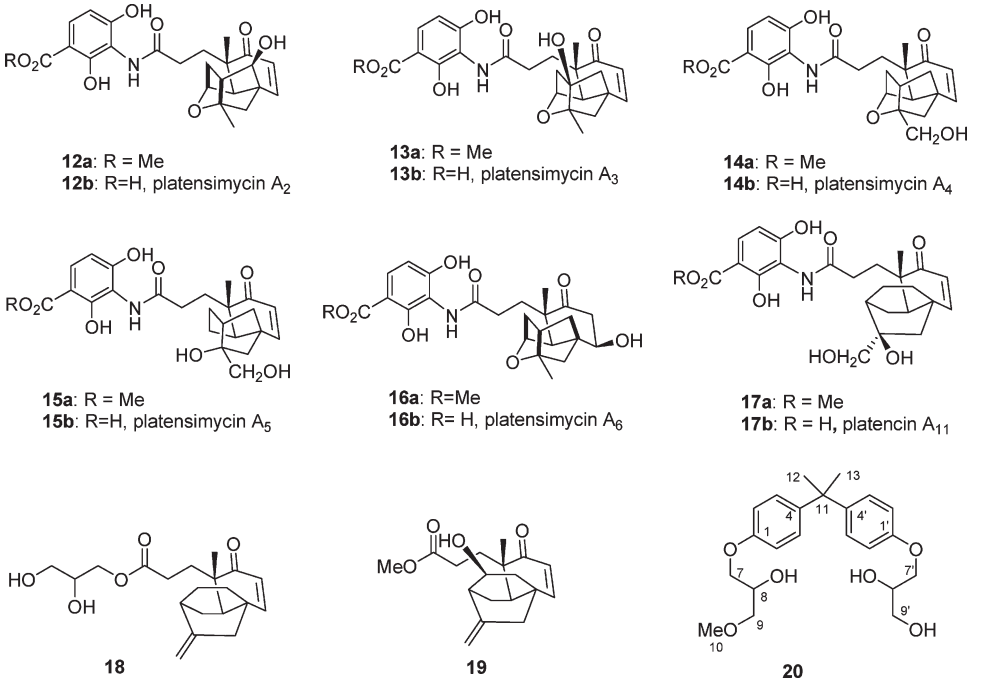
Chart 2



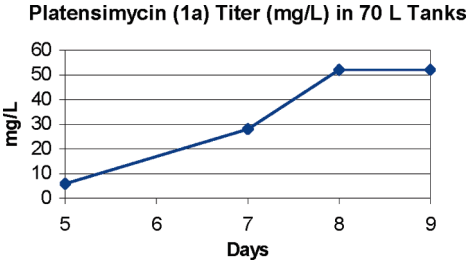


Figure 1. Time course of production of platensimycin (**1a**) by *S. platensis* in 70 L fermentation tanks.

80−90% aqueous MeOH were extracted with CH_2Cl_2 at pH 9 to give a neutral CH_2Cl_2 extract (A1012A). The resulting aqueous layer was acidified to pH 2 and extracted with CH_2Cl_2 to give an acidic extract (A1012B). The latter acidic CH_2Cl_2 extract was chromatographed on a silica gel column to afford three fractions, A1012B-1, -2, and -3. The middle fraction, A1012B-2, provided platensimycin (**1a**, 1.24 g, 28.8 mg/L, >50% isolation yield). Reversed-phase HPLC fractionations of fractions A1012B-1 and -3 yielded platensimycin B_2 (**4**, 24 mg, 0.56 mg/L) and platencin A_1 (**8a**, 3.3 mg, 0.077 mg/L), respectively. The drying of the CH_2Cl_2 extract (A1012A) on Na_2SO_4 retained platensimycin B_4 (**1d**, 9.2 mg, 0.21 mg/L) with the solid Na_2SO_4, which was recovered by washing with MeOH and purified by RP-HPLC. Sephadex LH-20 chromatography of the CH_2Cl_2 filtrate after Na_2SO_4 drying using a solvent gradient of hexane−CH_2Cl_2 and acetone−CH_2Cl_2, followed by MeOH wash, gave nine fractions (LH-20 Fr4 to Fr18). Reversed-phase HPLC of the MeOH wash (LH-20 Fr18) gave platensimycin B_4 methyl ester (**1e**, 7.2 mg, 0.17 mg/L). Each of the eight remaining LH-20 fractions was further purified by RP-HPLC to furnish one to three compounds each. These are listed in the order of elution from the LH-20 column: 12-hydroxyplatencinic acid methyl ester (**19**, 2.2 mg, 0.05 mg/L), platencin A_2 methyl ester (**8d**, 12.2 mg, 0.28 mg/L), platensimycin A_4 methyl ester (**14a**, 3.7 mg, 0.086 mg/L), platencin A_3 methyl ester (**8f**, 1.4 mg, 0.03 mg/L), 13-hydroxyplatencinic acid (**11**, 2.2 mg, 0.05 mg/L), 14-hydroxyplatensic acid methyl ester (**10d**, 1.3 mg, 0.03 mg/L), platensimycin A_3 methyl ester (**13a**, 0.8 mg, 0.02 mg/L), platensimycin A_6 methyl ester (**16a**, 3.2 mg, 0.07 mg/L), platensimycin A_2 methyl ester (**12a**, 15.7 mg, 0.37 mg/L), platensimycin A_1 methyl ester (**3b**, 35.2, 0.82 mg/L), glycerol ester of platencinic acid (**18**, 3.5 mg, 0.08 mg/L), platensimycin A_5 methyl ester (**15a**, 0.6 mg, 0.015 mg/L), platencin A_{11} (**17a**, 2 mg, 0.05 mg/L), platensimycin B_1 (**1c**, 11.2 mg, 0.26 mg/L), platensimycin B_3 (**5**, 3.7 mg, 0.087 mg/L), and **21** (3.7 mg, 0.087 mg/L). Finally, the Amberchrome fractions eluting with 90−100% MeOH (Fr13) was chromatographed on silica gel to yield platensimycin methyl ester (**1b**, 358 mg, 8.3 mg/L). RP-HPLC of the front and the back fractions from the silica gel column that yielded **1b** afforded platensic acid methyl ester (**10b**, 31 mg, 0.72 mg/L) and platencin A_1 methyl ester (**8b**, 0.8 mg, 0.02 mg/L), respectively.

Platencin A_2 and A_3 Methyl Esters (8d and 8f). Both compounds showed the same molecular formula ($C_{25}H_{29}NO_7$), and ESIMS fragmentation exhibited a common fragment ion at m/z 273 in each case. The ^1H and ^{13}C NMR spectroscopic analysis suggested the presence of an exocyclic methylene group indicative of compounds belonging to the hydroxylated platencin family. NMR data comparison (Table 2) of these compounds suggested **8d** is the methyl ester of 12β-hydroxyplatencin (platencin A_2, **8c**) and **8f** is the methyl ester of 14β-hydroxyplatencin (platencin A_3, **8e**).[25] The structures of these compounds were confirmed by comparison with the spectroscopic data of platencins A_2 and A_3.[25]

Platensimycin A_2 Methyl Ester (12a). Mass spectrometric analysis of **12a** using electrospray ionization produced ions at

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data of Platensimycin Methyl Esters (12a, 13a, 14a, 15a, and 16a)

