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Synthesis and biological evaluation of platensimycin analogs

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ABSTRACT

Platensimycin (**1**) displays antibacterial activity due to its inhibition of the elongation condensing enzyme (FabF), a novel mode of action that could potentially lead to a breakthrough in developing a new generation of antibiotics. The medicinal chemistry efforts were focused on the modification of the enone moiety of platensimycin and several analogs showed significant activity against FabF and possess antibacterial activity.

In 2006, the Merck Research Laboratories discovered platensimycin (**1**, Fig. 1), a structurally unique natural product, from *Streptomyces platensis*. Platensimycin exhibits remarkable antibacterial activity against Gram-positive bacteria including multiple drug-resistant strains such as vancomycin-resistant *Enterococcus faecalis* (VREF) and methicillin-resistant *Staphylococcus aureus* (MRSA).[1] The extraordinary antibacterial property of platensimycin is due to its selective inhibition of β-ketoacyl-(acyl-carrier-protein (ACP)) synthase I/II, also known as FabF/B condensing enzymes, which play an essential role in the bacterial type II fatty-acid synthesis (FASII). The novel scaffold and intriguing biological property of platensimycin immediately captured the interests of numerous research groups. Consequently, several elegant total syntheses have been reported.[2] In addition, medicinal chemistry studies have been conducted by the Nicolaou group.[3] Herein we report our efforts to interrogate structure–activity relationship (SAR) and pharmacophore of platensimycin, which could guide future design.

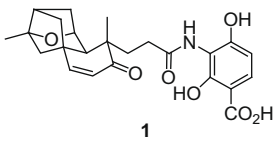

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Figure 1. Structure of platensimycin (**1**).

The X-ray crystallographic studies of platensimycin bound to modified enzyme ecFabF(C163Q) allows the identification of several critical hydrogen bonding and ionic interactions.[1] Regarding the benzoic acid fragment, carboxylate moiety formed salt bridge interactions with two amino acid residues: H340 and H303, the phenol hydroxy group showed water mediated H-bond interaction with D265, and the anilide N–H group exhibited hydrogen bond with T270 (Fig. 2). In addition, the benzene ring of platensimycin poses an edge-to-plane π-interaction with F400. Indeed, as the Nicolaou group[3a] and we discovered,[1c,d] the absence of any of these structural elements of platensimycin led to an almost complete loss of activity. Based on the SAR and X-ray finding, it has been hence concluded that the benzoic acid moiety acts as an essential polar warhead to block the malonate binding pocket of the enzyme. As shown in Figure 3, the ketolide portion of platensimycin is engaged in a hydrogen bond between the enone carbonyl oxygen and A309. Moreover, a hydrogen bond between the cage ether oxygen and T270 also contributes to the binding affinity.[4] Figure 4 reveals the surface of the binding pocket, in which the ketolide is exposed to solvent suggesting the feasibility of adding extra functional groups to the enone. This observation, in addition to a potential covalent protein binding issue associated with the enone group, led us to modify the enone moiety of platensimycin.

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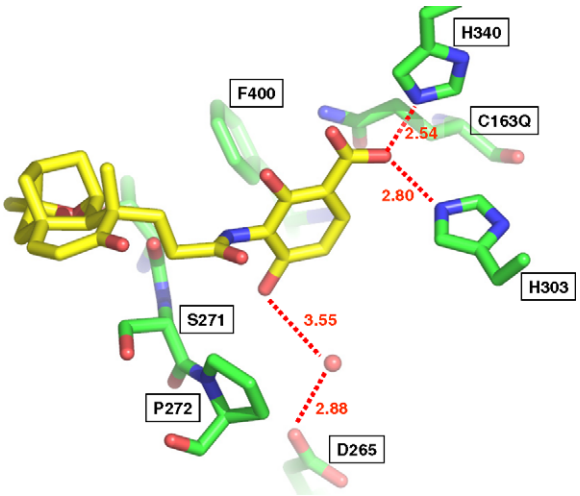


Figure 2. Co-crystal structure (2.5 Å) of platensimycin (**1**, yellow) and ecFabF (C163Q) showing the binding site. Key residues and hydrogen bonds involving the aminobenzoic acid motif are labeled. (PDB code: 2GFX).

