Isolation, structure and fatty acid synthesis inhibitory activities of platensimycin B_1–B_3 from *Streptomyces platensis*†

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Platensimycins B_1–B_3 are natural congeners of platensimycin with modest to significant changes in the benzoic acid portion of the molecule, leading to attenuation in the biological activities and thus confirming the significance of the free carboxylate for the potent activity.

Platensimycin (**1**) and platencin (**2**) are two novel antibiotics discovered recently from various strains of *Streptomyces platensis*.[1–4] They were discovered by application of a novel antisense differential sensitivity screening strategy in which *fabH*/*fabF* genes were sensitized.[3–6] Platensimycin (**1**) selectively inhibits the FabF acyl-enzyme intermediate of the elongation condensing enzyme whereas platencin inhibits both condensing enzymes, FabH and FabF, essentially with equal efficiency. Both compounds showed target selectivity and potent antibacterial activities in cell-free and whole-cell assays. They showed activity in a disseminated *Staphylococcus aureus* infection model when administered by intravenous (IV) continuous infusion route in mouse. However, poor *in vivo* activity was observed when administrated by conventional routes due to poor pharmacokinetic properties. In order to achieve higher and sustained systemic drug exposure, the PK of these compounds need to be improved or alternative delivery routes have to be developed. To meet this challenge multifaceted approaches were undertaken including chemical modifications,[7] biosynthesis,[8] and discovery of congeners.[9,10] The latter approach led to the isolation of three new congeners of platensimycin with modifications in the aromatic portion of the molecule, which validated the role of carboxylic acid for the potent activity of platensimycin. These compounds are platensimycin B_1 (**3**), B_2 (**4**) and B_3 (**5**). Isolation, structure elucidation and biological activities of these compounds are described.

Acidified (pH 2.7) fermentation broth of *Streptomyces platensis* MA7327 was extracted with MeOH and chromatographed on an Amberchrome column. The column was eluted with a step gradient of aqueous MeOH. Extraction of 80–90% MeOH eluent from the Amberchrome column with CH_2Cl_2 at pH 9.0 and 2.0 followed by silica gel, Sephadex LH-20 and reversed phase HPLC chromatography afforded platensimycin B_1 **3** (11.2 mg, 0.26 mg L^{-1}), B_2 **4** (24 mg, 0.56 mg L^{-1}), and B_3 **5** (3.7 mg, 0.09 mg L^{-1}), as amorphous powders.[±]

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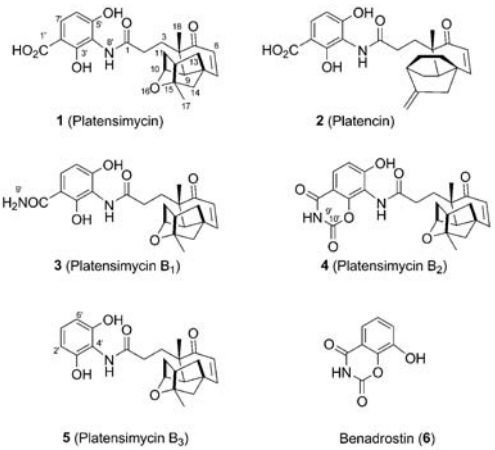
† Electronic supplementary information (ESI) available: Physical data, copies of ^1H and ^{13}C NMR, IR and UV spectra of all compounds, and Figure S1. See DOI: 10.1039/b810113b