	12a δ_C		**12a** δ_H (mult, *J* in Hz)	**13a** δ_C	**13a** δ_H (mult, *J* in Hz)	**14a** δ_C	**14a** δ_H (mult, *J* in Hz)	**15a** δ_C	**15a** δ_H (mult, *J* in Hz)	**16a** δ_C	**16a** δ_H (mult, *J* in Hz)
position	CD_3OD		CD_3OD	C_5D_5N	C_5D_5N	CD_3OD	CD_3OD	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N
1	175.6	C°		174.9		175.8		174.8		174.9	
2	32.0	CH_2	2.46, m	32.2	2.82, m	32.0	2.48, m	32.6	2.62, m	32.6	2.81, m
			2.28, m		2.72, m		2.30, m				
3	32.8	CH_2	2.28, m	32.6	2.71, m	32.7	2.31, m	34.0	2.62, m	34.5	2.42, ddd, 5.9, 10, 14.0
			1.81, m		2.06, m		1.85, m		2.11, m		
											2.13, m
4	47.6	C°		47.6		47.9		49.7		50.1	
5	205.0	C°		203.6		205.7		204.3		213.1	
6	128.7	CH	5.97, d, 10.2	127.8	5.97, d, 10.1	128.0	5.90, d, 10.4	127.5	5.96, d, 10.0	47.2	2.98, brt, 12.0, 2.87, dd, 12.0, 5.0
7	154.1	CH	6.88, d, 10.2	153.9	6.44, d, 10.1	155.9	6.67, d, 10.4	159.3	6.74, d, 10.0	70.2	4.03, dd, 12.0, 5.0
8	52.4	C°		44.6		46.9		46.1		51.2	
9	49.9	CH	2.48, brs	46.4	2.48, brs	47.8	2.48, brs	43.4	2.05, d, 8.9	48.3	2.16, brs
10	77.6	CH	4.42, brs	75.1	4.51, brs	78.0	4.55, brs	18.9	1.70, m	77.7	4.50, brt, 3.3
									1.59, m		
11	40.8	CH_2	2.14, d, 11.8	48.4	2.44, brd, 11.0	41.5	2.09, d, 11.9	26.5	1.91, m	42.2	1.97, m
			2.09, m		2.31, d, 11.0		2.02, m		1.46, m		
12	54.6	CH	2.24, d, 5.9	84.2		42.6	2.64, t, 6.6	45.9	2.50, brd, 4.0	45.0	2.26, brm
13	83.2	CH_2	4.37, brs	50.6	2.42, m	43.6	1.79, m	38.4	2.41, dd, 11.0, 4.3	36.1	2.23, m
					2.09, brd, 10.9		2.07, m		1.75, brd, 11.0		1.79, dd, 11.0, 3.2
14	49.9	CH_2	1.61, d, 10.9	54.9	1.82, brd, 11.0	51.5	1.77, m	53.6	2.13, brd, 13.6	52.0	2.23, m
			2.52, d, 10.8		1.78, brd, 11.0				1.90, brd, 13.6		1.67, dd, 11.0, 3.2
15	88.4	C°		86.1		92.0		82.9		86.3	
17	23.5	CH_2	1.41, s	21.1	1.57, s	65.7	3.72, d, 11.9	66.5	4.14, d, 10.8	24.0	1.44, s
							3.65, d, 11.9		4.07, d, 10.8		
18	25.8	CH_3	1.21, s	24.8	1.18, s	25.1	1.26, s	24.5	1.17, s	23.7	1.20, s
1′	171.8	C°		171.5		171.9		171.4		171.4	
2′	105.8	C°		105.5		105.7		105.5		105.5	
3′	160.0	C°		159.5		160.7		160.0		159.8	
4′	113.9	C°		115.4		113.7		115.6		115.7	
5′	158.7	C°		158.8		158.7		159.4		158.4	
6′	109.8	CH	6.43, d, 8.8	110.8	6.81, d, 9.0	109.8	6.45, d, 8.7	110.9	6.82, d, 8.8	110.8	6.82, d, 9.0
7′	130.0	CH	7.61, d, 8.8	129.3	7.71, d, 9.0	130.1	7.64, d, 8.7	129.3	7.72, d, 8.8	129.3	7.71, d, 9.0
8′		NH			10.54, s				10.57, s		10.52, s
OMe	52.6	CH_3	3.88, s	52.5	3.74, s	52.6	3.89, s	52.5	3.73, s	52.5	3.74, s

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Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data of Esters of Platencin (8d, 8f, and 17a) and Platencinic Acids (18 and 19)

	8d δ_C		8d δ_H (mult, *J* in Hz)	8f δ_C	8f δ_H (mult, *J* in Hz)	17a δ_C	17a δ_H (mult, *J* in Hz)	18 δ_C	18 δ_H (mult, *J* in Hz)	19 δ_C	19 δ_H (mult, *J* in Hz)
position	C_5D_5N		C_5D_5N	CD_3OD	CD_3OD	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N	C_5D_5N
1	175.0	C°		176.0		175.2		174.2		174.4	
2	32.2	CH_2	2.72, m	31.8	2.31, m	32.4	2.30, m	30.3	2.35, m	30.1	2.40, m
					2.14, m		2.81, m				
3	31.9	CH_2	2.54, m	32.0	2.31, m	32.1	2.59, m	30.9	2.35, m	31.0	2.36, m
			1.93, m		1.71, m		2.00, m		1.71, m		1.73, m
4	48.1	C°		48.3		48.1		47.8		47.7	
5	204.2	C°		206.4		204.8		204.0		204.0	
6	127.0	CH	5.95, d, 10.0	127.4	5.91, d, 10.4	126.6	5.92, d, 10.2	126.8	5.92, d, 9.8	126.8	5.97, d, 10.0
7	154.5	CH	6.46, d, 10.0	154.9	6.97, d, 10.4	155.8	6.39, d, 10.2	155.0	6.36, d, 9.8	154.4	6.47, d, 10.0
8	37.7	C°		41.4		36.2		36.6		37.7	
9	39.4	CH	2.04, t, 9.8	38.7	1.87, t, 9.7	39.0	2.70, dd, 9.9, 9.5	40.3	1.95, t, 10.0	39.2	2.05, t, 9.6
10	26.2	CH_2	1.68, m	28.5	1.73, m	24.0	2.31, m	28.5	1.53, m	26.1	1.68, m
			1.39, dd, 12.5, 10.0		1.62, t, 11.2		1.22, m		1.34, m		1.40, dd, 12.9, 10.1
11	45.0	CH	2.63, t, 3.8	37.2	2.47, brs	32.6	2.81, m	36.7	2.27, brs	45.0	2.69, t, 4.3
12	68.7	CH	4.26, dt, 9.2, 3.2	26.8	1.74, m	23.3	1.46, m	26.5	1.55, m	68.6	4.29, ddd, 9.0, 3.7, 2.6
13	39.3	CH_2	2.42, ddd, 14.2, 9.1, 3.1	20.8	1.96, m	26.6	1.77, m	27.1	1.78, m	39.2	2.43, m
			1.64, m		1.77, m		1.18, m		1.31, m		1.65, m
14	45.1	CH_2	2.54, m	76.7	3.73, brs	49.5	1.53, d, 13.8	45.0	2.20, d, 15.7	45.0	2.57, dt, 16.0, 2.7
			2.03, m				1.46, m		1.99, d, 15.7		2.08, brd, 16.1
15	146.6	C°		154.5		74.2		149.9		146.7	
16	111.2	CH_2	5.06, brs	112.2	5.06, d, 4.2	68.8	3.93, d, 11.2	107.8	4.90, brs	111.1	5.11, dd, 4.4, 1.9
			4.97, brs				3.82, d, 11.2		4.74, brs		4.99, dd, 3.8, 1.8
17	21.7	CH_3	1.08, s	22.3	1.22, s	21.6	1.12, s	21.5	1.04, s	21.6	1.08, s
1′	171.4	C°		171.9		171.4		67.5	4.70, dd, 11.6, 5.0		
									4.61, dd, 11.1, 6.6		
2′	105.5	C°		105.6		105.4		71.3	4.43, m		
3′	160.0	C°		160.5		159.5		64.7	4.11, brd, 5.4		
4′	115.7	C°		113.8		115.8					
5′	158.6	C°		158.4		159.1					
6′	110.8	CH	6.83, d, 8.8	109.8	6.43, d, 8.9	110.9	6.82, d, 8.9				
7′	129.4	CH	7.72, d, 8.8	130.0	7.62, d, 8.9	129.2	7.71, d, 8.9				
8′		NH	10.50, s				10.49, s				
OMe	52.6	CH_3	3.76, s	52.6	3.88, s	52.5	3.73, s			51.8	3.62, s

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m/z 494 [M + Na] and *m/z* 472 [M + H] in the positive-ion mode and at *m/z* 470 [M − H] in the negative-ion mode, indicating a molecular mass of 471 Da for this compound. HRESIMS analysis produced a molecular formula of $C_{25}H_{29}NO_8$, consistent with the presence of an additional CH_2O unit compared to platensimycin (**1a**) and additional oxygen when compared with platensimycin methyl ester (**1b**). The UV spectrum of **12a** was identical to platensimycin/platencin, suggesting that it belongs to this family of compounds. Examination of the ^1H and ^{13}C NMR spectrum of **12a** showed the presence of an oxymethine resonance for C-10, confirming the platensimycin family (Table 1). ESIMS of **12a** produced a fragment ion at *m/z* 289 characteristic of a hydroxyplatensic acid derivative.