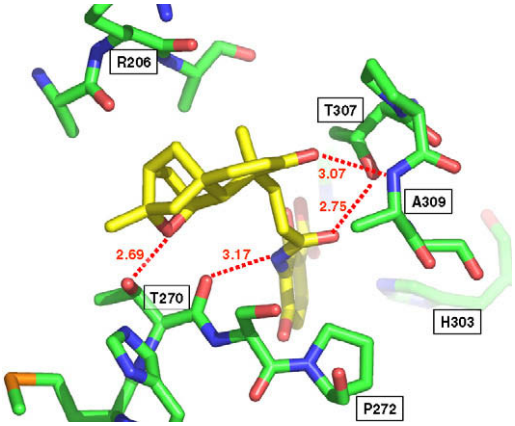


Figure 3. Co-crystal structure (2.5 Å) of platensimycin (**1**, yellow) and ecFabF (C163Q) showing the binding site. Key residues and hydrogen bonds involving the tetracyclic enone acid and linker are labeled. (PDB code: 2GFX).

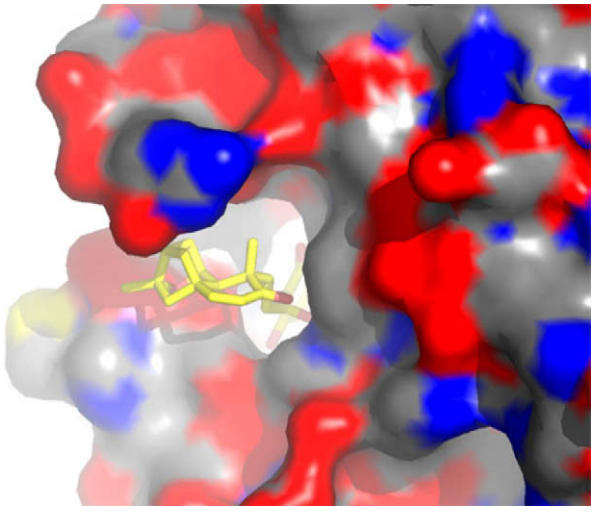


Figure 4. The binding pocket of platensimycin (**1**) in ecFabF (C163Q) (surface coloring: red, oxygen; gray, carbon; blue, nitrogen; yellow, sulfur) (PDB code: 2GFX).

Furthermore, we envision that the poor pharmacokinetic profile of platensimycin may be due in part to the enone component, which may act as an electrophile in vivo causing its high clearance. Thus by modifying the enone group, we hope to minimize the undesirable properties of platensimycin whereas retaining its antibacterial activity.

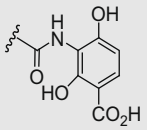
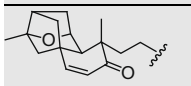
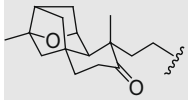
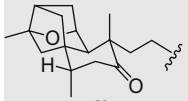
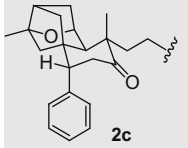
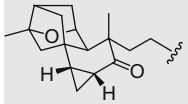
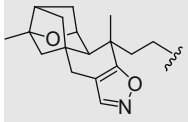
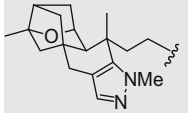
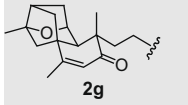
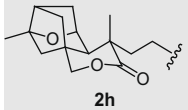
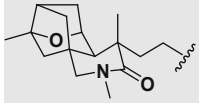
We used three assays to monitor SAR study focusing on the enone modification of platensimycin. The assays were (a) whole cell antibacterial activity using the wild type *S. aureus*, (b) inhibition of FabF enzyme using cell free FASII assay,[1f] and (c) antisense *fabH*/*fabF* sensitized strain of *S. aureus* (saFabF2 assay is the most sensitive *S. aureus* antisense two-plate differential sensitivity cellular assay.[1e] The data reported as MDC which is defined as the minimum concentration of the compound showing differential zone of clearance between antisense plates compared to control plate). The data are summarized in Table 1. Occasional discrepancy among these three assays can be attributed to the different nature of these three assays. The data from *sa*FabF2 (MDC) assay is the cumulative inhibition result of both FabH and FabF, which share common operon and as a result antisense knock down of one gene knocks down both genes. Therefore, better sensitivity is generally observed for the *sa*FabF2 assay. On the other hand, FASII is a cell free enzyme assay and does not capture this effect. MIC is the inhibitory data from wild type *sa* whole cell assay and is much coarser but most important.

