NOTE

Isolation and structural elucidation of glucoside congeners of platencin from *Streptomyces platensis* SB12600

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Platensimycin (PTM, **1**)^{1,2} and platencin (PTN, **2**)^{3,4} are recently discovered natural products, which are potent and selective inhibitors of bacterial and mammalian fatty acid synthases (Figure 1a). They have emerged as promising drug leads for both antibacterial and antidiabetic therapies.^{1–5} Although the efficacy of PTM and PTN has been demonstrated in mouse models, they suffer from poor pharmacokinetic properties, mainly a high rate of clearance, thereby requiring continuous infusion to ensure adequate exposure, a delivery method that is not desirable to most patients.^{6,7} Consequently, significant effort has been undertaken to prepare PTM and PTN analogues by organic synthesis,^{8,9} and to isolate PTM and PTN congeners by fermentation optimization.^{10–21} Although these analogues have helped define the structure–activity relationship of PTM and PTN, none of them however can be argued to have significantly improved therapeutic properties.

PTM and PTN were originally isolated from *Streptomyces platensis* MA7327 and MA7339, respectively.^{1–4} We subsequently cloned and sequenced the gene clusters encoding PTM and PTN biosynthesis from both *S. platensis* MA7327 and MA7339, unveiling MA7327 as a PTM and PTN dual producer.^{18,20} Inactivation of *ptmR1* and *ptnR1*, which encode a GntR-like transcriptional regulator within the PTM and PTN clusters,^{18,20} in *S. platensis* MA7327 and MA7339 afforded mutant recombinant strains that dramatically overproduce PTM¹⁸ and PTN.¹⁹ Although PTN was produced in very low titre in the wild-type *S. platensis* MA7339 strain,^{3,4} 17 congeners were isolated upon medium optimization and large-scale fermentation.^{14,16,17} From the *ΔptnR1* mutant strain of *S. platensis* SB12600 that significantly overproduces PTN, nine additional congeners were isolated, four of which were also isolated from the wild-type strains, albeit at significantly lower yield.¹⁹ Finally, expression of the *ptn* cluster from *S. platensis* MA7339 in the heterologous host *Streptomyces lividans* K4-114 afforded the production and isolation of six

additional PTN congeners.²¹ The 29 PTN congeners isolated to date (summarized in Supplementary Figure S1) could be divided structurally into three subclasses—(i) biosynthetic intermediates, (ii) shunt metabolites *en route* to PTN and (iii) hydroxylated or glucosylated congeners, most likely resulted from adventitious P-450 enzymes or glucosyltransferases, respectively.

Inspired by our early findings that the PTN-overproducing *ΔptnR1* mutant strain SB12600 provided an excellent opportunity to isolate PTN congeners that would be produced in titres too low to be detected in the wild-type strain,¹⁹ we have now subjected *S. platensis* SB12600 to large-scale fermentation to isolate the minor congeners. Crude extract made from approximately 15 l of SB12600 fermentation was subjected to multiple steps of SiO₂, C-18 and Sephadex LH-20 (Sigma-Aldrich, St Louis, MO, USA) chromatography, affording four new PTN congeners, **3** (11.8 mg), **4** (10.2 mg), **5** (3.6 mg) and **6** (4.1 mg; Supplementary Information). Structural elucidation of **3–6** as glucoside congeners of PTN (Figure 1b) was based mainly on HR-ESI-MS and ¹H and ¹³C NMR spectroscopic analyses, as well as comparison with previously reported glucoside congeners of PTM (**7** and **8**)¹⁵ and of PTN (**9** and **10**)¹⁹ (Figure 1c).

HR-ESI-MS analysis of **3** yielded [M + H]⁺ and [M + Na]⁺ ions at *m/z* 588.2439 and *m/z* 610.2259, respectively, predicting a molecular formula of C₃₀H₃₇NO₁₁ (calculated [M + H]⁺ and [M + Na]⁺ ions at *m/z* 588.2445 and *m/z* 610.2265, respectively). The ¹³C NMR data of **3** (Table 1) was very similar to those of **2**,^{4,19} with the exception of an extra six resonances, which indicated a hexose moiety. The structure of the PTN aglycone moiety was further confirmed by key COSY and HMBC correlations (Figure 1d). The hexose moiety was established by a 1D-TOCSY experiment, verified to be a glucoside by gHSQC, gHMBC and TOCSY correlations, and further confirmed to be a glucoside by comparison with previously reported NMR data for the PTM glucosides (**7** and **8**)¹⁵ and the PTN glucosides