The NMR spectra of **12a** exhibited a singlet resonance at δ_H 3.88 (δ_C 52.6), which showed a HMBC correlation to the C-1′ signal (δ_C 175.6), confirming the presence of a methyl ester. Further comparison of the ^{13}C NMR spectrum with platensimycin suggested that **12a** was a methyl ester of 13-hydroxyplatensimycin, which was confirmed by full NMR assignments by COSY, TOCSY, HSQC, HMBC, and NOE difference spectra. The hydroxy substitution at C-13 caused 5.4 and 8.9 ppm downfield shifts of the adjacent carbons C-8 and C-12, respectively. Irradiation of H-13 showed a NOE enhancement of H_3-18, indicating that the proton was on the back side and proximal to the axial methyl H_3-18. On the basis of this evidence, a 13β-hydroxyplatensimycin (named platensimycin A_2, **12b**) methyl ester structure was assigned to **12a**. Platensimycin A_2 (**12b**) was not isolated from these broths. The methyl ester **12a** is likely an artifact of isolation formed either during extraction by MeOH at pH 2 or by direct methylation of the thio-ester biosynthetic intermediate.[28]

Platensimycin A_3 Methyl Ester (13a). ESIMS analysis of **13a** showed a parent ion at *m/z* 472 [M + H], which was analyzed by HRESIFTMS, yielding the molecular formula $C_{25}H_{29}NO_8$, suggesting this compound to be a member of the platensimycin family. The UV spectrum was identical to that of platensimycin (**1a**). Comparison of the molecular formulas and ^{13}C NMR spectra of **13a** and platensimycin suggested that the C-12 methine carbon (δ_C 45.7) in the latter was replaced by an oxygen-bearing quaternary carbon (δ_C 84.2) possessing a methyl ester in **13a**. The ESIMS of **13a** produced a fragment ion at *m/z* 289, consistent with hydroxy substitution in the platensic acid unit. The NMR shifts were fully assigned by COSY, TOCSY, HMQC, and HMBC correlations. The HMBC correlation of the methoxy methyl protons (δ_H 3.74; δ_C 52.5) to the carboxyl carbon (δ_C 171.5) was used to confirm a methyl ester unit. Hydroxy group substitution at C-12 led to discernible downfield chemical shifts of the adjacent carbons, C-11 and C-13. A β configuration was assigned to the C-12 hydroxy group on the basis of steric crowding of the highly rigid tetracyclic caged structure. On the basis of these data a 12β-hydroxyplatensimycin [named platensimycin A_3 (**13b**) methyl ester] structure was assigned to **13a**.

Platensimycin A_4 Methyl Ester (14a). The mass spectrum of **14a** was identical to the mass spectra of **12a** and **13a**, and HRESIMS analysis showed the same molecular formula. The ESIMS also showed a fragment ion at *m/z* 289 characteristic of a hydroxyplatensic acid derivative. The UV spectrum of this compound was identical to the platensimycins. Examination of the ^1H and ^{13}C NMR spectra (Table 1) showed the absence of resonances for one of the methyl groups and the presence of an oxymethylene resonance (δ_C 65.7; δ_H 3.72, 3.75), suggesting hydroxylation of the methyl group. These oxymethylene protons showed HMBC correlations to C-15 (δ_C 92.0), C-12 (δ_C 42.6), and C-14 (δ_C 51.5), confirming the hydroxylation of the C-17 methyl group. The methoxy protons (δ_H 3.89) showed HMBC correlations to C-1′, thus confirming structure **14a** for the platensimycin A_4 methyl ester, i.e., 17-hydroxyplatensimycin methyl ester.

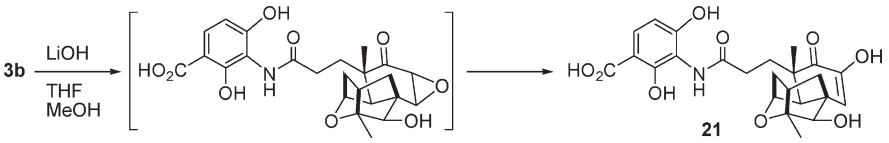
Platensimycin A_5 Methyl Ester (15a). ESIMS analysis of **15a** showed a sodiated molecular ion peak at *m/z* 473, providing a molecular formula of $C_{25}H_{31}NO_8$, indicating the presence of a molecule of hydrogen when compared to the hydroxyplatensimycin methyl esters. The characteristic fragment ion for the hydroxyplatensic acid unit was shifted by 2 amu and appeared at *m/z* 291. Comparison of the ^{13}C NMR spectrum with the other compounds described above indicated the absence of the C-10 methine resonance and the presence of an additional highly shielded methylene resonance appearing at δ_C 18.9, suggesting the opening of the ether ring. This led to an about 15 ppm upfield shift of C-11. The remaining ^1H and ^{13}C NMR spectroscopic data were similar to platensimycin A_4 methyl ester (**14a**) and were assigned by the COSY, TOCSY, HSQC, and HMBC experiments (Table 1). These data established structure **15a** for platensimycin A_5 and **15b** for its methyl ester.

Platensimycin A_6 Methyl Ester (16a). This compound showed pseudomolecular ions at *m/z* 496 [M + Na] and 474 [M + H] in the ESIMS. HRESIFTMS analysis of **16a** gave a molecular formula of $C_{25}H_{31}NO_8$, indicating the compound to be isomeric to **15a**. The UV spectrum of **16a** showed three distinct maxima at 228, 255, and 299 nm and was different from that of platensimycin but similar to 6,7-dihydroplatensimycin,[4,9] indicating the loss of a 6,7-olefin and an enone conjugation in compound **16a**. The ESIMS exhibited fragment ions at *m/z* 291 and also at *m/z* 273, indicating a conjugate addition of water to the olefin, which would produce the former fragment ion by cleavage of the amide bond and the latter ion after elimination of a molecule of water. The ^1H and ^{13}C NMR spectra of this compound showed the absence of resonances for an olefin and instead displayed additional resonances for a methylene (δ_C 47.2; δ_H 2.98, 2.87) coupled to an oxymethine (δ_C 70.2; δ_H 4.03), and a downfield shifted ketone C-5. These methylene and methine resonances were assigned to C-6 and C-7, respectively, and were confirmed by HMBC correlations of H-7 to C-13 (δ_C 36.1) and H-6 (axial) to C-8 (δ_C 51.2). The H-7 signal showed a large (J = 12 Hz) coupling constant with the H-6 proton, indicating that it is axial. This structural assignment was corroborated by a NOE enhancement of H-9 on irradiation of H-7. On the basis of these data, 7β-hydroxydihydroplatensimycin methyl ester (**16a**) was assigned to platensimycin A_6 methyl ester.

Platencin A_{11} Methyl Ester (17a). ESIMS of **17a** showed a parent ion at *m/z* 474, which was analyzed for the molecular formula, $C_{25}H_{31}NO_8$, by HRESIFTMS. The ESIMS produced fragment ions at *m/z* 291 and 273 due to cleavage at the amide bond and subsequent loss of water, respectively. The ^1H and ^{13}C NMR spectra of **17a** (Table 2) showed the absence of any characteristic C-10 resonance of platensimycin and resonances for an exocyclic methylene group and showed the presence of an oxymethylene and a quaternary oxygenated carbon, suggesting the addition of a molecule of water at the exocyclic methylene group followed by hydroxylation of either the resultant methyl group or C-15. The structure **17a** was assigned by the COSY, TOCSY, gHSQC, and gHMBC NMR spectroscopic data as platencin A_{11} methyl ester.

