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Isolation, enzyme-bound structure and antibacterial activity of platencin A_1 from *Streptomyces platensis*

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ABSTRACT

Natural products continue to serve as one of the best sources for discovery of antibacterial agents as exemplified by the recent discoveries of platensimycin and platencin. Chemical modifications as well as discovery of congeners are the main sources for gaining knowledge of structure–activity relationship of natural products. Screening for congeners in the extracts of the fermentation broths of *Streptomyces platensis* led to the isolation of platencin A_1, a hydroxy congener of platencin. The hydroxylation of the tricyclic enone moiety negatively affected the antibacterial activity and appears to be consistent with the hydrophobic binding pocket of the FabF. Isolation, structure, enzyme-bound structure and activity of platencin A_1 and two other congeners have been described.

Emergence of drug resistance bacteria is becoming serious cause for concern leading to real need for discovery and development of new antibiotics with new mechanism of action. Recent discovery of platensimycin (**1**) and platencin (**2**) from various strains of *Streptomyces platensis* is certainly very timely and exciting.[1–4] They were discovered by a novel antisense differential sensitivity screening strategy in which FabH/FabF was sensitized.[3–6] Platensimycin is a selective inhibitor of the FabF acyl-enzyme intermediate of the elongation condensing enzyme FabF whereas platencin is a balanced inhibitor of both the initiation condensing enzyme (FabH) and elongation enzymes (FabF). Both compounds demonstrated potent in vitro activity against both cell-free and whole-cell systems. Though there remains discordance between the in vitro and in vivo activities particularly when administered by conventional routes. However, when administered by continuous infusion the drugs were highly efficacious against *Staphylococcus aureus*. The paucity of in vivo activity under conventional administration is due to poor pharmacokinetic properties. In order to achieve higher levels of systemic drug exposure which is required for antibacterial agents PK improvement is a must for these compounds. To answer this challenge two approaches—chemical modification[7,8] and discovery of congeners—were undertaken.[9–11] Platensimycin[12–18] and platencin[19–22] have been subjected to a number of total and formal total syntheses. The congener discovery approach led to the isolation of three new congeners platencin A_1 (**3a**), methyl ester (**3b**) and hydroxy tricyclic enone methyl ester (**4**). Isolation, structure elucidation, ecFabF-bound structure, and biological activity of these compounds is described.

S. platensis MA7327 was grown in stirred tanks on CLA production medium as described earlier[2] and was acidified with 2 N HCl to pH 3.0. It was extracted with methanol and chromatographed on an Amberchrome column eluting with a step gradient of 40–100% aqueous CH_3OH gradient. Amberchrome column fractions eluting with approximately 80–90% CH_3OH were extracted with CH_2Cl_2 at pH 9. The aqueous layer was acidified with 6 N HCl to pH 2 and extracted with CH_2Cl_2 and chromatographed on a silica gel column eluting with a 80:20:0.5:0.5:0.5 mixture of hexane–EtOAc–water–AcOH–MeOH. The fractions eluting in the middle of the column run were further chromatographed by RP HPLC (Zorbax C_8 column eluting with an aqueous CH_3CN + 0.1% TFA) to yield platencin A_1 (**3a**) (3.3 mg, 0.077 mg/L) as a beige powder. $[\alpha]_D^{23}$ −43.0 (*c* 0.5, CH_3OH), UV (CH_3OH) λ_{max} 227 (ε, 15,000), 240 (sh), 295 (sh) nm, IR (ZnSe) ν_{max} 3266, 2929, 1648, 1535, 1390, 1202, 1151, 1056, 1023, 790 cm^{-1}. Amberchrome fractions eluting with MeOH was extracted with CH_2Cl_2 and the organic extract was chromatographed on a silica gel column eluting with a step gradient of 10–100% of hexane–EtOAc. The fractions eluting with 50%