First the saturation of the olefin of the platensimycin enone provided analogs **2a–d**. Although the olefin does not appear to be directly involved in interacting with the enzyme based on the X-ray structure, the saturation of the double bond, however, caused a minor conformational change as revealed by co-crystal structures of **2a**·ecFabF(C163Q) and **2c**·ecFabF(C163Q). Figures 5 and 6 show overlay of the crystal structures of platensimycin and dihydroplatensimycin (**2a**) and platensimycin and phenyl dihydroplatensimycin (**2c**), respectively. The comparison suggest that the boat conformation of platensimycin changed to the chair conformation in the former case, and the twist chair in the latter case both resulting in a small but appreciable change of the positioning of ketolide carbonyl as well as the aminobenzoic acid moiety. Consequently, these changes led to a 4–8 fold loss of antibacterial activity against wild type *S. aureus* and cell free FASII assay. These compounds showed much profound loss of activity against the FabF2 antisense assay. Thus, the binding pocket of platensimycin appears to be quite rigid and does not accommodate substantial conformational changes. It was then postulated that to increase of the sp^2 character of the α and/or β carbon of the ketolide carbonyl would be beneficial to activity. Indeed, cyclopropyl analog **2d** was fourfold more active in FabF2 antisense assay than compound **2a** and two fold better in killing wt *S. aureus*. However, this substitution has fivefold opposite effect against FASII assay.

Next, fused heterocyclic analogs containing a pyrazole or isoxazole were examined to replace the electrophilic enone. The rational was to use hetero atoms of heterocycles to mimic the enone carbonyl oxygen, and maintain the sp^2 character of the ketone α-carbon in platensimycin. Unfortunately, this strategy generated analogs **2e** and **2f** with much weaker activity. It is worth noting that analog **2e** was more active than **2f** suggesting the pyrazole methyl substituent may disrupt the hydrogen bonding between the analog **2f** and A309.

Using an heteroatom to replace the α-carbon of the enone in platensimycin, we also prepared lactone **2h** and lactam **2i** that displayed only very weak activities in all the assays presumably due to the change of the carbonyl's orientation. Finally, the fine-tuning of platensimycin with minimum conformational change by adding a methyl group at the β-position of enone resulted in analog **2g** that was equipotent as platensimycin in the antisense saFabF2 assay. The advantage of compound **2g** is the more steric hindrance of

Table 1
In vitro activity of platensimycin analogs

Compds	saMIC (μg/mL)[a]	IC_{50}, sa FASII (μg/mL)	saFabF2 (MDC), antisense, (μg/mL)
			
 **1**	0.5	0.5	0.01–0.04
 **2a**	2	2	0.4
 **2b**	4	13	<20
 **2c**	4	4	<20
 **2d**	1	9.5	0.1
 **2e**	16	10.5	0.3
 **2f**	>64	75.3	>2.7
 **2g**	4	2.4	0.03
 **2h**	>64	73.6	>2.7
 **2i**	>64	>200	>2.7

[a] sa = *Staphylococcus aureus*.

the enone β-position would render the potential in vivo 1,4-addition pathway to enone less likely. Our SAR study strongly suggests that the intricate interaction between platensimycin and the enzyme does not allow a significant change of the cyclohexenone conformation. Future design should maximize the conformational similarity of analogs to the cyclohexenone of platensimycin.