Platencinic Acid Glycerol Ester (18). The mass spectrometric analysis of **18** showed a sodiated molecular ion at *m/z* 371 and an elemental formula of $C_{20}H_{28}O_5$, which was confirmed

Scheme 1. Basic Hydrolysis of Platensimycin Methyl Ester (3b)



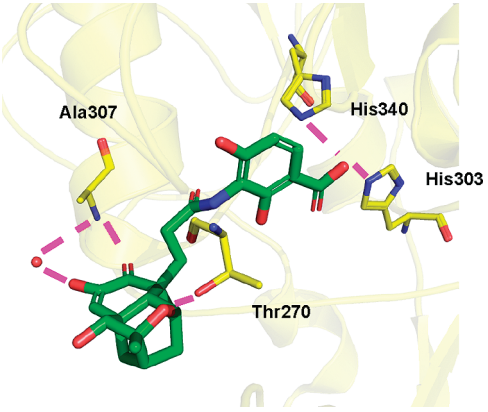


Figure 2. Compound **21** docked in the active site of ecFabF.

from the ^{13}C NMR spectrum. The lack of a C-10 methine resonance and the presence of the exocyclic methylene group in the NMR spectra suggested that **18** is a platencin-type enone. Careful inspection of the ^{13}C NMR spectrum of **18** showed the presence of all resonances for platencinic acid and three oxygenated carbon resonances typical of a glycerol unit, which were confirmed by the COSY and TOCSY correlations. The H_2-1′ signal of the glycerol moiety of **18** showed a strong HMBC correlation to C-1 and confirmed the esterification (IR ν_{max} 1731 cm^{-1}). Accordingly structure **18** for platencin glycerol ester was assigned.

12β-Hydroxyplatencinic Acid Methyl Ester (19). The HRESIMS of **19** was consistent with a molecular formula of $C_{18}H_{25}O_4$. The UV spectrum showed a maximum at 234 nm. In the ^{13}C NMR spectrum, 18 resonances were evident, consistent with the molecular formula proposed. The ESIMS showed a fragment ion at m/z 273, in a similar manner to platensic acid and the hydroxyplatencinic acids. Analysis of ^{1}H and ^{13}C NMR spectra indicated the absence of a characteristic resonance for C-10 of platensic acid (**10a**) and the presence of an exocyclic methylene group, suggesting that compound **19** belonged to the hydroxyplatencinic acid class. The ^{1}H NMR spectrum showed a methine doublet of doublets of doublets at δ_H 4.29 (J = 9.0, 3.7, 2.6 Hz) that correlated to a resonance at δ_C 68.6 in the HSQC spectrum. This methine showed a correlation to a methylene and a methine group in the COSY spectrum, suggesting hydroxylation to occur at C-12, which was confirmed by a HMBC correlation with the H_2-10 resonance. The confirmation of β-hydroxy group substitution at C-12 was established by NOE difference experiments. Irradiation of H-12 showed enhancements of H-11, H-13 (δ_H 2.43) and H-10 axial (δ_H 1.40), confirming the axial *R* configuration of H-12. In this manner, 12*R*-hydroxyplatencinic acid methyl ester (**19**) was assigned structurally.

Compound 20. Mass spectrometric analysis of **20** provided a molecular mass of 390 Da and a molecular formula of $C_{22}H_{30}O_6$. The UV spectrum suggested that this compound was structurally different from the platensimycin compound series. The ^{13}C NMR spectrum showed the presence of 16 resonances, suggesting the overlap of a number of resonances. In the ^{1}H NMR spectrum, signals were observed for two methyl singlets, a methoxy group, four *ortho*-coupled methines each integrating for two protons, four oxymethylenes, and two oxymethines, which were corroborated by the ^{13}C NMR and HSQC correlations determined. These assignments suggested the presence of three exchangeable protons. The signal integration and chemical shift distribution indicated that compound **20** was dimeric in nature and consists of two *para*-substituted aromatic rings with oxygen substitution on one side and a carbon substitution on the other. The methyl singlet (δ_H 1.64) showed HMBC correlations to the only quaternary carbon (δ_C 42.3, C-11) and two aromatic quaternary carbons [δ_C 143.9 (C-4) and 144.0 (C-4′)], thus confirming the connectivities of the two aromatic rings via C-11. The methyl singlet showed a HMBC correlation to an attached carbon, indicating the overlap of *gem*-dimethyl groups. The H-3, 5 (H-3′, 5′) signals showed HMBC correlations to C-11, further confirming the assignments made. Analysis of the ^{1}H, ^{13}C, DEPT, COSY, and HSQC NMR indicated the presence of a pair of glycerol units. The methylene protons of glycerol (H-2 and H-7; H-2′ and H-7′) showed HMBC correlations to the most downfield aromatic carbons (δ_C 158.1 and 158.0), confirming the ether linkages of the aromatic rings with two glycerol units. The methoxy group (δ_H 3.36) showed a HMBC correlation to C-9 (δ_C 75.5), while the H_2-9 signals (δ_H 3.76, 3.77) showed HMBC correlations to the methoxy carbon C-10 (δ_C 59.5) and C-7 (δ_C 71.3 and 69.7), confirming the methylation of the glycerol unit connected at C-4. On the basis of these data structure **20** was assigned to this dimeric compound.

The actual natural products present in the fermentation broth of *S. platensis* are likely free carboxylic acids, and the methyl esters described herein are perhaps the isolation byproduct. In fact, during the time course analysis of platensimycin using MeOH as an extraction solvent at pH 2−3, the formation of methyl esters increased over time. However, what is not clear is why in some cases the compounds present in much lower concentrations were exclusively isolated as methyl esters. In fact, when acetone was used as an extraction solvent, no methyl esters of platensimycin were observed, suggesting that the carboxylic acids, e.g., **12b**, **13b**, **14b**, **15b**, **16b**, and **17b**, are likely actual natural products. In addition, it is most noteworthy that this particular fermentation batch processed produced a number of hydroxylated congeners of platencin (**2**), but **2** itself was not isolated. Therefore, perhaps platencin, being more hydrophobic, has a better propensity for oxidation by cytochrome P450 enzymes than platensimycin.

Basic hydrolysis (LiOH in THF−water) of **3b** produced the 6-hydroxy acid, **21** (Scheme 1). The hydroxylation at C-6 likely proceeds through epoxidation from peroxides present in THF followed by hydrolysis of the epoxide and dehydration of the C-7 hydroxy group. Substitution of THF with freshly distilled THF

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prevented the oxidation at C-6 and produced platensimycin A_1 (**3a**), analogous to the hydrolysis results of platensimycin and analogues.[9,10]

Biological Activity. All new compounds were evaluated in various in vitro cell-free and whole-cell assays, and the results are summarized below. None of the compounds except **21** showed any inhibitory activity in the *Staphylococcus aureus* cell-free fatty acid synthesis inhibitory (FASII) assay[31] at 167 μg/mL. The dihydroxy compound **21** gave an IC_{50} of 16 μg/mL, in the FASII assay, which is 12.8-fold lower than the 6-deoxy compound, **3a** (IC_{50} 1.25 μg/mL).[23] The lower activity exhibited by the dihydroxy compound **21** could be explained by molecular docking of **21** at the active site of the crystal structure of ecFabF in reference to platensimycin A_1 (**3a**). The crystal structure of **3a** bound to ecFabF shows strong H-bond interaction of the NH group of Ala-307 with the C-7 carbonyl group of **3a**. Compound **21** was docked (Figure 2) to the active site of ecFabF with reference to platensimycin A_1 (**3a**). Both the C-7 carbonyl and C-6 hydroxy groups are within the H-bonding distance with the NH group of Ala-307. In order to understand the interactions better, atomic charges of **3a** and **21** were calculated by Gaussian Hartree−Fock 3-21G* geometry optimization. The charge of the oxygen atom of the C-7 carbonyl of **3a** was −0.59 and that of **21** was −0.50. Therefore, the H-bonding ability of the C-7 carbonyl group of **21** with the backbone NH of Ala-307 is expected to be reduced and likely competes with water for the same backbone interactions with NH of Ala-307, leading to reduced binding and lower activity.

In the most sensitive cellular *S. aureus* antisense two-plate differential sensitivity assay, all new compounds except **14a** and **21** showed MDC (minimum detection concentrations) of 1000 μg/mL, or 25 000-fold less activity than platensimycin.[3] Platensimycin A_4 methyl ester **14a** showed a MDC value of 100 μg/mL, 10-fold more than all other compounds in this study. Compound **21** exhibited a MDC of about <20 μg/mL, which appears to be identical to the 6-deoxy compound **3a**.[23] The MDC is defined as the minimum concentration of the compound showing a 5 mm differential zone of clearance between the antisense plates compared to control plates. All of the compounds in this study were tested for their antibacterial activities against the Gram-positive pathogens *S. aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *E. faecium* and the Gram-negative pathogens *E. coli* and *Haemophilus influenzae*. None of these compounds inhibited the growth of these pathogens at 64 μg/mL. The lack of activity of all these compounds is consistent with the activities observed with the compounds reported earlier. Methylation of the carboxyl group causes the disruption of the essential salt bridge interactions between the carboxyl and two active site histidines. It has also been reported that any polarity addition in the terpenoid portion of the molecule significantly reduces activity, particularly in the cellular assays, perhaps due to poor entry to the cell. The dimeric compound **20** did not exhibit any antibacterial activity or activities in the cell-free assays described in this report.

