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Structure of homoplatensimide A: a potential key biosynthetic intermediate of platensimycin isolated from *Streptomyces platensis*

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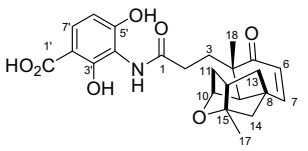
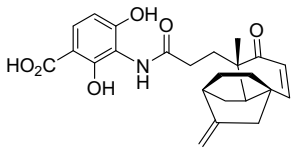
Abstract

Platensimycin and platencin are novel natural product antibiotics that inhibit bacterial growth by inhibiting fatty acid biosynthesis enzymes FabF and FabF/FabH, respectively. Continued search for the natural congeners for structure activity relationship studies led to the isolation of a congener which possesses all of the twenty carbons of diterpenoid unit, a potential biosynthetic intermediate of platensic acid unit of platensimycin. Isolation, structure, and activity of homoplatensimide A and biosynthetic relationship to platensimycin have been described.

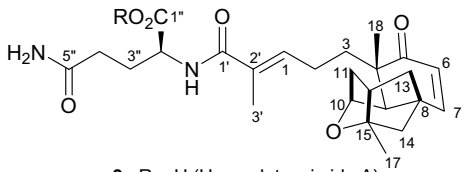
Platensimycin (**1**) and platencin (**2**) are two novel antibiotics isolated from various strains of *Streptomyces platensis*.[1–4] They were discovered by a novel antisense differential sensitivity screening strategy in which FabH/FabF was sensitized.[3–6] Platensimycin selectively inhibits the elongation 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 pharmacokinetic properties which could potentially be improved by chemical modification or by the discovery of the natural congeners from the fermentation broth. Continued studies on the isolation of congeners led to isolation of homoplatensimide A (**3a**) and its methyl ester **3b**. Discovery of these compounds provides critical link of biosynthetic origin of C-17 tetracyclic enoic acid (platensic acid, **4**) of platensimycin.



1 (Platensimycin)



2 (Platencin)



3a R = H (Homoplatensimide A)
3b R = Me (Homoplatesimide A methyl ester)

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Fermentation broth of *S. platensis* MA7327 grown in 70 L fermentation tanks on the original media[2] was extracted with methanol at pH 3.0 and chromatographed on an Amberchrome column eluting with a 40–100% aqueous CH_3OH gradient. The fractions eluting with approximately 50% CH_3OH were extracted with CH_2Cl_2 at pH 9 and pH 2. The acidic and neutral extracts were pooled and chromatographed on a silica gel column eluting with 5–25% step gradient of CH_2Cl_2–MeOH. Fractions eluting with 5% MeOH were further chromatographed by two successive steps of reversed-phase HPLC using Zorbax RX C_8 column eluting with a gradient of aqueous CH_3CN containing 0.1% TFA. This afforded 0.4 (0.01 mg/L) and 1.6 mg (0.04 mg/L) of homoplatensimide A (**3a**) and methyl ester (**3b**), respectively, both as a gum, **3b**: $[\alpha]_D^{23}$ –16 (*c* 0.5, CH_3OH), UV (CH_3OH) λ_{max} 236 nm, IR (ZnSe) ν_{max} 3287, 2967, 1651, 1535, 1379, 1308, 1202, 1154, 11061, 924, 831 cm^{-1}.

HRESIFTMS analysis of **3b** provided a parent ion at *m/z* 473.2641 and afforded a formula $C_{26}H_{36}N_2O_6$ (calcd for M+H, 473.2646). The formula was supported by the ^{13}C NMR spectral analysis in C_5D_5N which showed 26 lines distributed throughout the ^{13}C chemical shift ranges. The UV spectrum showed an absorption maxima at λ_{max} 236 nm indicative of the presence of an α,β-unsaturated ketone. The ^{13}C NMR spectrum along with DEPT (Table 1) showed the presence of four ketones including one enone, three olefinic methines, an olefinic quaternary, four methyls including one methoxy, four methines (one oxymethine), seven methylenes, three aliphatic quaternary carbon including one being oxygenated. The ^1H NMR spectrum of **3b** together with COSY, TOCSY, and HMQC (Fig. 1 and Table 1) suggested the presence of a molecular fragment consisting of two methylenes and α-methine (δ_H 5.01, δ_C 54.1) reminiscent of glutamic acid type residues which was confirmed by HMBC correlations of the methine pro-