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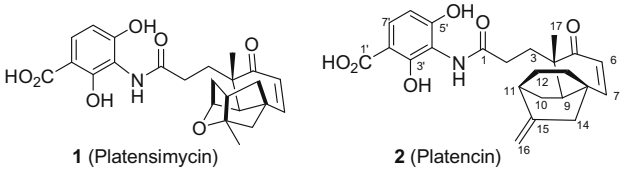
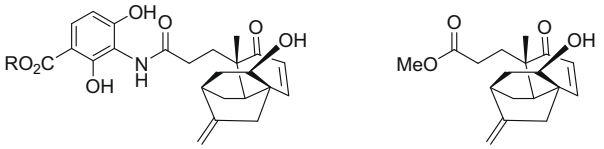
EtOAc were further chromatographed by RP HPLC (Zorbax RX C_8 column eluting with an aqueous CH_3CN + 0.1% TFA gradient) to afford methyl ester **3b** (0.8 mg, 0.018 mg/L), as a colorless powder. $[\alpha]_D^{23}$ −109.2 (*c* 0.5, CH_3OH), UV (CH_3OH) λ_{max} 236 nm, IR (ZnSe) ν_{max} 3444 (br), 2952, 1734, 1670, 1439, 1380, 1354, 1303, 1230, 1194, 1173, 1092 cm^{-1}. Amberchrome fractions eluting with 80–90% MeOH was basified to pH 9 and extracted with CH_2Cl_2 which was chromatographed on Sephadex LH20 followed by RP HPLC to afford methyl ester **4** (2.2 mg, 0.05 mg/L) as a gum. $[\alpha]_D^{23}$ −96.0 (*c* 0.5, CH_3OH), UV (CH_3OH) λ_{max} 232 (ε 8974) nm, IR (ZnSe) ν_{max} 3455, 2937, 1735, 1667, 1537, 1438, 1381, 1294, 1270, 1201, 1176, 1154, 1085, 1033, 993, 885, 852 cm^{-1}.



1 (Platensimycin) **2** (Platencin)



3a: R = H (Platencin A_1)
3b: R = Me

4

Analysis of platencin A_1 (**3a**) by HRESIFTMS produced a parent ion at *m/z* 442.1869 and afforded a formula of $C_{24}H_{27}NO_7$ (calcd for M+H, 442.1866). Similar analysis of the methyl ester **3b** gave molecular formula $C_{25}H_{29}NO_7$ (found: 456.2018, calcd for M+H, 456.2022). These formulae differ by a methyl group and were fully supported by the ^{13}C NMR spectral analysis (Table 1). The UV spectrum was identical to the platensimycin and platencin. The examination of the molecular formula suggested that **3a** was isomeric to platensimycin and was up by an oxygen atom from platencin. However ^1H and ^{13}C NMR analysis indicated the presence of an exocyclic methylene group and only one of the two angular methyls, a characteristic of platencin. The absence of the resonances for C-10 oxygenated methine characteristically present in platensimycin indicated that **3a** and **3b** belonged to the platencin class. Comparison of the NMR spectra of **3a**, **3b** and platencin indicated that the absence of one of the methylene carbons and the presence of a relatively upfield shifted oxygenated methine (δ_C 65.8, δ_H 4.2 for **3a** and δ_C 68.4, δ_H 4.50 for **3b**). The methine proton in each compound showed COSY correlations to the methylene protons at C-12 which showed correlations to H-11. H-13 also showed W-couplings with the downfield shifted methylene proton of C-14 in all cases. Finally H-9 and H_2-14 showed strong HMBC correlations to the methine carbon at C-13 thus establishing the structure of **3a** as platencin A_1 (13-hydroxy platencin). The substitution at C-13 was further corroborated by ~5 and 10 ppm downfield shift of the adjacent carbons C-8 and C-12, respectively, and ~7 ppm upfield shift of C-14.

