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Structure and Semisynthesis of Platensimide A, Produced by *Streptomyces platensis*

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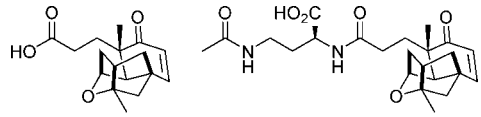
ABSTRACT



Platensimycin and platencin are novel natural product antibiotics that inhibit bacterial growth by inhibiting condensing enzymes FabF and FabF/FabH of fatty acid biosynthesis pathways, respectively. Continued search for the natural congeners of these compounds led to the isolation of platensic acid, the free C-17 tetracyclic enoic acid, and platensimide A, a 2,4-diaminobutyric acid amide derivative. Isolation, structure, semisynthesis, and activity of these compounds are described.

Platensimycin (**1**) and platencin (**2**) are two novel antibiotics isolated from various strains of *Streptomyces platensis*.[1–4] They were discovered by utilization of a novel antisense differential sensitivity screening strategy in which *fabH*/*fabF* expression was reduced, thereby making these strains hypersensitive to target-based inhibitors.[3–6] Platensimycin selectively inhibits the elongation condensing enzyme FabF of the bacterial fatty acid synthesis pathway by interacting with the malonyl binding site of the catalytic triad of the FabF acyl-enzyme intermediate. Platencin is a balanced inhibitor of both the initiation condensing enzyme (FabH) and elongation enzymes (FabF). They exhibited potent in vitro activity against both cell-free and whole-cell systems. The in vitro activities could not be directly translated to an in vivo mouse model when the drug was administered by conventional routes. However, when administered by continuous infusion, the drug was highly efficacious. The poor in vivo activity under conventional administration is attributed to its poor pharmacokinetic properties which could potentially be improved by chemical modification[7] or by discovery of the natural congeners from the fermentation broth which are devoid of the PK offending groups. The present study describes the discovery of three new congeners of platensimycin: methylplatensinoate (**3**), platensic acid (**4**), and platensimide A (**5**) from the fermentation broth by chemical screening.

S. platensis MA7327 was grown in 70 L fermentation tanks in the same media that was described for the original production of platensimycin yielding 43 L of broth.[2] The

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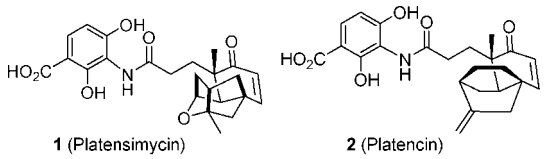
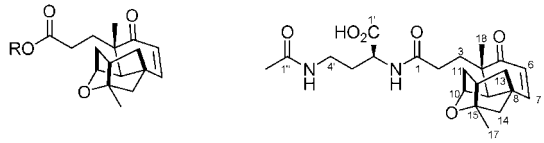
broth containing 52 mg/L of platensimycin was extracted with 1/2 volume of MeOH at pH 3.0, and the filtered broth was chromatographed on reversed-phase Amberchrome resin eluting with 40−100% aqueous MeOH gradient. One of the fractions eluting with a 100% MeOH was chromatographed on silica gel and eluted with a step gradient of hexane−EtOAc. Fractions eluting with 30% EtOAc afforded **3** (31 mg, 0.8 mg/L) as a gum: $[\alpha]^{23}_D$ −26.6 (c 3.5, MeOH); UV (MeOH) λ_{max} 234 (ϵ 10078) nm; IR (ZnSe) ν_{max} 2952, 2869, 1734, 1664, 1534, 1438, 1341, 1262, 1149 cm^{-1}.





Sephadex LH20 chromatography of one of the polar fractions eluting with 50% MeOH from the Amberchrome column followed by reversed-phase C_8 HPLC and lyophilization furnished platensic acid **4** (10 mg, 0.25 mg/L) as an amorphous powder: $[\alpha]^{23}_D$ −30 (*c* 1.5, MeOH); UV (MeOH) λ_{max} 234 (ϵ 8405) nm; IR (ZnSe) ν_{max} 3300, 2945, 1718, 1656, 1444, 1406, 1382, 1333, 1297, 1272, 1229, 1182, 1094, 1020, 991, 952, 914 cm^{-1}.