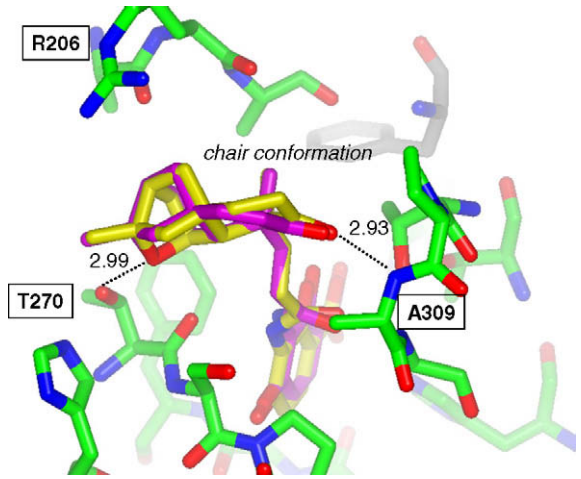


Figure 5. Overlay of 2.5 Å co-crystal structures of platensimycin (**1**, purple) and **2a** (yellow) and ecFabF (C163Q) showing the binding site. (PDB code: 3G0Y).

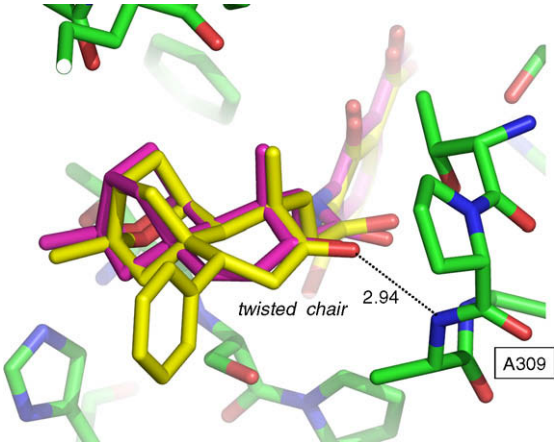
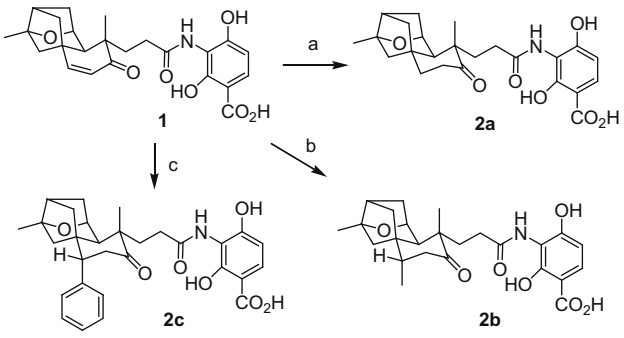


Figure 6. Overlay of 2.5 Å co-crystal structures of platensimycin (**1**, purple) and **2c** (yellow) and ecFabF (C163Q) showing the binding site. (PDB code: 3G11).

To obtain dihydroplatensimycin, a Pd/C-catalyzed hydrogenation provided analog **2a** in good yield (Scheme 1). Direct 1,4-addition to the enone of platensimycin with a large excess of Grignard reagents provided the desired adducts **2b** and **2c**, respectively with an excellent control of diastereoselectivity (>10:1). The observed



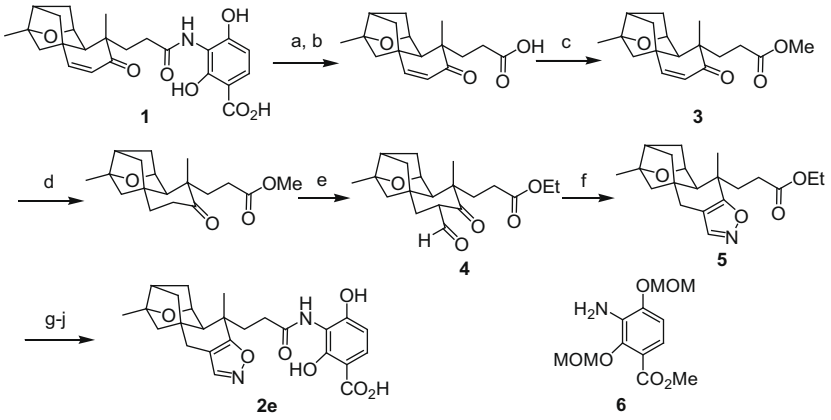
Scheme 1. Reagents and conditions: (a) 10% Pd/C, MeOH, rt, 16 h, 99%; reaction performed as a Na salt (see Ref. 1c); (b) MeMgBr (10 equiv), THF–DME (2:1), rt, 10%; (c) PhMgBr (15 equiv), THF–DME (1:1), rt to 50 °C, 16 h, 10%.

diastereoselectivity is due to the bulky cage fragment that sterically biased the 1,4-addition to proceed exclusively from the bottom face.