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) assignment of homoplatensimide A (**3a**) and methyl ester (**3b**) in C_5D_5N

	3b				**3a**
No.	δ_C	Type	δ_H, mult, *J* in Hz	HMBC	δ_H, mult, *J* in Hz
1	136.2	CH	6.69, t, 7.0	C-1′, C-2′, C-3′	6.73, t, 7.0
2	23.8	CH_2	2.04, m		2.05, m
			2.13, m		2.13, m
3	34.7	CH_2	1.33, m	C-18	1.33, ddd, 15, 7.5, 6
			2.02, m		2.02, m
4	46.5	C°			
5	203.7	C°			
6	127.8	CH	6.07, d, 10.0	C-4, C-8	6.01, d, 10.0
7	154.4	CH	6.41, d, 10.0	C-5, C-8, C-9, C-14	6.41, d, 10.0
8	47.2	C°			
9	46.6	CH	2.51, br s	C-4, C-10, C-11, C-13, C-14, C-18	2.53, br s
10	77.0	CH	4.36, t, 3.5	C-12, C-15	4.38, t, 3.5
11	41.3	CH_2	1.98, m	—	1.98, m
			1.85, br d, 11.5	C-10, C-12, C-13	1.85, br d, 11.5
12	45.5	CH	2.23, t, 6.5	C-8, C-10, C-14, C-17	2.23, t, 6.5
13	43.5	CH_2	1.82, br d, 11.5, 3.0		1.82, br d, 12, 3.0
					1.59, dd, 12, 7
			1.59, dd, 11.5, 6.5		
14	55.5	CH_2	1.51, d, 11.5	C-7, C-8, C-9, C-12, C-17	1.51, d, 11
			1.83, dd, 11.5, 3.5		
					1.83, dd, 11, 3
15	87.4	C°			
17	23.7	CH_3	1.42, s	C-12, C-14, C-15	1.43, s
18	25.1	CH_3	1.10, s	C-3, C-4, C-5, C-9	1.09, s
1′	170.5	C°			
2′	131.7	C°			
3′	13.4	CH_3	2.00, s	C-1, C-1′, C-2′	2.04, s
1″-OMe	52.2	CH_3	3.62, s	C-1″	–
1″	173.6	C°			
2″	54.1	CH	5.01, ddd, 9.0, 7.5, 5.0	C-1″, C-2″, C-3″	5.25, br m
3″	27.2	CH_2	2.46, m,	C-2″, C-4″, C-5″	2.63, m,
			2.59, m		2.82, m
4″	32.5	CH_2	2.74, m	C-2″, C-3″, C-5″	2.90, m
5″	175.1	C°			
5″-NH_2		NH	8.38, br s,		8.38, br s,
					7.80, br s
			7.84, br s		
2′-NH		NH	8.90, d, 7.5	C-1′	8.68, d, 7.5

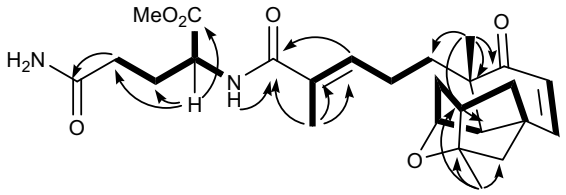
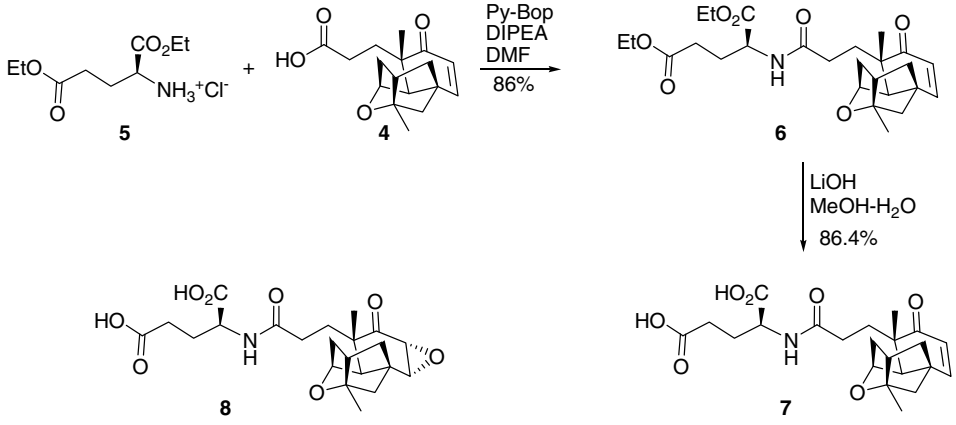