Compound **4** exhibited a molecular ion at *m/z* 305.1751 in HRESIFTMS and afforded a molecular formula of $C_{18}H_{24}O_4$ (calcd for M+H; 305.1752) which was corroborated by the ^{13}C NMR spectrum (Table 1). It was quite clear from the molecular formula, UV and ^{13}C

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) assignment of platencin A_1 (**3a**), methyl ester (**3b**) and **4**

	3a		**3a**	**3a**	**3b**	**3b**	**4**	**4**
	DMSO-d_6		DMSO-d_6	C_5D_5N	CD_3OD	CD_3OD	C_5D_5N	C_5D_5N
no	δ_C	Type	δ_H	δ_H	δ_C	δ_H	δ_C	δ_H
1	171.7	C°			176.1		174.3	
2	30.2	CH_2	2.05, m	2.8, m	32.1	2.36, m	30.2	2.38, m
			2.15, m					
3	30.2	CH_2	1.45, m	1.90, m	31.8	1.73, m	31.1	2.37, m
			1.90, m	2.60, m		2.19, m		1.71, m
4	46.5	C°			48.1		47.6	
5	202.9	C°			206.4		203.7	
6	126.1	CH	5.8, d, 10	6.1, d, 10	127.6	5.97, d, 10	127.3	6.11, d, 10.2
7	154.2	CH	6.8, d, 10	7.1, d, 10	155.6	6.94, d, 10	154.8	7.14, d, 10.2
8	40.0	C°			42.5		42.3	
9	40.7	CH	2.0, t, 10	2.2, t, 10	41.3	2.20, t, 10.5	40.7	2.20, t, 10.1
10	27.0	CH_2	1.44, m	1.4, m	28.4	1.77, m	28.5	1.61, dddd, 12.5, 10.0, 5.0, 2.6
			1.60, m	1.6, m		1.60, m		1.42, m
11	36.4	CH	2.40, m	2.40, m	38.3	2.48, m	37.9	2.44, m
12	37.8	CH_2	1.35, 2.15	2.27, m	38.8	1.58, m	39.2	2.29, ddd, 13.3, 9.6, 3.6
				1.8, m		2.30, m		1.82, m
13	65.8	CH	4.2, br m	4.66, br d, 10	68.4	4.45, dd, 10, 4.5, 2	67.3	4.67, ddd, 9.6, 4.3, 2.0
14	36.2	CH_2	1.70, d, 16	1.80, d, 15.5	37.4	1.80, br d, 16.5	37.4	3.33, br d, 16.3, 2.0
			2.90, br d, 16	3.32, br d, 15.5		3.05, br d, 16.5		1.84, m
15	149.1	C°			150.2		150.3	
16	106.3	CH_2	4.6, br s	4.75, br s	107.3	4.6, br s	107.2	4.93, dd, 2.1, 4.1
			4.8, br s	4.90, br s		4.8, br s		4.77, dd, 1.9, 3.8
17	22	CH_3	1.05, s	1.12, s	22.9	1.20, s	22.7	1.10, s
1′	172.2	C°			171.8			
2′	112.8	C°			115.2			
3′	158.9	C°			159.4			
4′	104.3	C°			105.2			
5′	159.2	C°			159			
6′	107.8	CH	6.40, d, 9	6.90, d, 9	109.8	6.4, d, 9		
7′	128.9	CH	7.54, d, 9	8.10, d, 9	130	7.6, d, 9		
NH			8.91	10.55				
3′-OH			10.1					
5′-OH			11.7					
OMe					52.6	3.9, s	51.8	3.61, s