In summary, the isolation, structure elucidation, and biological characterization of nine new platensimycin and platencin congeners and methyl esters (**11**−**20**) produced by *S. platensis* during large-scale fermentation have been described. These hydroxylated compounds are likely produced by cytochrome P450 enzymes post biosynthesis of platensimycin (**1a**) and platencin (**2**). Such compounds, although less active, should provide valuable structure activity insight and serve as reference substances for the in vivo metabolites produced by oxidative metabolism of platensimycin and platencin during animal experiments.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Beckman DU-70 spectrophotometer. IR spectra were recorded with a Perkin-Elmer Spectrum One FT-IR spectrophotometer. All NMR spectra were recorded with a Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) spectrometer. Residual protons in deuterated solvents were used as internal reference (DMSO-d_6 δ_H 2.49, δ_C 39.51; C_5D_5N δ_H 8.74, δ_C 150.35). ^{1}H−^{1}H COSY, DEPT, TOCSY, HMQC, and HMBC spectra were measured using standard Varian pulse sequences. ESIMS data were recorded on an Agilent 1100 MSD with ESI ionization. HRESIFTMS was acquired on a Thermo Finnigan LTQ-FT mass spectrometer with the standard Ion Max API source (without the sweep cone) and ESI probe. Three scan events were used. The ion trap was scanned from 150 to 2000 first in the negative-ion mode and then in the positive-ion mode. The FT was scanned from 200 to 2000 in the positive-ion mode only. In all cases the SID was set to 18 V to try to reduce multiple ion clusters. Instrument resolution was set to 100 000 at m/z 400. No internal calibration was required since the instrument was calibrated once a week and checked daily to ensure accuracy. An Agilent HP1100 instrument was used for analytical HPLC.

Fermentation Condition. A 1 mL frozen suspension of *S. platensis* MA7327 (ATCC PTA-5316) was inoculated in 50 mL of ATCC-2 seed medium (consisting of, in g/L, starch, 20, dextrose, 10, NZ amine type E, 5, beef extract, 3, peptone, 5, yeast extract, 5, calcium carbonate, 1) with 2% inoculum in a 250 mL baffled Erlenmeyer flask and incubated at 28 °C at 220 rpm on a shaker for 48 h. The second stage seed was developed by aseptic transfer of 8 mL of broth from the stage-1 seed into three 2 L nonbaffled Erlenmeyer flasks, containing 500 mL of ATCC-2 medium each with 1.6% inoculum, and cultivated for 48 h at 180 rpm shaker speed at 28 °C. Pooled contents from all three flasks (1.5 L) were transferred into 50 L of "CLA" production medium (consisting of g/L, yellow corn meal, 40, lactose, 40, and ambrerex pH, 5, antifoaming agent (P2000), 0.8 mL/L; 3% inoculum) in a 70 L fermentation tank. The production batch (43 L volume) was harvested after nine days at 5 psig pressure, 300 rpm agitation, 7 slpm airflow, at 28 °C.

Extraction and Isolation. The fermentation broth (43 L) was diluted with 29 L of MeOH, acidified with 2 N HCl to pH 2.7, shaken for 60 h at room temperature, and filtered. The filtrate (72 L, aqueous−MeOH ratio 60:40) was charged directly onto a 1.5 L Amberchrome column. The column was eluted with a step gradient of 40−100% aqueous MeOH with 10% increments of MeOH. Two 600 mL fractions were collected from each 10% MeOH increment, affording 14 fractions (Amberchrome fractions 1−14).

Combined Amberchrome fractions 6 and 7, eluted with 60−70% MeOH, were concentrated under vacuum to 700 mL, and the pH was adjusted to 8.5 by addition of saturated aqueous $NaHCO_3$. The basic fraction was extracted with CH_2Cl_2 (3 × 500 mL). The aqueous layer was acidified to pH 2.0 by addition of 6 N HCl and extracted again with CH_2Cl_2 (3 × 500 mL). The latter CH_2Cl_2 extract was concentrated under reduced pressure to yield 400 mg of an oily material. This material was purified by four identical runs of reversed-phase HPLC on a Zorbax RX C_8 column (21 × 250 mm), eluting with a 37 min gradient of 30−90% aqueous CH_3CN containing 0.1% TFA at a flow of 12 mL/min. The fractions eluting at 9−10 min were pooled and lyophilized to yield 80 mg of a solid material. A 20 mg aliquot of this material was further purified by reversed-phase HPLC using a Zorbax SB phenyl column (21 × 250 mm), eluting with a 37 min gradient of 30−80% CH_3CN−water containing 0.1% TFA at a flow rate of 12 mL/min.

Fractions eluting at 26 and 31 min were lyophilized to yield 1 mg (0.1 mg/L) and 2.4 mg (0.22 mg/L) of 14-hydroxyplatensic acid (**10c**) and platensimide A (**6**), respectively, as gums.

Amberchrome fractions 8 and 9, eluted with 70−80% MeOH, were pooled and concentrated under reduced pressure to remove most of the MeOH and adjusted to pH 9 by addition of solid $NaHCO_3$, leading to a volume of 300 mL. The basic solution was extracted with CH_2Cl_2 (300 mL) followed by EtOAc (300 mL). The aqueous extract was adjusted to pH 3 by addition of 4 N HCl and extracted once with equal volumes of CH_2Cl_2 followed by EtOAc. The CH_2Cl_2 extracts at pH 3 was concentrated under reduced pressure to give 700 mg of material, which was purified on a small silica gel column eluting with a 5, 10, 15, and 25% step gradient of MeOH in CH_2Cl_2. The fractions were collected into five pools according to their analytical HPLC profile. The pooled fractions eluting with 5% MeOH in CH_2Cl_2 were concentrated to give 188 mg of semipurified material. An 80 mg portion of this material was further purified using reversed-phase HPLC using Zorbax RX C_8 (21 × 250 mm) with 35% aqueous CH_3CN containing 0.1% TFA at a flow rate of 12 mL/min. Lyophilization of the fraction eluting at 16 min afforded 9 mg of material, which was further chromatographed by semipreparative HPLC on a Zorbax RX C_8 (75 × 4.6 mm) column operating with a 30 min gradient of 20−90% aqueous CH_3CN containing 0.1%TFA at a flow rate of 3 mL/min. Fractions eluting between 17 and 19 min from the semipreparative column were lyophilized to give 0.4 mg (0.01 mg/L) and 1.6 mg (0.04 mg/L) of homoplatensimide A (**7a**) and homoplatensimide A methyl ester (**7b**), respectively, as gums. The pooled fraction eluting with 15% MeOH in CH_2Cl_2 from the silica gel column was concentrated to give 35 mg of a gum, which was purified further using reversed-phase HPLC on Zorbax RX C_8 (21 × 250 mm) with 35% aqueous CH_3CN + 0.1% TFA at a flow rate of 12 mL/min. Lyophilization of the fraction containing the major peak gave 4 mg (0.1 mg/L) of platensimycin A_1 (**3a**) as an amorphous powder.

Amberchrome fractions 10−12, eluted with 80−90% MeOH, were pooled and concentrated to 200 mL, containing mostly aqueous solution. Then, 300 mL of water was added to give a final volume of 500 mL, to which was added $NaHCO_3$ to adjust the pH to ∼9. This solution was extracted three times with CH_2Cl_2 (3 × 450 mL, fraction A1012A). The aqueous layer was acidified to pH 2 with 6 N HCl and extracted four times with CH_2Cl_2, and the extracts were combined (1900 mL) to give fraction A1012B. The latter fraction (A1012B) was concentrated under reduced pressure to afford 2.6 g of solid, which was dissolved in a small volume of EtOAc and charged to a 500 cm^3 silica gel column packed in 20% EtOAc−hexane. The column was washed with four column volumes of 20% EtOAc, collecting four 500 mL fractions each (fraction A1012B-1). The column was eluted with four column volumes each of 80:20:0.5:0.5:0.5 of hexane−EtOAc−water−AcOH−MeOH, collecting 200 mL fractions each. Fractions 6−10 and 11/12 were pooled to give fractions A1012B-2 and A1012B-3, respectively. Fraction A1012B-2 was concentrated under reduced pressure to give 1.24 g (50% isolation yield) of platensimycin (**1a**), as a buff-colored powder.