Fig. 1. The COSY, TOCSY (bold) and HMBC correlations of **3b**.

ton to a carboxyl (δ_C 173.6, C-1″) and the α-methylene protons to other carboxyl (δ_C 175.1, C-5″). The methoxy methyl protons showed HMBC correlation to the carboxyl at (δ_C 173.6) assigned as C-1″. The ^{1}H NMR spectrum displayed two broad resonances integrating for one proton each self coupled exchangeable singlets (δ_H 8.38 and δ_H 7.84) characteristic of primary amide which was assigned to the C-5″ NH_2 of glutamine. The subtraction of the glutamine methyl ester from the formula of **3b** and the comparison of the remaining signals of the ^{13}C NMR spectrum of **3b** with platensimycin suggested that the tetracyclic terpenoid cores of the two molecules were identical. The differences between the two were the presence of an additional CH_3–C=CH-unit in the structure of **3b**. The COSY and TOCSY correlation of **3b** produced a structural fragment CH_3–C=CH–CH_2–CH_2-suggesting that these three additional carbons were connected in between the two methylenechains and the carboxyl group of the side chain of platensimycin. This structural unit was confirmed by HMBC correlations from the olefinic methyl group to C-1′, C-2′, and C-1 and olefinic methine to the carboxyl C-1′. The HMBC correlation from C-18 methyl protons to the C-3 methylene confirmed the connectivity of this six carbon chain to the tetracyclic enone core. HMBC correlations from the α-NH group of glutamine to the carboxyl C-1′ established the amide bond and connected the two pieces together. Mass spectral analysis of homoplatensimide A (**3a**) produced a molecular formula $C_{26}H_{36}N_2O_6$ (*m*/*z* 459.2487, calcd for M+H, 459.2490) which was a methylene unit less than **3b**. The ^{1}H NMR spectrum indicated a loss of the methoxy signal and slight downfield shift of glutamine signals due to formation of pyridinium salt and significant signal broadening of the α-proton. Acid hydrolysis (6 N HCl in a sealed tube at 116 °C for 15 h) of **3a** and **3b** followed by preparation of Marfey's derivatives of each of the reaction products and comparison with the Marfey's derivatives prepared from standard (*S*)- and (*R*)- glutamine as well as (*S*)- and (*R*)-glutamic acid confirmed the presence of (*S*)-glutamic acid in the reaction mixture which is derived from (*S*)-glutamine and (*S*)-glutamine methyl ester by the acid hydrolysis. Marfey's derivative of the hydrolysis product of the standard glutamines accordingly produced glutamic acid derivatives. Based on this analysis and known absolute configuration of tetracyclic enone of platensimycin, structure **3a** was assigned for homoplatensimide A and **3b** to the methyl ester which is likely an isolation artifact.

To test whether the addition of negative charge would improve the activity of a non-benzoic acid substituted analog of platensimycin, (*S*)-glutamic acid amide (**7**) of platensic acid was prepared, as follows: Coupling of platensic acid (**4**)[7] with (*S*)-glutamic acid diethyl ester hydrochloride (**5**) using Py-Bop reagent in DMF and DIPEA yielded amide **6** in 86% yield (Scheme 1). Amide **6** was hydrolyzed with LiOH in MeOH–H_2O to yield diacid **7** in 86.4% yield. When hydrolysis was performed in THF–H_2O particularly when THF was not freshly distilled the hydrolysis reaction yielded a mixture of desired product **7** along with epoxide **8** (*m*/*z* 436.1966, M+H, $\delta_{H\text{-}6}$ 3.45, d, $J = 4.5$ Hz, $\delta_{H\text{-}7}$ 3.17, d, $J = 4.5$ Hz). The ratio of the products was dependent on the quality of THF.