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Figure 1 Structures of platensimycin (1), platencin (2) and their glucosides (3–10) isolated from wild-type and engineered *S. platensis* strains. (a) Platensimycin (1) from wild-type *S. platensis* MA7327²² and platencin (2) from wild-type *S. platensis* MA7339.⁴ (b) Platencin A12 (3), A13 (4), A14 (5) and A15 (6) from engineered *S. platensis* SB12600 reported in the current study. (c) Platensimycin B4 (7) and its methyl ester (8) from wild-type *S. platensis* MA7327,¹⁵ and platencin A9 (9) and A10 (10) from engineered *S. platensis* SB12600.¹⁹ (d) Key COSY, NOESY and HMBC correlations supporting the structures of 3–6 as exemplified by 3.

(9 and 10).¹⁹ The glucosidic linkage was established at position C-5' by the gHMBC correlation of the anomeric proton H-1'' (δ_H 5.71) to C-5' (δ_C 158.7). This was further supported by the NOESY correlations between H-6' (δ_H 7.19) and H-1'' (δ_H 5.71), which also showed correlations with H-3'' (δ_H 4.36) and H-5'' (δ_H 4.15), confirming the β -D-glucoside configuration (Figure 1d). On the basis of these data, 3 was assigned as platencin A12, a platencin-5'- β -D-glucoside (Figure 1b).

The molecular formula of 4 was deduced as $C_{30}H_{39}NO_{11}$ based on the HR-ESI-MS spectrum that gave $[M + H]^+$ and $[M + Na]^+$ ions at m/z 590.2596 and m/z 612.2416 (calculated $[M + H]^+$ and $[M + Na]^+$ ions at m/z 590.2602 and m/z 612.2421), respectively. Comparison of the 1H and ^{13}C data (Table 1) of 3 and 4 indicated a close identity between the two compounds, with the double bond of the enone moiety reduced in 4. The latter was supported by the 2 mass unit difference between 3 and 4, and further confirmed by the disappearance of olefinic moiety in 3 (H-6/H-7, δ_H 5.90/ δ_H 6.33 and C-6/C-7, δ_C 126.7/ δ_C 154.9), with the concomitant appearance of a $-CH_2-CH_2-$ moiety (H₂-6/H₂-7, δ_H 1.91, 1.04/ δ_H 1.40, 1.34 and C-6/C-7, δ_C 28.4/ δ_C 35.4) in 4, as well as the gHMBC correlations of H₂-6 (δ_H 1.91, 1.04) to C-8 (δ_C 32.2) and H₂-7 (δ_H 1.40, 1.34) to C-5 (δ_C 215.7) in 4. 1H and ^{13}C NMR data also confirmed the identity of the sugar moiety to that of 3 (Table 1), and thus 4 was assigned as platencin A13, a 6,7-dihydroplatencin-5'- β -D-glucoside (Figure 1b).

The molecular formula $C_{30}H_{37}NO_{12}$ for 5 was established on the basis of HR-ESI-MS analysis that afforded $[M + H]^+$ and $[M + Na]^+$ ions at m/z 604.2392 and m/z 626.2209 (calculated $[M + H]^+$ and $[M + Na]^+$ ions at m/z 604.2395 and m/z 626.2214), respectively. Careful comparison of the 1H and ^{13}C NMR data with previously reported PTN analogues^{14,16,17,19} revealed that the aglycone moiety is identical to that of platencin A1,^{14,19} which is also supported by the 16 mass unit difference from 3. Hydroxylation at C-13 was further confirmed by the gCOSY correlations of H-13 (δ_H 4.62) to H₂-12 (δ_H 1.36, 1.24), and NOESY correlation between H-13 (δ_H 4.62) and H₃-17 (δ_H 1.05) verified the configuration of the hydroxyl group at C-13. 1H and ^{13}C NMR data also confirmed the identity of the sugar moiety to that of 3 and 4 (Table 1), and hence, 5 was similarly assigned as platencin A14, a platencin A1-5'- β -D-glucoside.