Acid−base extraction of one of the subsequent polar Amberchrome fractions eluting with 50−60% MeOH followed by two successive steps of reversed-phase C_8 and phenyl-bonded HPLC using aqueous CH_3CN + 0.1% TFA gradient of 1/4th aliquot gave **5** (2.4 mg, 1 mg/L) as a gum: $[\alpha]^{23}_D$ +29.2 (*c* 1.3, MeOH); UV (MeOH) λ_{max} 230 (ϵ 4867) nm; IR (ZnSe) ν_{max} 3300 (br), 3095, 2964, 1657 (br), 1545, 1445, 1380, 1348, 1309, 1198, 1136, 1106 cm^{-1}.

HRESIFTMS analysis of methylplatensinoate (**3**) produced a parent ion at *m*/*z* 305.1738 analyzed for a molecular formula $C_{18}H_{24}O_4$ (calcd for M + H, 305.1753). This compound showed a UV maximum at 234 nm different from platensimycin. The ^{13}C NMR spectrum (Table 1) of **3** showed the presence of 18 carbon signals distributed between δ_C 23.2 and δ_C 205.3 corroborating the molecular formula. The ^{13}C NMR, DEPT, and HMQC spectral analysis indicated that the molecule consisted of an enone, a carboxyl, an oxymethine, two aliphatic methines, two methyls, a methoxy, five methylenes, and two quaternary carbons one bearing an oxygen atom. The ^1H NMR spectrum (Table 1) revealed the presence of a secondary methyl, an angular methyl, a methoxy methyl, a pair of olefinic protons with J = 10 Hz, and an oxymethine broad singlet. These signals were characteristic of C-17 tetracyclic enone acid portion of platensimycin. The ^{13}C NMR chemical shift comparison of **3** with platensimycin together with HMBC correlation of the methoxy group to the C-1 (δ_C 175.4) suggested that this molecule was the methyl ester of the C-17 tetracyclic enone acid (platensic acid) piece of platensimycin, named herein methylplatensinoate (**3**). The structure was confirmed by HMBC correlations just like platensimycin. The mass spectral analysis of the free carboxylic acid (platensic acid, **4**) produced a molecular formula of $C_{17}H_{22}O_4$ (*m*/*z* 291.1588 calcd for M + H, 291.1591) and was characterized by NMR spectral analyses (Table 1) and the structure was confirmed by the direct comparison of a sample prepared by alkaline

Scheme 1. Semisynthesis of Platensimide A (**5**)