Compounds **3a** was a poor inhibitor of *Staphylococcus aureus* fatty acid synthesis in cell free system measuring FASII assay[8] (IC_{50} of >167 μg/mL) and did not inhibit *S. aureus* growth at 250 μg/mL. In more sensitive antisense two-plate differential sensitivity assay[5] it showed MDC 400 μg/mL. MDC is defined by minimum concentration of the compound showing differential zone of clearance between antisense plates compared to control plate. This activity was about 10000-fold lower than platensimycin. The carboxyl group of 3-amino-2,4-dihydroxy benzoic acid



Scheme 1. Synthesis of (*S*)-Glu-platensimide (**8**) analog of homoplatensimide A.

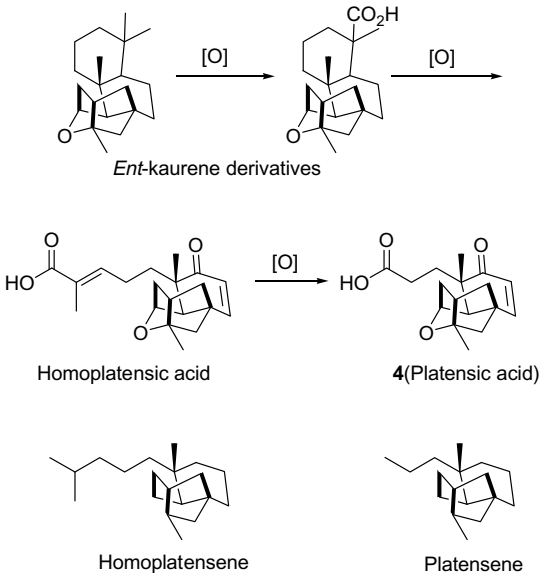


Fig. 2. Biogenetic relationship of platensic and homoplatensic acids.

unit of platensimycin showed strong interaction with both active site histidines. To test whether the addition of negative charge will improve the activity of a non-benzoic acid substituted analog of platensimycin, (*S*)-glutamic acid amide (**7**) of platensic acid was prepared, and tested against *S. aureus* whole cell assay. None of the compounds (compounds **7**, the diester derivative **6**, and the epoxy derivative **8**) show any growth inhibition at 64 μg/mL (the highest concentration tested). Platensimycin showed MIC value of 0.5 μg/mL against this strain.

The discovery of homoplatensimide A is highly significant for the study of the biosynthesis of platensimycin. We have recently shown that the tetracyclic C-17 acid is derived from a non-mevalonate terpenoid pathway likely derived from *ent*-kaurene.[9] The existence of an A-ring open diterpenoid, homoplatensic acid (the carbon skeleton hydrocarbon named here is homoplatensene) in the same broth provides a strong evidence for its intermediacy for the biosynthesis of platensic acid (the carbon skeleton hydrocarbon named here is platensene). The oxidative excision of the three carbons (Fig. 2) from homoplatensic acid would lead to platensic acid. The likely source of the homoplatensic acid is *ent*-kaurene (not seco-*ent*-kaurene)[3] or its analogs which is derived from bicyclic diterpenoid intermediate copalyl diphosphate.[10,11] Significant differences of both amino and acid components between platensimycin and homoplatensimide A would suggest the presence of more than one amidase for the amide formation.

In summary, we have described the isolation, structure, and activities of homoplatensimide A, a new congener of platensimycin that contains a C-20 diterpenoid tetracyclic enoic acid coupled with glutamine. The C-20 diterpenoid, homoplatensic acid is the likely biosynthetic source of platensic acid, the C-17 acid of platensimycin. This report also validated the importance of the 3-amino-2,4-dihydroxy-benzoic acid for the activity of platensimycin.

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