Fraction A1012B-1 was concentrated at reduced pressure to yield 350 mg of a gum, which was dissolved in 5 mL of EtOAc and charged to a 70 cm^3 silica gel column packed in 1:1 EtOAc−hexane. The column was eluted with one column volume each of 1:1, 6:4, 7:3, 8:2 EtOAc−hexane followed by 8:2:0.05:0.05:0.05 hexane−EtOAc−water−AcOH−MeOH, to furnish seven fractions. The last fraction was concentrated under reduced pressure, affording 36.5 mg of a solid, which was dissolved in 300 μL of MeOH, purified on a Zorbax RX C_8 (250 × 21 mm) column, and eluted with a 30 min gradient of 10−90% CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. Fractions eluting at 19−20 min were pooled and lyophilized to afford 24 mg (0.56 mg/L) of platensimycin B_2 (**4**), as an amorphous powder.

Fraction A1012B-3 was concentrated under reduced pressure to give 43.5 mg of a solid material, which was purified by reversed-phase HPLC on a Zorbax RX C_8 column eluting with a 30 min gradient of 10−90% aqueous CH_3CN + 0.1% TFA at a flow rate of 8 mL/min. Lyophilization of the fraction eluting at 20 min gave 3.3 mg (0.077 mg/L) of platencin A_1 (**8a**) as an amorphous powder.

The CH_2Cl_2 extract (fraction A1012A) containing essentially neutral compounds was dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 1.77 g of a solid (fraction A1012A-1). Solid Na_2SO_4 was washed with MeOH and filtered. The MeOH filtrate was concentrated under reduced pressure to give 0.314 g of a further solid (fraction A1012A-2). Fraction A1012A-2 was chromatographed by reversed-phase HPLC (Zorbax C_8, 21 × 250 mm), eluting with a 40 min gradient of 20−80% aqueous CH_3CN + 0.05% TFA at a flow rate of 10 mL/min. The fraction eluting at 13 min was lyophilized to yield 9.2 mg (0.21 mg/L) of platensimycin B_4 (**1d**), as an amorphous powder.

Fraction A1012A-1 (1.77 g) was subjected to Sephadex LH-20 chromatography (1 L column). The column was eluted with a step gradient of one column volume each of hexane−CH_2Cl_2 (1:4), acetone−CH_2Cl_2 (1:4), and acetone−CH_2Cl_2 (3:2) at a flow rate of 10 mL/min. After elution with these solvents the column was unpacked and Sephadex LH-20 was washed with MeOH (2 L). The fractions were analyzed by silica gel TLC and HPLC, producing 22 pooled fractions.

The MeOH wash from the LH-20 column was concentrated to give 224.5 mg of a fraction, which was chromatographed by reversed-phase HPLC on Zorbax C_8 (21.2 × 250 mm), eluting at 10 mL/min with 25% aqueous CH_3CN + 0.05% TFA for 40 min, followed by a 40 min gradient of 25−100% aqueous CH_3CN. Fractions eluting at 37−40 min were pooled and extracted with EtOAc. The EtOAc extract was concentrated to furnish 7.2 mg (0.17 mg/L) of the methyl ester of platensimycin B_4 (**1e**), as an amorphous powder.

The LH-20 column pooled fraction 4 (elution at 0.8 column volume) was concentrated to give 40.2 mg of material, which was chromatographed by reversed-phase HPLC on a Zorbax C_8 (21.2 × 250 mm) column and eluted at 5 mL/min with 35% aqueous CH_3CN+0.05% TFA for 60 min, then a 10 min gradient from 35% to 100% aqueous CH_3CN. Fractions eluting at 36 and 58 min were lyophilized to give 2.2 mg (0.05 mg/L) and 12.2 mg (0.28 mg/L) of 12-hydroxyplatencinic acid methyl ester (**19**) and platencin A_2 methyl ester (**8d**), respectively, as gums.

The LH-20 column pooled fraction 5 (elution at one column volume), after concentration, produced 21.9 mg of a gum, which was purified by reversed-phase HPLC (Zorbax C_8, 21.2 × 250 mm, 5 mL/min, 40 min gradient of 40−70% aqueous CH_3CN + 0.05% TFA followed by a 10 min gradient to 100% CH_3CN detection). Two major compounds eluted in fractions 22 and 34. These fractions were freeze-dried to afford 3.7 mg (0.086 mg/L) and 1.4 mg (0.03 mg/L) of methyl esters of platensimycin A_4 (**14a**) and platencin A_3 (**8f**), respectively, as amorphous powders.

The LH-20 column pooled fraction 6 (elution at 1.1 column volume) afforded 17.4 mg of a residue, after concentration, which was chromatographed by a similar reversed-phase HPLC using a Zorbax C_8 (21.2 × 250 mm) column, eluted at 5 mL/min, with a 40 min gradient of 35−65% aqueous CH_3CN + 0.05% TFA followed by a 10 min gradient to 100% CH_3CN. The fraction eluting at 34 min was lyophilized to produce 2.2 mg (0.05 mg/L) of the methyl ester of 13-hydroxyplatencinic acid (**11**), as an amorphous powder.

The LH-20 column pooled fraction 11 (elution at 1.5 column volume) provided 32.6 mg of a residue, which was chromatographed on a Zorbax C_8 column (21.2 × 250 mm, eluted at 5 mL/min, with 30% aqueous CH_3CN + 0.05% TFA for 60 min, followed a 10 min gradient to 100% CH_3CN). Fractions eluting at 44, 49, and 68 min were lyophilized to give 1.3 (0.03 mg/L), 0.8 (0.02 mg/L), and 3.2 mg (0.07 mg/L) of the methyl esters of 14-hydroxyplatensic acid (**10d**), platensimycin A_3 (**13a**), and platensimycin A_6 (**16a**), respectively, as gums.

The pooled LH-20 column fraction 12 (1.6 elution volume) gave 89.2 mg of a gum, which was subjected to chromatographic separation by

reversed-phase HPLC on a Zorbax C_8 column (21.2 × 250 mm), eluted at 10 mL/min with a 40 min gradient of 20−60% aqueous CH_3CN + 0.05% TFA, followed by a 10 min gradient to 100% CH_3CN. Two major compounds eluted at 27 and 32 min. These two fractions were lyophilized to furnish amorphous powders of the methyl esters of platensimycins A_2 (**12a**, 15.7 mg, 0.37 mg/L) and A_1 (**3b**, 35.2 mg, 0.82 mg/L) listed in the order of elution.

The pooled LH-20 column fraction 14 (1.8 elution volume) gave 256.0 mg of a residue, which was chromatographed by reversed-phase HPLC on a Zorbax C_8 column (21.2 × 250 mm), eluted at 10 mL/min with a 40 min gradient of 30−60% aqueous CH_3CN + 0.05% TFA, followed by a 10 min gradient to 100% CH_3CN. The fraction eluting at 25 min was freeze-dried to yield 3.5 mg (0.08 mg/L) of the glycerol ester of platencinic acid (**18**), as a gum.

The pooled LH-20 column fraction 15 (elution 2 column volumes) afforded 116.2 mg of a residue, which was similarly chromatographed by preparative HPLC on a Zorbax C_8 column (21.2 × 250 mm), eluted at 10 mL/min, with 30% aqueous CH_3CN + 0.05% TFA for the first 60 min, followed by a 20 min linear gradient to 100% CH_3CN. The fractions eluting at 24 and 32 min were lyophilized to yield 0.6 mg (0.01 mg/L) and 2.0 mg (0.05 mg/L) of the methyl esters of platensimycin A_5 (**15a**) and platencin A_{11} (**17a**), respectively, as gums.