NMR spectrum that it did not contain the 3-amino-2,4-dihydroxy benzoic acid unit. The ^{13}C NMR spectrum showed the presence of an exocyclic methylene and only one angular methyl suggesting that it represented the terpenoid portion of the platencin class.[23,24] From the analysis of the ^{1}H and ^{13}C NMR spectral data and comparison with the corresponding data of platencin (**2**) and platencin A_1 (**3a**) it became clear that **4** was the methyl ester of 13-hydroxy platencinic acid. The structure was confirmed by COSY, HMQC and HMBC correlations. Like platencin A_1, H-13 showed COSY correlations to H-12 and W-coupling with downfield shifted proton (δ_H 3.33) of C-14. The W-coupling would be consistent if H-13 and H-14 are in α orientation in the current drawing which was confirmed by NOE difference experiment. Irradiation of H-13 produced enhancements of H-12 (δ_H 2.29) and the H_3-17 methyl protons (δ_H 1.10) thus establishing the orientation of H-13 towards the methyl group and placing the hydroxy group on the β face, confirming *R* configuration at C-13 and structure of **4** as 13*R*-hydroxy platencinic acid. Both methyl esters are most likely isolation artifacts and carboxylic acids are true natural products.

Compounds **3a**, **3b** and **4** were evaluated for their ability to inhibit *S. aureus* fatty acid synthesis in cell free system in FASII assay.[25] Platencin A_1 (**3a**) showed IC_{50} of 7.12 μg/mL (16.1 μM). The other two compounds were inactive at 167 μg/mL. In this assay platensimycin and platencin exhibited IC_{50} values of 0.5 and 12 μg/mL, respectively. Platencin A_1 inhibited saFabH assay with IC_{50} value of 15 μg/mL (34 μM). Both FabH and FASII activity of platencin A_1 is in the same range of platencin. However the cellular activity of platencin A_1 was significantly reduced even in the most sensitive antisense two-plate differential sensitivity assay[5] where it showed MDC of 20 μg/mL compared to platencin (MDC of 0.2 μg/mL). MDC is defined by minimum concentration of the compound showing differential zone of clearance between antisense plates compared to control plate. This lower cellular activity was also translated in the MIC assay. Platencin A_1 showed MIC values of 32 μg/mL (*S. aureus* Smith), 8 μg/mL (*Enterococcus faecalis*, CL8516) and >64 μg/mL (*Streptococcus pneumoniae*, CL2883). These values are 4–64-fold higher (less active) than platencin.[4] The other two compounds did not show any activity at 64 μg/mL against any strains. In the ecFabF (C163A) direct binding assay,[1] **3a** showed a binding IC_{50} of 789 nM which is approximately sevenfold lower affinity than platencin (**2**, IC_{50} 113 nM), and 40-fold lower affinity than platensimycin (**1**, IC_{50} 19 nM).

X-ray crystallographic studies of *Escherichia coli* FabF (C163A) in complex with **1**, **2** and **3a** were performed using previously described methodologies[1] to final resolutions and R/R free values of 2.75 Å (18.5/27.0) (**1**) PDB code (3HNZ); 2.0 Å (16.3/20.8) (**2**) PDB code (3HO2); and 1.9 Å (15.6/19.0) (**3a**) PDB code (3HO9). The structures show that compounds containing the platencin terpenoid ring are missing the ether oxygen present in the platensimycin structure to make a direct hydrogen bond to Thr270 in the protein (Fig. 1). The lack of this hydrogen bond may account for the sixfold weaker binding of **2** compared to platensimycin. Substitution of ether bridge with a CH_2 in platensimycin led to carboplatensimycin with a similar 6–8-fold loss of whole cell activity (no enzyme activity was reported).[26]

Interestingly, the structures of **2** and **3a** are extremely similar, both in protein conformation and relative positioning of the inhibitor. The hydroxyl group of **3a** is seen to project into solvent, with the nearest contacting residue being Arg206 which is positioned 3.2 Å away making an extremely weak hydrogen bond. Because of the similarity of the structures, they do not explain the sevenfold weaker binding of **3a** compared to **2**. It is possible that unappreciated effects on charge distribution, solvation/desolvation energies, and other kinetic and/or thermodynamic parameters may contribute to the weaker binding affinity of the hydroxylated platencin variant. The additional polarity in the hydrophobic terpenoid unit[9,24] of the molecule has a detrimental effect on the biological activity. Future studies could be designed to address these questions more fully.