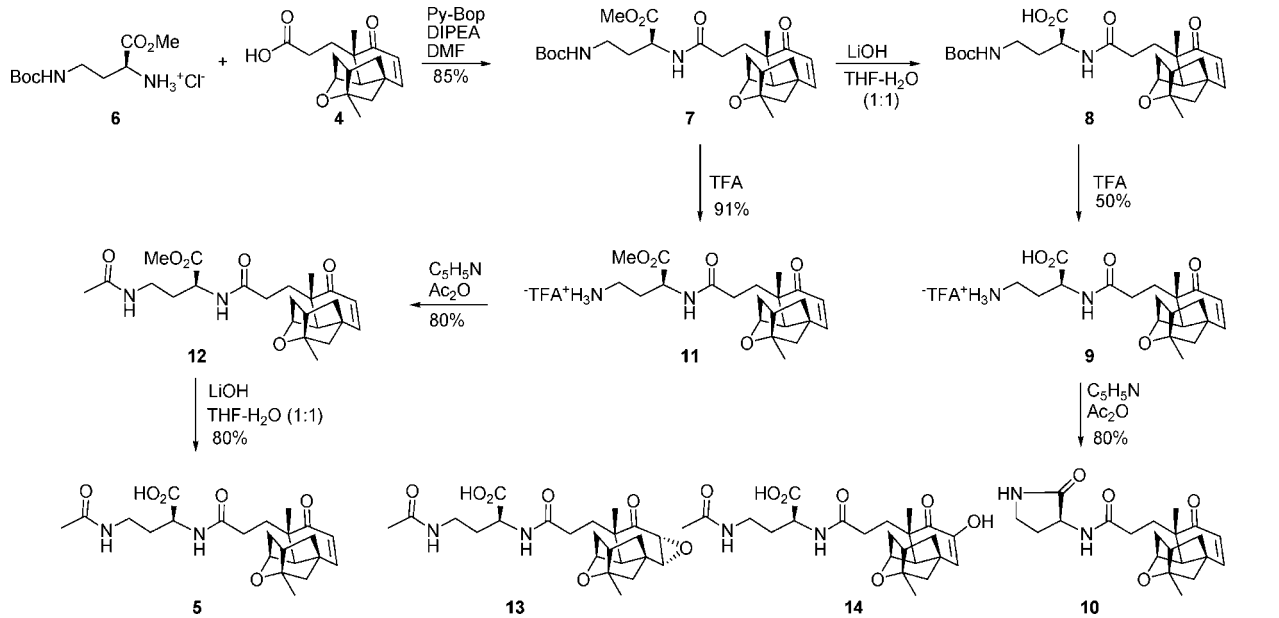


Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Assignments of **3** (CD_3OD), **4** (C_5D_5N), and **5** (C_5D_5N)

	3			**4**		**5**	
no.	δ_C	type	δ_H, mult, J in Hz	δ_C	δ_H, mult, J in Hz	δ_C	δ_H, mult, J in Hz
1	175.4	C^0		176.3		173.0	
2	30.2	CH_2	2.10, m	30.9	2.56, m	32.2	2.47, m
			2.30, m		2.65, m		2.57, m
3	31.9	CH_2	2.25, m	32.3	2.67, m	32.7	2.08, m
			1.70, m		2.00, m		2.59, m
4	47.6	C^0		47.2		47.2	
5	205.3	C^0		203.8		203.8	
6	127.8	CH	5.87, d, 10	127.8	6.00, d, 10	127.7	5.94, d, 10
7	155.9	CH	6.65, d, 10	154.6	6.44, d, 10	154.5	6.35, d, 10
8	47.2	C^0		47.0		46.6	
9	47.2	CH	2.37, brs	46.7	2.54, brs	46.8	2.43, brs
10	78.0	CH	4.41, brt, 3.0	77.1	4.57, brt, 3.0	76.9	4.47, t, 3.5
11	41.5	CH_2	2.08, m	41.4	1.96, m	41.2	1.76, m
					1.87, brd, 11.5		1.87, m
12	46.0	CH	2.44, t, 6	45.6	2.25, t, 6.5	45.5	2.18, t, 6.6
13	43.9	CH_2	2.05, m	43.6	1.62, ddt, 11.5, 7.0, 1.9	43.5	1.55, m
			1.84, ddt, 12, 7.0, 1.5		1.81, dd, 11.5, 3.5		1.78, m
14	55.8	CH_2	1.78, dd, 11.5, 1.5	55.5	1.85, d, 11.5,3.0	55.1	1.46, d, 10.8
			1.74, d, 11.5		1.53, d, 11.5		1.72, dd, 10.8, 3.4
15	88.7	C^0		87.4		87.2	
17	23.2	CH_3	1.43, s	23.8	1.43, s	23.7	1.37, s
18	25.0	CH_3	1.24, s	25.2	1.20, s	25.07	1.12, s
1′	52.2	CH_3	3.66, s			175.9	
2′		CH				51.7	5.1, dt, 5, 8.5
3′		CH_2				33.5	2.24, m
							2.57, m
4′		CH_2				37.1	3.57, dq, 12, 6
							3.87, dq, 12, 6
4′-NH							8.5, t, 6.2
1″		C°				170.7	
2″		CH_3				23.6	2.0, s
1′-NH							8.9, d, 8.8

hydrolysis (LiOH in either THF−water or MeOH−water) of the methyl ester **3**.