The pooled LH-20 column fraction 17 (elution at 2.3 column volumes) gave 231.0 mg of a residue, which likewise was chromatographed by preparative HPLC (Zorbax C_8, 21.2 × 250 mm, 10 mL/min, 30% aqueous CH_3CN + 0.05% TFA isocratic elution for the first 60 min, followed by a 20 min linear gradient to 100% CH_3CN). Fractions eluting at 36, 44, and 55 min were lyophilized to give platensimycin B_1 (**1c**, 11.2 mg, 0.26 mg/L), platensimycin B_3 (**5**, 3.7 mg, 0.087 mg/L), and **20** (3.7 mg, 0.086 mg/L), respectively, as amorphous powders.

Amberchrome column fraction 13, eluted in 90−100% MeOH, was concentrated under reduced pressure to remove most of the MeOH, redissolved in 200 mL of 10% MeOH−water, and extracted with CH_2Cl_2 (4 × 200 mL). The CH_2Cl_2 extract was concentrated under reduced pressure to yield 1.2 g of an oily material. This material was dissolved in 5 mL of CH_2Cl_2, adsorbed onto 2.5 g of silica gel, and chromatographed on a 150 g silica gel flash column packed in 10% EtOAc−hexane and eluted with EtOAc−hexane with increasing percentages of EtOAc [i.e., EtOAc−hexane 10:90 (500 mL), 20:80 (600 mL), 25:75 (600 mL), 30:70 (1.2 L), 40:60 (500 mL), 50:50 (1 L), 75:25 (500 mL), 100:0 (500 mL)] at a flow rate of 20 mL/min. Fractions eluting with 30% EtOAc−hexane were pooled and concentrated under reduced pressure to afford 31 mg (0.72 mg/L) of platensic acid methyl ester (**10b**) as a colorless oil. Concentration of fractions, eluting with 40% EtOAc, afforded 358 mg (8.3 mg/L) of platensimycin methyl ester (**1b**). A fraction eluting with 50% EtOAc−hexane from the silica gel column was concentrated to dryness under reduced pressure to yield 28 mg of an oily material, which was purified by reversed-phase HPLC using Zorbax RX C_8 [(21 × 250 mm), eluting with a 37 min gradient of 10−95% CH_3CN−water containing 0.1% TFA at a flow of 12 mL/min]. The fraction eluting at 26 min was lyophilized to furnish 3 mg of a solid material, which was further purified by semipreparative reversed-phase HPLC using a Zorbax RX C_8 column [(9.1 × 250 mm), eluting with a 25 min gradient of 30−60% CH_3CN−water containing 0.1% TFA at a flow of 4 mL/min]. The fraction eluting at 12.1 min was lyophilized to yield 0.8 mg (0.02 mg/L) of platencin A_1 methyl ester (**8b**) as a colorless powder.

Platencin A_2 Methyl Ester (8d): colorless gum; $[\alpha]^{23}_D$ −16 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 228 (ε 22 006), 256 (13 968) 299 (4110) nm; FTIR (ZnSe) ν_{max} 3283, 2932, 1662 (brs), 1594, 1536, 1439, 1341, 1262, 1198, 1143, 1063, 1014, 791 cm^{-1}; for ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 478 [M + Na], 456 [M + H], 273; HRESIFTMS *m/z* 456.2027 (calcd for $C_{25}H_{29}NO_7$ + H, 456.2022).

Platencin A_3 Methyl Ester (8f): colorless, amorphous powder; $[\alpha]^{23}_D$ −2 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 227 (ε 14 863) 298 (2990) nm; FTIR (ZnSe) ν_{max} 3331, 2934, 2867, 1663 (brs), 1597, 1535, 1449, 1376, 1341, 1292, 1262, 1202, 1146, 1094, 1060, 1031, 955, 904 cm^{-1}; for ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 456 [M + H], 273; HRESIFTMS *m/z* 456.2024 (calcd for $C_{25}H_{29}NO_7$ + H, 456.2022).

Platensimycin A_2 Methyl Ester (12a): colorless, amorphous powder; $[\alpha]^{23}_D$ −82 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 216 (ε 17 766) 227 (18 538), 256 (13 995), 298 (5,113) nm; FTIR (ZnSe) ν_{max} 3331, 2958, 1666 (brs), 1536, 1440, 1379, 1340, 1264, 1202, 1147, 1108, 1062, 956 cm^{-1}; for ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 494 [M + Na], 472 [M + H], 470 [M − H], 289; HRESIFTMS *m/z* 472.1981 (calcd for $C_{25}H_{29}NO_8$ + H, 472.1971).

Platensimycin A_3 Methyl Ester (13a): colorless, amorphous powder; $[\alpha]^{23}_D$ −34 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 228 (ε 14 309, 245 (sh), 298 (2618) nm; FTIR (ZnSe) ν_{max} 3332, 2960, 1661 (brs), 1537, 1440, 1380, 1340, 1312, 1263, 1201, 1144, 1101, 1062, 994, 834, 789 cm^{-1}; for ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 472 [M + H], 289; HRESIFTMS *m/z* 472.1972 (calcd for $C_{25}H_{29}NO_8$ + H, 472.1966).

Platensimycin A_4 Methyl Ester (14a): colorless gum; $[\alpha]^{23}_D$ −28 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 227 (ε 22 853), 245 (sh), 298 (5033) nm; FTIR (ZnSe) ν_{max} 3320, 2955, 1665 (br, strong), 1597, 1538, 1439, 1341, 1263, 1200, 1147, 1064, 1024, 953 cm^{-1}; for ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 494 [M + Na], 472 [M + H], 289; HRESIFTMS *m/z* 472.1986 (calcd for $C_{25}H_{29}NO_8$ + H, 472.1971).

Platensimycin A_5 Methyl Ester (15a): colorless, amorphous powder; $[\alpha]^{23}_D$ −12 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 228 (ε 14 556), 255 (sh), 299 (3204) nm; FTIR (ZnSe) ν_{max} 3320, 2938, 1648 (brs), 1535, 1438, 1374, 1336, 1263, 1200, 1146, 1063, 1008, 789 cm^{-1}; for ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 496 [M + Na], 474 [M + H], 472 [M − H], 291; HRESIFTMS *m/z* 474.2123 (calcd for $C_{25}H_{31}NO_8$ + H, 474.2127).

Platensimycin A_6 Methyl Ester (16a): colorless gum; $[\alpha]^{23}_D$ −14 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 225 (ε 16 187), 255 (12 162), 299 (3784) nm; FTIR (ZnSe) ν_{max} 3387, 2959, 1666, 1535, 1439, 1380, 1339, 1262, 1201, 1143, 1105, 1064, 1037, 936, 792 cm^{-1}; for ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 496 [M + Na], 474 [M + H], 291, 273; HRESIFTMS *m/z* 474.2133 (calcd for $C_{25}H_{31}NO_8$ + H, 474.2127).

Platencin A_{11} Methyl Ester (17a): colorless oil; $[\alpha]^{23}_D$ −26 (*c* 0.5, MeOH); UV (CH_3OH) λ_{max} 229 (ε 16 555) 298 (3356) nm; FTIR (ZnSe) ν_{max} 3333, 2943, 1662 (brs), 1534, 1439, 1375, 1340, 1263, 1201, 1143, 1064, 1044, 1001, 955 cm^{-1}; for ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 474 [M + H], 456 [M − H_2O], 291, 273; HRESIFTMS *m/z* 474.2114 (calcd for $C_{25}H_{31}NO_8$ + H, 474.2128).

Platencinic acid glycerol ester (18): colorless oil; $[\alpha]^{23}_D$ −10 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 230 (ε 8108) nm; FTIR (ZnSe) ν_{max} 3407, 3068, 2932, 2868, 1731, 1668, 1536, 1454, 1390, 1291, 1262, 1226, 1177, 1124, 1093, 1077, 1048, 1001, 926 cm^{-1}; for ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 371 [M + Na], 331 [M − H_2O]; HRESIFTMS *m/z* 349.2020 (calcd for $C_{20}H_{28}O_5$ + H, 349.2015).

12*R*-Hydroxyplatencinic Acid Methyl Ester (19): colorless gum; $[\alpha]^{23}_D$ −4 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 229 (ε 9594) nm; FTIR (ZnSe) ν_{max} 3444, 2929, 1731, 1667 (brs), 1438, 1380, 1290, 1262, 1195, 1174, 1132, 1099, 1085, 1072, 1017, 896, 825 cm^{-1}; for ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 327 [M + Na], 305 [M + H], 273; HRESIFTMS *m/z* 305.1757 (calcd for $C_{18}H_{24}O_4$ + H, 305.1752).