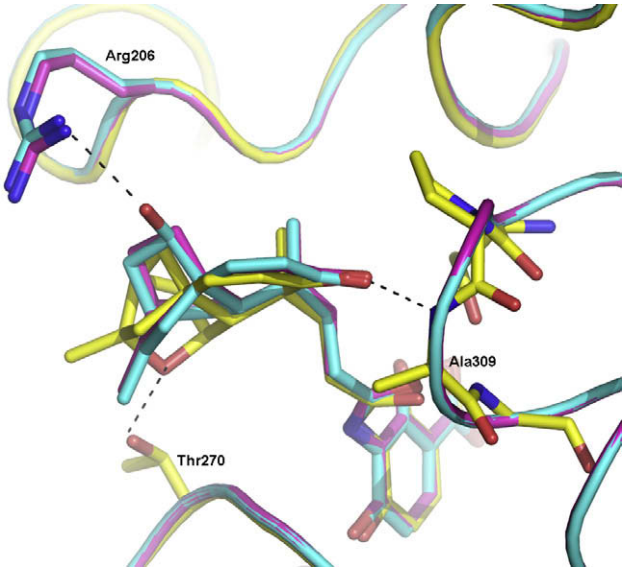


Figure 1. Superposition of the structures of platensimycin (yellow), platencin (magenta) and platencin A_1. Residues discussed in the text are labelled.

In summary, we have described the isolation, structure, activities and inhibitor-bound structure of ecFabF of platencin and platencin A_1. The latter compound is a new congener of platensimycin/platencin. The methyl esters of platencin A_1 and hydroxy tricyclic enone acid are also reported.

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References and notes

1. Wang, J.; Soisson, S. M.; Young, K.; Shoop, W.; Kodali, S.; Galgoci, A.; Painter, R.; Parthasarathy, G.; Tang, Y.; Cummings, R.; Ha, S.; Dorso, K.; Motyl, M.; Jayasuriya, H.; Ondeyka, J.; Herath, K.; Zhang, C.; Hernandez, L.; Alloco, J.; Basilio, Á.; Tormo, J. R.; Genilloud, O.; Vicente, F.; Pelaez, F.; Colwell, L.; Lee, S. H.; Michael, B.; Felcetto, T.; Gill, C.; Silver, L. L.; Hermes, J.; Bartizal, K.; Barrett, J.; Schmatz, D.; Becker, J. W.; Cully, D.; Singh, S. B. *Nature* **2006**, *441*, 358.
2. Singh, S. B.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K. B.; Zhang, C.; Zink, D. L.; Tsou, N. N.; Ball, R. G.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Pelaez, F.; Young, K.; Wang, J. *J. Am. Chem. Soc.* **2006**, *128*, 11916. and 15547.
3. Jayasuriya, H.; Herath, K. B.; Zhang, C.; Zink, D. L.; Basilio, A.; Genilloud, O.; Diez, M. T.; Vicente, F.; Gonzalez, I.; Salazar, O.; Pelaez, F.; Cummings, R.; Ha, S.; Wang, J.; Singh, S. B. *Angew. Chem., Int. Ed.* **2007**, *46*, 4684.
4. Wang, J.; Kodali, S.; Lee, S. H.; Galgoci, A.; Painter, R.; Dorso, K.; Racine, F.; Motyl, M.; Hernandez, L.; Tinney, E.; Colletti, S.; Herath, K.; Cummings, R.; Salazar, O.; Gonzalez, I.; Basilio, A.; Vicente, F.; Genilloud, O.; Pelaez, F.; Jayasuriya, H.; Young, K.; Cully, D.; Singh, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7612.
5. Young, K.; Jayasuriya, H.; Ondeyka, J. G.; Herath, K.; Zhang, C.; Kodali, S.; Galgoci, A.; Painter, R.; Brown-Driver, V.; Yamamoto, R.; Silver, L. L.; Zheng, Y.; Ventura, J. I.; Sigmund, J.; Ha, S.; Basilio, A.; Vicente, F.; Tormo, J. R.; Pelaez, F.; Youngman, P.; Cully, D.; Barrett, J. F.; Schmatz, D.; Singh, S. B.; Wang, J. *Antimicrob. Agents Chemother.* **2006**, *50*, 519.
6. Singh, S. B.; Phillips, J. W.; Wang, J. *Curr. Opin. Drug Disc. Dev.* **2007**, *10*, 160.
7. Singh, S. B.; Herath, K. B.; Wang, J.; Tsou, N. N.; Ball, R. G. *Tetrahedron Lett.* **2007**, *48*, 5429.
8. Shen, H. C.; Ding, F. X.; Singh, S. B.; Parthasarathy, G.; Soisson, S. M.; Ha, S. N.; Chen, X.; Kodali, S.; Wang, J.; Dorso, K.; Tata, J. R.; Hammond, M. L.; Maccoss, M.; Colletti, S. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1623.
9. Herath, K. B.; Zhang, C.; Jayasuriya, H.; Ondeyka, J. G.; Zink, D. L.; Burgess, B.; Wang, J.; Singh, S. B. *Org. Lett.* **2008**, *10*, 1699.