Platensimide A (**5**) was analyzed by HRESIFTMS to afford a molecular formula $C_{23}H_{32}N_2O_6$ (observed *m/z* 433.2324, calcd for M + H, 433.2339). The UV spectrum showed an absorption maximum at 230 nm similar to platensic acid. The ^{13}C NMR spectrum (Table 1) of **5** revealed the presence of 23 carbons and confirmed the molecular formula. The ^{1}H NMR spectrum of **5** showed the presence of all of the signals present in **4** along with the presence of a number of additional signals including an acetoxy group at δ_H 2.0 (δ_C 23.6). Comparison of ^{1}H and ^{13}C NMR spectra of **5** with **3** and platensimycin suggested that **5** consisted of platensic acid ($C_{17}H_{21}O_3$) with an additional six carbons, three oxygens, and two nitrogens ($C_6H_{11}N_2O_3$). The acetate group accounted for two carbons leaving behind $C_5H_8N_2O_2$ comprised of two methylenes, one methine, and a carboxyl group. This fragment was consistent with a 2,4-diaminobutyric acid residue which was confirmed by the COSY correlations of the methine proton (δ_H 5.1) with the α-NH (δ_H 8.9, d, J = 8.8 Hz) and the β-methylene protons (δ_H 2.24 and δ_H 2.57), which showed correlations to the γ-methylene protons (δ_H 3.6 and δ_H 3.9), which in turn showed COSY correlation to a NH triplet at δ_H 8.5. This structural unit was confirmed by the TOCSY correlations which showed extended correlations of the methine protons to the CH_2−NH and thus corroborated the COSY fragment. The structure of this unit was further confirmed by HMQC and HMBC correlations (Figure 1). For example, the H_2-4′ showed HMBC correlations to C-2′ (δ_C 51.7) and C-3′ (δ_C 33.5); H-2′ exhibited HMBC correlations to C-1′ (δ_C 175.9) and C-3′. The α-NH (δ_H 8.9) displayed HMBC correlation to C-1 (δ_C 173.0), thus establishing the connectivity of the 2,4-diaminobutyric acid to platensic acid via an amide bond. The HMBC correlation of H_2-4′ and the acetate methyl at δ_H 2.0 to the same carbonyl C-1″ (δ_C 170.7) confirmed the capping of the γ-amino group of the 2,4-diaminobutyric acid with the acetate group and thus establishing the structure **5** for platensimide A. The

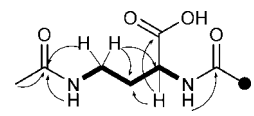


Figure 1. Selected COSY, TOCSY, and HMBC correlations of **5**.

structure and absolute configuration of **5** was confirmed by semisynthesis.

Coupling of platensic acid (**4**) with Boc-(*S*)-2,4-diaminobutyric acid methyl ester hydrochloride (**6**) using Py-Bop in DMF and DIPEA yielded bis-protected amide **7** in 85% yield. The amide **7** was first hydrolyzed with LiOH in THF−water to yield compound **8** which was treated with excess TFA without workup to give TFA salt of the free acid **9**. Acetylation of the amino acid **9** led to only lactam **10** and no acetylated product **5**. Therefore, the deprotection routine was reversed. The Boc deprotection of **7** with TFA produced **11** in excellent yield which was acetylated with acetic anhydride and pyridine to afford the acetylated product **12**. Basic (LiOH in THF−water or MeOH−water hydrolysis) of **12** afforded the semisynthetic compound **5** which was identical to the natural platensimide A (co-HPLC, NMR, LCMS).

When hydrolysis was performed using older lots of THF that contained peroxide, epoxide **13** was formed in significant amounts, at times exclusively. The concentration of the epoxide **13** in the presence of aqueous TFA produced the hydroxy enone **14** presumably after epoxide hydrolysis followed by dehydration. The formation of epoxide in these conditions is interesting since epoxidation under standard conditions led to Bayer−Villiger oxidation followed by a rearrangement.[7]

Compounds **3** inhibited *S. aureus* fatty acid synthesis in cell free system using FASII assay[8] with an IC_{50} of 167 μg/mL. Platensic acid did not show any activity at >333 μg/mL in this assay. Platensimide A (**5**) inhibited FASII assay with an IC_{50} value of 80 μg/mL. None of these compounds inhibited *S. aureus*, *Streptococcus pneumoniae*, or *Enterococcus faecalis* growth at 64 μg/mL. Platensimide A exhibited minimum detection concentration (MDC, minimum concentration of the compound showing differential zone of clearance between antisense plate versus control plate) at 100 μg/mL in the antisense two-plate differential sensitivity assay[6] demonstrating about 2500-fold lower activity than platensimycin.[1] The poor activity of these compounds underscores the importance of the 3-amino-2,4- dihydroxybenzoic acid present in platensimycin and platencin. None of the synthetic compounds showed better activity than **5** and showed MIC >64 μg/mL against *S. aureus*. Platensimycin exhibited MIC value of 0.5 μg/mL against the same *S. aureus* strain.

In summary, we have described the isolation, structure, and activities of three congeners of platensimycin including the aliphatic tetracyclic enone carboxylic acid unit. The amidation of the platensic acid with an amino acid drastically different than the one present in platensimycin indicates that likely there are likely more than one amidase present in *S. platensis*.[9]

Supporting Information Available: ^{1}H and ^{13}C NMR data of compounds **3**−**5**, ^{1}H NMR spectra of **7**−**14**, and experimental details of synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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