Compound 20: colorless, amorphous powder; $[\alpha]^{23}_D$ −0 (*c* 0.5, MeOH); UV (CH_3OH) λ_{max} 228 (ε 14 040), 276 (2371) nm; FTIR (ZnSe) ν_{max} 3378, 2933, 1674 (br), 1608, 1509, 1457, 1296, 1245, 1182, 1132, 1040, 1012, 829, 802 cm^{-1}; ^1H NMR 500 MHz (C_5D_5N) δ_H 7.06 (2H, d, *J* = 8.5 Hz, H-2, 6), 7.24 (2H, d, *J* = 8.5 Hz, H-3, 5), 4.51 (1H, dd, *J* = 9.5, 5.0 Hz, H-7), 4.35 (1H, dd, *J* = 9.5, 5.0 Hz, H-7), 4.53 (1H, pentet, *J* = 5.0 Hz, H-8), 3.78 (1H, dd, *J* = 10, 5 Hz, H-9), 3.77 (1H, dd, *J* = 10, 5 Hz, H-9), 3.36 (3H, s, OMe-10), 1.64 (6H, s, CH_3-12, 13), 7.07

(2H, d, J = 8.5 Hz, H-2′, 6′), 7.26 (2H, d, J = 8.5 Hz, H-3′, 5′), 4.43 (1H, dd, J = 9.5, 6 Hz, H-7′), 4.32 (1H, dd, J = 9.5, 6 Hz, H-7′), 4.58 (1H, pentet, J = 5 Hz, H-8′), 4.25 (1H, dd, J = 11, 5 Hz, H-9′), 4.22 (1H, dd, J = 11, 5 Hz, H-9′); ^{13}C NMR 125 MHz (C_5D_5N) δ_H 158.1 (C-1), 114.9 (C-2, 6), 128.6 (C-3, 5), 143.9 (C-4), 71.3 (C-7), 69.7 (C-8), 75.5 (C-9), 59.5 (C-10), 42.3 (C-11), 31.7 (C-12, 13), 158.0 (C-1′), 114.9 (C-2′, 6′), 128.7 (C-3′, 5′), 144.0 (C-4′), 71.3 (C-7′), 72.0 (C-8′), 64.8 (C-9′); HRESIFTMS m/z 391.2114 (calcd for $C_{22}H_{30}O_6$ + H, 391.2121).

Hydrolysis of Platensimycin A_1 Methyl Ester (3b). To a solution of compound **3b** (5 mg) in 1:1 THF−water (1 mL) was added 10 mg of LiOH, and the mixture was stirred overnight. An aliquot of 65 μL of 4 N HCl was added, then 2 mL of H_2O. The product was extracted with 3 mL of CH_2Cl_2, and the solvent was removed under a stream of nitrogen. LC-MS analysis indicated formation of oxidized product with intact methyl ester. This product was dissolved in THF−water (1.4 mL, 5:9), and 18 mg of LiOH was added. This mixture was heated at 50 °C overnight and quenched with 200 μL of 4 N HCl. Then, the solution was extracted with 3 × 3 mL of CH_2Cl_2. The organic extract was concentrated, and the product was purified by semipreparative HPLC (Zorbax C_8, 9.4 × 250 mm, 40 min gradient of 20−50% aqueous CH_3CN with 0.05% TFA, detection at 215 nm). Lyophilization of the fraction eluting at 22 min afforded 1.2 mg of the hydroxylated hydrolyzed product, **21**, as a powder: $[\alpha]^{23}_D$ +6 (c 0.5, MeOH); UV (MeOH) λ_{max} 227(ε 14 635), 255 (12 648), 295 (5676) nm; FTIR (ZnSe) ν_{max} 3316, 2963, 1647 (brs), 1607, 1536, 1449, 1393, 1347, 1308, 1246, 1202, 1148, 1075, 1052, 1032, 993 cm^{-1}; ^{1}H NMR 500 MHz (C_5D_5N) δ_H 10.50 (1H, s, NH), 8.10 (1H, d, J = 9.2 Hz, H-7′), 6.86 (1H, d, J = 8.8 Hz, H-6′), 6.75 (1H, s, H-7), 4.47 (1H, brs, H-10), 3.92 (1H, s, H-14), 2.90 (1H, m, H-2), 2.80 (1H, m, H-3), 2.76 (1H, m, H-2), 2.14 (1H, m, H-3), 2.71 (1H, m, H-13), 2.61 (1H, brs, H-9), 2.30 (1H, t, J = 6.5 H Hz, H-11), 1.98 (1H, m, H-11), 1.93 (1H, brd, J = 11.3 Hz, H-11), 1.88 (1H, d, J = 11.7 Hz, H-13), 1.66 (3H, s, H_3-17), 1.31 (3H, s, H_3-18); ^{13}C NMR 125 MHz (C_5D_5N) δ_C 175.3 (C-1), 32.3 (C-2), 33.4 (C-3), 47.3 (C-4), 200.4 (C-5), 148.1 (C-6), 120.9 (C-7), 50.0 (C-8), 47.9 (C-9), 76.8 (C-10), 41.3 (C-11), 44.7 (C-12), 40.1 (C-13), 86.1 (C-14), 90.4 (C-15), 19.8 (C-17), 23.1 (C-18), 174.7 (C-1′), 107.5 (C-2′), 158.7 (C-3′), 115.7 (C-4′), 158.4 (C-5′), 110.4 (C-6′), 129.9 (C-7′); HRESIFTMS m/z 474.1776 (calcd for $C_{24}H_{27}NO_9$ + H, 474.1764).

FabF$_{2P}$ Assay. *Staphyloccus aureus* cells (RN450) carrying plasmid S1-1941 bearing antisense to FabF (*fabF* AS-RNA strain) or vector (control strain) were inoculated from a frozen vial source into a tube containing 3 mL of Miller's LB broth (Invitrogen) plus 34 μg/mL chloramphenicol. Tubes were incubated at 37 °C at 220 rpm for 18−20 h and kept at room temperature until use. Miller's LB broth was supplemented with 1.2% Select agar (Invitrogen), 0.2% glucose, 15 μg/mL chloramphenicol, and 50 mM xylose (only for the antisense strain). The OD_{600} of the culture was measured and 1:1000 of OD 3.0 inoculated. Next, 100 mL was poured into each NUNC plate, well-caster templates were placed into the agar, and the agar was allowed to solidify. Then, 20 μL of test samples was added to the wells, the plates were incubated at 37 °C for 18 h, and zones of inhibition were measured.[7] MDC (minimum detection concentration) values were determined by 2-fold serial dilution.

FASII Assay. The assay was performed in a phospholipid-coated 96-well flash plate. Briefly, 1.26 μg of the partially purified protein from *S. aureus* containing all the required fatty acid synthesis enzymes was preincubated with a serial dilution of natural products at room temperature for 20 min in 50 μL of buffer containing 100 mM sodium phosphate (pH 7.0), 5 mM EDTA, 1 mM NADPH, 1 mM NADH, 150 μM DTT, 5 mM β-mercaptoethanol, 20 μM *n*-octanoyl-CoA (or lauroyl-CoA), 4% Me_2SO, and 5 μM ACP. The reaction was initiated by addition of 10 μL of water-diluted [2-^{14}C]-malonyl-CoA, giving a final concentration of 4 μM malonyl-CoA with total counts of about 10 000 cpm. The reaction was incubated at 37 °C for 90 min. The reaction was terminated by adding 100 μL of 14% perchloric acid. The plates were sealed and incubated at room temperature overnight and counted for 5 min using a Packard TopCount NXT scintillation counter. Further details of the assay are described in ref 31.

Antibiotic Assay (MIC). The MIC (minimum inhibitory concentration) against each of the strains was determined by 2-fold dilution, as previously described, and conducted under Clinical Laboratory Standards Institute (CLSI) guidelines.[731] Cells were inoculated at 10^5 colony-forming units/mL followed by incubation at 37 °C with a 2-fold serial dilution of compounds in the growth medium for 20 h. MIC is defined as the lowest concentration of antibiotic inhibiting visible growth.

■ ASSOCIATED CONTENT

Supporting Information. Table S1, Scheme S1, and ^{1}H and ^{13}C NMR spectra of **8d**, **8f**, **12a**, **13a**, **14a**, **15a**, **16a**, and **18**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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