10. Jayasuriya, H.; Herath, K. B.; Ondeyka, J. G.; Zink, D. L.; Burgess, B.; Wang, J.; Singh, S. B. *Tetrahedron Lett.* **2008**, *49*, 3648.
11. Zhang, C.; Ondeyka, J.; Zink, D. L.; Burgess, B.; Wang, J.; Singh, S. B. *Chem. Commun. (Camb)* **2008**, 5034.
12. Nicolaou, K. C.; Li, A.; Edmonds, D. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 7086.
13. Nicolaou, K. C.; Edmonds, D. J.; Li, A.; Tria, G. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 3942.
14. Kaliappan, K. P.; Ravikumar, V. *Org. Lett.* **2007**, *9*, 2417.
15. Lalic, G.; Corey, E. J. *Org. Lett.* **2007**, *9*, 4921.
16. Nicolaou, K. C.; Pappo, D.; Tsang, K. Y.; Gibe, R.; Chen, D. Y. *Angew. Chem., Int. Ed.* **2008**, *47*, 944.
17. Tiefenbacher, K.; Mulzer, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 2548.
18. Ghosh, A. K.; Xi, K. *J. Org. Chem.* **2009**, *74*, 1163.
19. Nicolaou, K. C.; Tria, G. S.; Edmonds, D. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 1780.
20. Hayashida, J.; Rawal, V. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 4373.
21. Tiefenbacher, K.; Mulzer, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 6199.
22. Waalboer, D. C.; Schaapman, M. C.; van Delft, F. L.; Rutjes, F. P. *Angew. Chem., Int. Ed.* **2008**, *47*, 6576.
23. Herath, K. B.; Attygalle, A. B.; Singh, S. B. *J. Am. Chem. Soc.* **2007**, *129*, 15422.
24. Herath, K.; Attygalle, A. B.; Singh, S. B. *Tetrahedron Lett.* **2008**, *49*, 5755.
25. Kodali, S.; Galgoci, A.; Young, K.; Painter, R.; Silver, L. L.; Herath, K. B.; Singh, S. B.; Cully, D.; Barrett, J. F.; Schmatz, D.; Wang, J. *J. Biol. Chem.* **2005**, *280*, 1669.
26. Nicolaou, K. C.; Tang, Y.; Wang, J.; Stepan, A. F.; Li, A.; Montero, A. *J. Am. Chem. Soc.* **2007**, *129*, 14850.