ESIMS analysis of platensimycin B_1 (**3**) produced ions at *m*/*z* 463 [M + Na] and 441 [M + H] in the positive and *m*/*z* 439 [M − H] in the negative ion detection modes suggesting a molecular weight of 440. HRESIFTMS analysis produced a parent ion at *m*/*z* 441.2012 analyzed for a molecular formula $C_{24}H_{28}N_2O_6$ (calcd for M + H, 441.2025). The IR and UV spectrum of **3** was similar to the UV spectrum of platensimycin (**1**). Comparison of the formula of **3** with platensimycin indicated the substitution of one of the oxygen atoms with a NH group. The ESIMS produced a fragment ion at *m*/*z* 273 characteristic of the fragment derived from the C-17 tetracyclic enone acid part of the platensimycin indicating that the nitrogen substitution was in the anilide portion of the molecule (ESI, Fig. S1†). The ^{13}C NMR spectrum of **3** was essentially identical to that of **1** except for minor differences in the carboxyl resonances indicating the substitution of the carboxylic acid with a carboxy amide. This was confirmed by the presence of a pair of broad amide proton singlets at δ_H 8.84 and δ_H 9.21 that correlated to each other in the TOCSY. The carboxyamide carbon C-1′ (δ_C 174.7) exhibited strong HMBC correlation to H-7′ (δ_H 8.10) thus confirming the structure **3** for platensimycin B_1.



Platensimycin B_2 (**4**) produced ions at *m*/*z* 489 [M + Na] and *m*/*z* 467 [M + H] and *m*/*z* 465 [M − H] in ESIMS indicating a molecular weight of 466. In HRESIFTMS it showed a parent ion at *m*/*z* 467.1828 providing a molecular formula $C_{25}H_{26}N_2O_7$ (calc. for M + H, 467.1818) consisting of an extra carbon and nitrogen with loss of a hydrogen atom from platensimycin (**1**). The ^{13}C NMR spectrum (DMSO-d_6) displayed 25 resonances and corroborated the molecular formula. The UV spectrum of **4** (λ_{max} = 215 and 234 nm) showed slight differences from platensimycin (**1**). The IR spectrum showed absorption bands for hydroxy and ketone stretches

and a carbamate (ν_{max} 1761 cm^{-1}). ESIMS produced a fragment ion at m/z 273 suggesting that C-17 tetracyclic enone acid was unchanged. This observation was supported by the comparison of ^{13}C NMR spectrum with platensimycin (**1**) (Table 1). However, the aromatic portion of the ^{13}C NMR spectrum of **4** differed significantly from **1**. The aromatic methine carbon C-6′ was shifted downfield by 5 ppm, C-7′ was shifted upfield by 3 ppm and the acid carbonyl was shifted upfield by 12 ppm indicating that the additional carbon and nitrogen was attached to this part of the molecule. The ^{13}C NMR spectrum of **4** showed the presence of an additional resonance at δ_C 147.3 which accounted for the additional carbon in the molecule. The ^{1}H NMR spectrum showed the presence of a pair of doublets for *ortho*-coupled aromatic protons and three exchangeable proton singlets δ_H 11.9, δ_H 10.8 and δ_H 9.3. One of these protons was assigned to the amide NH-8′ (δ_H 9.30) which showed HMBC correlations to the amide carbonyl C-1 (δ_C 171.3). This amide carbonyl was assigned using its HMBC correlations with H_2-2 and H_2-3. The aromatic methine H-7′ showed strong three-bond HMBC correlations to C-1′ (δ_C 160.1), C-3′ (δ_C 151.0) and C-5′ (δ_C 160.9) and H-6′ showed correlations to C-2′ (δ_C 112.4) and C-4′ (δ_C 106.1). The exchangeable proton at δ_H 11.9 showed HMBC correlations to δ_C 106.1. The third exchangeable proton δ_H 10.8 showed strong HMBC correlation with C-2′ and a weak HMBC correlation to C-1′. These assignments accounted for all carbons except for a carbon resonating at δ_C 147.3 which was assigned to a carbamate carbonyl at C-10′. Based on these HMBC correlations, observed upfield shifts of C-1′ (δ_C 160.1), carbonyl carbon C-10′ and C-2′ ($-\Delta\delta$ 7, due to acylation), molecular formula and the UV spectrum, a cyclic carbamate structure **4** was established for platensimycin B_2. Six-membered cyclic carbamates are highly rare natural products. A substructure search in SciFinder returned benadrostin (**6**) as the only natural product hit which was isolated from *Streptomyces flavovirens* as an inhibitor of poly(ADP ribose) synthetase.[11,12] The ^{13}C NMR chemical shifts of **4** and **6** are consistent.