HR-ESI-MS analysis of 6 provided $[M + H]^+$ and $[M + Na]^+$ ions at m/z 604.2393 and m/z 626.2211, respectively, indicating a molecular formula of $C_{30}H_{37}NO_{12}$ (calculated $[M + H]^+$ and $[M + Na]^+$ ions at m/z 604.2395 and m/z 626.2214, respectively), which had the same molecular formula as 5. Careful analysis of the 1H and ^{13}C NMR data (Table 1) revealed that 6 was a regioisomer of 5. Hydroxylation at C-14 was confirmed by the gHMBC correlations of H₂-16 (δ_H 5.27, 5.09) to C-14 (δ_C 76.2), and NOESY correlation between H-14 (δ_H 3.99) and H-9 (δ_H 1.93) established the configuration of the hydroxyl group at C-14, affording the PTN aglycone that is identical to platencin A3.^{17,18} Finally, 1H and ^{13}C NMR data of 6 similarly indicated the same hexose moiety as 3–5, and this allowed the final assignment of 6 as platencin A15, a platencin A3-5'- β -D-glucoside (Figure 1b).

PTM and PTN congeners isolated to date have helped define the structure–activity relationship of the PTM and PTN scaffolds as promising drug leads for both antibacterial and antidiabetic therapies.^{6,7,10–19} The current work demonstrated once again the power and utility of engineering overproducers by manipulating pathway regulation to isolate natural product congeners that would be produced in titres too low to be detected in the wild-type strains.²² Glucosides of both PTM (7 and 8)¹⁵ and PTN (9 and 10)¹⁹ have been isolated previously (Figure 1c). Glucosylation of the C-5' hydroxyl group of PTM and PTN completely abolished their FabF/H inhibitory activity *in vitro* and antibacterial activity *in vivo*.^{15,19} This is consistent with the importance of the C-5' hydroxyl group in mediating hydrogen bonding to the FabF/H active sites as observed in the PTM- and PTN-bound X-ray structures.^{1,3,10–19} The newly isolated four PTN glucosides (3–6) also showed no antibacterial activity as 7–10 reported previously,^{15,19} further supporting the emerging structure–activity relationship that the C-5' hydroxyl group is critical

Table 1 Summary of ^1H (700 MHz) and ^{13}C (175 MHz) NMR Data for platencin A12–A15 (3–6) in d_6 -pyridine^a