Platensimycin B_3 (**5**) showed pseudo-molecular ions at m/z 420 [M + Na] and 398 [M + H] in ESIMS. No ions were observed in the negative ion mode. HRESIFTMS analysis of **5** produced a parent ion at m/z 398.1961 and a molecular formula $C_{23}H_{27}NO_5$ (calc. for M + H, 398.1967) indicating a loss of CO_2 from platensimycin. The UV spectrum of **5** was visibly different

Table 1 ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignment of platensimycins B_1 (**3**), B_2 (**4**) and B_3 (**5**)[a]

Solvent No.	**3** C_5D_5N δ_C	Type	**3** C_5D_5N δ_H	**4** DMSO-d_6 δ_C	**4** DMSO-d_6 δ_H	**5** C_5D_5N δ_C	**5** C_5D_5N δ_H
1	175.1	C°		171.3		174.8	
2	32.2	CH_2	2.83, ddd, 5.4, 14.5, 11.3 2.75, ddd, 4.4, 14.5, 11.3	30.3	2.10, m 2.36, m	32.2	2.81, ddd, 5.3, 14.8, 11.7 2.74, ddd, 4.3, 14.8, 11.5
3	32.6	CH_2	2.66, ddd, 4.4, 14.0, 11.5 2.03, ddd, 5.1, 13.8, 11.0	31.1	1.70, m 2.10, m	32.6	2.64, ddd, 4.3, 13.6, 11.7 2.00, ddd, 5.3, 13.6, 11.5
4	47.2	C°		45.8		47.2	
5	203.8	C°		202.9		203.7	
6	127.7	CH	5.96, d, 10.4	126.5	5.83, d, 10	127.7[b]	5.93, d, 10.0
7	154.5	CH	6.39, d, 10.4	154.5	6.67, d, 10	154.5	6.36, d, 10.0
8	46.6	C°		45.6		46.6	
9	47.0	CH	2.44, br s	45.7	2.27, br s	47.0	2.43, br s
10	76.9	CH	4.48, br s	75.5	4.38, br s	76.9	4.47, br s
11	41.3	CH_2	1.91, m 1.81, br d, 11.3	40.2	1.90, m 2.09, m	41.2	1.90, m 1.81, br d, 11.4
12	45.5	CH	2.21, t, 6.5	44.2	2.34, m	45.5	2.19, t, 6.5
13	43.5	CH_2	1.81, br d, 11.3 1.58, dd, 6.6, 11.7	42.4	1.79, m 1.95, m	43.5	1.80, br d, 11.4 1.56, dd, 6.6, 11.6
14	55.4	CH_2	1.73, dd, 10.9, 3.1 1.48, d, 11.0	54.2	1.7, br s	55.4	1.72, dd, 11.2, 3.4 1.47, d, 11.2
15	87.3	C°		86.4		87.3	
17	23.7	CH_3	1.40, s	22.9	1.35, s	23.7	1.38, s
18	24.9	CH_3	1.14, s	24.8	1.15, s	24.9	1.11, s
1′	174.7	C°		160.1		—	—
2′	107.9	C°		112.4		109.6	6.83, d, 8.1
3′	158.7	C°		151.0		152.8	
4′	116.2	C°		106.1		117.1	—
5′	157.4	C°		160.9		152.8	—
6′	110.1	CH	6.84, d, 8.8	113.5	6.89, d, 9	109.6	6.83, d, 8.1
7′	127.9	CH	8.10, d, 8.8	125.6	7.68, d, 9	127.7[b]	7.09, t, 8.2
8′-NH			10.49, s		9.3, s		10.39, s
9′-NH			9.21, br s 8.84, br s		10.8, s		—
3′-OH			—		—		—
5′-OH			—		11.9, s		—
10′			—		147.3		—