Position	Platencin A12 (3)		Platencin A13 (4)		Platencin A14 (5)		Platencin A15 (6)	
	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)	δ_{C}	δ_{H} (mult, J in Hz)
1	173.9, C		173.9, C		173.8, C		173.9, C	
2	32.7, CH ₂	2.76, t (11.0)	32.7, CH ₂	2.75, t (11.1)	32.8, CH ₂	2.77, t (11.6)	32.8, CH ₂	2.78, m
		2.57, m		2.63, m		2.59, m		2.58, m
3	32.2, CH ₂	2.60, m	33.3, CH ₂	2.36, m	32.3, CH ₂	2.62, m	32.3, CH ₂	2.65, m
		2.06, t (10.8)		2.00, m		2.04, m		2.11, m
4	48.1, C		50.7, C		47.9, C		48.1, C	
5	204.3, C		215.7, C		204.0, C		204.2, C	
6	126.7, CH	5.90, d (9.8)	28.4, CH ₂	1.91, t (12.2)	127.2, CH	6.06, d (10.0)	127.1, CH	6.04, d (10.2)
				1.04, m				
7	154.9, CH	6.33, d (9.9)	35.4, CH ₂	1.40, m	154.8, CH	7.10, d (10.0)	153.8, CH	7.18, d (10.2)
				1.34, m				
8	36.5, C		32.2, C		42.1, C		38.3, C	
9	40.1, CH	2.02, t (9.7)	43.2, CH	1.75, t (9.3)	40.9, CH	2.19, t (10.5)	38.3, CH	1.93, t (9.6)
10	28.3, CH ₂	1.64, m	28.4, CH ₂	1.57, m	28.3, CH ₂	1.65, m	28.1, CH ₂	1.62, m
		1.33, m		1.32, m		1.36, m		1.40, m
11	36.5, CH	2.19, m	36.5, CH	2.15, m	37.7, CH	2.32, br s	36.5, CH	2.34, m
12	27.0, CH ₂	1.75, m	27.0, CH ₂	1.58, m	28.3, CH ₂	1.36, m	26.5, CH ₂	1.82, m
		1.28, m		1.51, m		1.24, m		1.53, m
13	26.4, CH ₂	1.52, m	25.4, CH ₂	1.33, m	67.3, CH	4.62, d (8.5)	20.6, CH ₂	2.23, t (11.5)
		1.49, m						1.69, m
14	44.8, CH ₂	2.16, d (16.1)	46.5, CH ₂	2.16, d (15.9)	46.2, CH ₂	1.64, m	76.2, CH	3.99, m
		1.95, d (16.2)		1.98, d (16.0)		1.36, m		
15	149.7, C		149.7, C		149.7, C		149.9, C	
16	107.7, CH ₂	4.83, br s	106.6, CH ₂	4.83, br s	107.1, CH ₂	4.85, br s	111.4, CH ₂	5.27, br s
		4.70, br s		4.70, br s		4.74, br s		5.09, br s
		1.03, s		1.05, s		1.05, s		1.13, s
17	21.5, CH ₃		20.5, CH ₃		22.7, CH ₃		22.1, CH ₃	
1'	174.9, C		174.9, C		175.2, C		175.1, C	
2'	111.3, C		111.8, C		114.8, C		115.4, C	
3'	159.9, C		159.0, C		160.6, C		160.4, C	
4'	115.8, C		115.6, C		115.4, C		115.5, C	
5'	158.7, C		158.3, C		157.5, C		157.8, C	
6'	106.2, CH	7.19, d (8.9)	105.8, CH	7.17, d (8.9)	104.9, CH	7.10, d (8.9)	105.1, CH	7.12, d (8.7)
7'	130.3, CH	8.18, d (8.9)	130.1, CH	8.17, d (8.9)	130.2, CH	8.33, d (8.9)	130.2, CH	8.28, d (8.8)
1''	103.7, CH	5.71, d (7.4)	103.5, CH	5.69, d (7.6)	103.7, CH	5.65, d (7.5)	103.7, CH	5.68, d (7.6)
2''	75.4, CH	4.29, t (7.7)	75.2, CH	4.28, t (7.7)	75.4, CH	4.26, t (7.5)	75.4, CH	4.28, t (7.7)
3''	78.2, CH	4.36, m	78.0, CH	4.35, m	78.2, CH	4.32, m	78.2, CH	4.36, m
4''	71.2, CH	4.34, m	71.1, CH	4.34, m	71.3, CH	4.33, m	71.3, CH	4.34, m
5''	79.4, CH	4.15, m	79.2, CH	4.13, m	79.2, CH	4.09, m	79.3, CH	4.11, m
6''	62.7, CH ₂	4.56, d (11.8)	62.5, CH ₂	4.54, dd (12.0, 4.1)	62.7, CH ₂	4.52, d (11.4)	62.7, CH ₂	4.52, d (11.7)
		4.42, dd (11.7, 4.5)		4.41, dd (12.0, 4.1)		4.40, dd (11.6, 4.2)		4.41, dd (11.7, 4.3)
NH		10.82, s		10.69, s		10.68, s		10.70, s

^a ^1H and ^{13}C assignments were based on COSY, HMBC, HSQC and TOCSY spectra, as well as in comparison with the assignments for other platensimycin and platencin glucosides reported previously.

and the C-5' position cannot tolerate large group for biological activity.

Antibiotic-producing *Streptomyces* must have co-evolved, with their antibiotic biosynthetic machinery, strategies for self-protection from their own biologically active metabolites. Bioinformatics analysis of the genes within the *ptm* and *ptn* biosynthetic gene clusters from *S. platensis* MA7327 and MA7339 have previously unveiled several candidates, potentially conferring PTM and PTN self-protection via FabF/H alteration and PTM and PTN efflux.²⁰ Although no gene encoding glucosyltransferase is identified within the cloned *ptm* and *ptn* clusters,²⁰ it is tempting to speculate that glucosylation may

represent an additional mechanism of self-protection when *S. platensis* overproduces PTM or PTN.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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