[a] All assignments were confirmed by COSY, TOCSY, HMQC and HMBC experiments as reported for platensimycin (C° = quaternary carbon). [b] These resonances appeared at δ_C 127.69 and δ_C 127.66 ppm.

from **1** suggesting the loss of the conjugation. The ESIMS produced the fragment ion at m/z 273 corroborating that the structural change was in the aromatic part of the molecule. The ^{13}C NMR spectrum of **5** displayed only 21 resonances and showed the presence of only one of the two carboxyl type carbons. The spectrum displayed the presence of only four resonances for aromatic carbons with two resonances showing doubling of the signal height. The ^{1}H NMR spectrum showed an *ortho* coupled doublet at δ_H 6.83 (δ_C 109.6) integrating for two protons which was coupled to a triplet δ_H 7.09 integrating for a single proton. These resonances were assigned to H-2′, H-6′ and H-7′, respectively. The NMR assignment was confirmed by COSY, TOCSY, HMQC and HMBC experiments. The aromatic protons H-7′ (δ_H 7.09) showed strong HMBC correlations to C-3′ (C-6′) and weak correlation to C-4′. H-2′ (H-6′) showed strong HMBC correlations to C-4′ and weak correlation to C-3′ (C-5′). H-2′ (H-6′) showed HMBC correlation to δ_C 109.6, the carbon to which they are attached to, thus confirming the symmetry. Based on these data structure **5** was assigned to platensimycin B_4 which is 2′-decarboxy platensimycin. The assignment of the absolute configuration of **3–5** is based on the absolute configuration of platensimycin (**1**) which was determined by a single X-crystallographic studies[2] of the 6′-bromo derivative and confirmed by asymmetric synthesis.[13]

Platensimycins B_1–B_3 (**3–5**) were first evaluated for their ability to inhibit *Staphylococcus aureus* fatty acid synthesis in a cell-free FASII assay.[14] Compounds **3** and **4** showed inhibition in this assay with an IC_{50} value of 100 μg ml^{-1} indicating a 200-fold lower potency than platensimycin (IC_{50} 0.5 μg ml^{-1}). Both these compounds inhibited saFabH assay with IC_{50} values of 78 and 100 μg ml^{-1}, respectively.[1] This activity was only ~2–3 fold lower than platensimycin. Platensimycin B_3 (**5**) was inactive in both FASII and saFabH assays at 1000 and 167 μg ml^{-1}, respectively. None of the three compounds inhibited growth of *S. aureus* Smith or other bacterial strains at 64 μg ml^{-1} or less. In the most sensitive *S. aureus* antisense two-plate differential sensitivity cellular assay,[5] **3** and **4** showed MDC (MDC is defined by minimum concentration of the compound showing differential zone of clearance between antisense plates compared to control plate) of 100 μg ml^{-1}, exhibiting a 2500 fold lower activity than platensimycin. The poor activities of these compounds could be attributed to the disruption of the salt bridge interaction of the carboxylic acid with the active site histidines present in platensimycin as confirmed by the enzyme-bound X-ray crystal structure (Fig. 1). Clearly without negative charge, the ionic interactions between these inhibitors and the enzyme would be absent which would account for the loss of binding and activity. This observation confirms the critical role played by the carboxylic acid for the potent FabF inhibitory activity of platensimycin and platencin.

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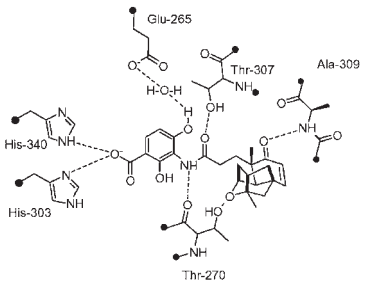

Fig. 1 Graphical representation of platensimycin binding with ecFabF and its impact of activity.

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