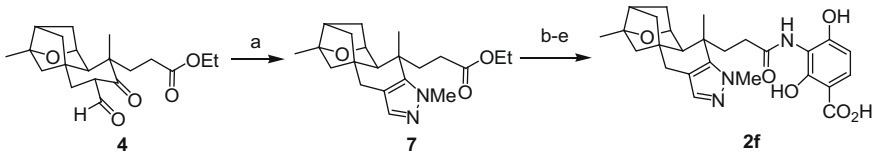
The synthesis of other analogs commenced with the common intermediate **3** derived from platensimycin via a mild amide hydrolysis[5] and methyl ester formation (Scheme 2). Subsequently, the introduction of an α-formyl group with a concomitant *trans*-esterification set the stage for condensation reactions of intermediate **4** with hydroxylamine and methyl hydrazine to generate isoxazole **5** (Scheme 2) and pyrazole **7** (Scheme 3), respectively. The following hydrolysis, amide formation and deprotection sequence then led to analogs **2e** and **2f** (Schemes 2 and 3). It should be noted that the use of pyridine as the base for the amide formation instead of triethyl amine gave substantially higher yields.

The incorporation of a cyclopropyl group was achieved using the Corey-Chaykovsky cyclopropanation (Scheme 4).[6] The resulting cyclopropane intermediate **8** was obtained as a single diastereomer, serving as a key intermediate leading to analog **2d** (Scheme 4).

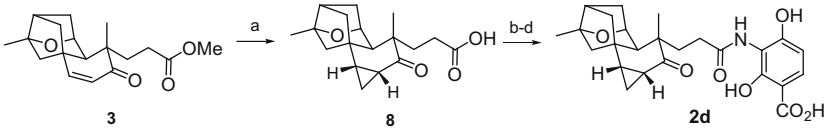
To install a β-methyl group to platensimycin, the 1,4-methyl addition to intermediate **8** followed by a Nicolaou's dehydrogenation protocol[7] generated compound **9**, which was eventually converted to analog **2g** over four steps (Scheme 5).[8]



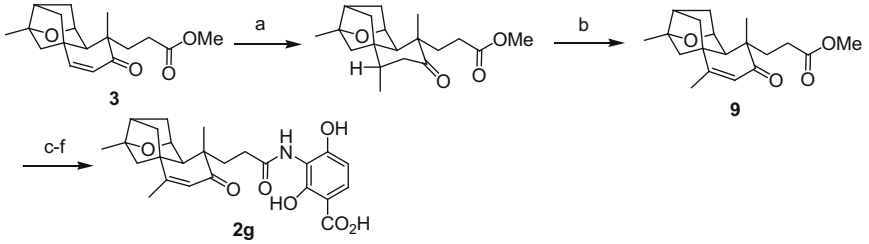
Scheme 2. Reagents and conditions: (a) $NaNO_2$, HOAc, Ac_2O, 0 °C to rt, 5 h, 60%; (b) LiOH, THF, H_2O, rt, 16 h, 99%; (c) $TMSCHN_2$, MeOH, 0 °C, 5 min, 90%; (d) 10% Pd/C, MeOH, rt, 2 h, 97%; (e) NaH, EtOCHO, Et_2O, rt, 16 h, 74%; (f) NH_2OH HCl salt, EtOH, reflux, 16 h, 47%; (g) LiOH, THF, MeOH, H_2O, rt, 2 h, 90%; (h) **6**, HATU, pyridine, DMF, rt, 16 h, 29%; (i) LiOH, THF, H_2O, rt, 16 h; (j) HCl, THF, 40 °C, 24 h, 86% over two steps.



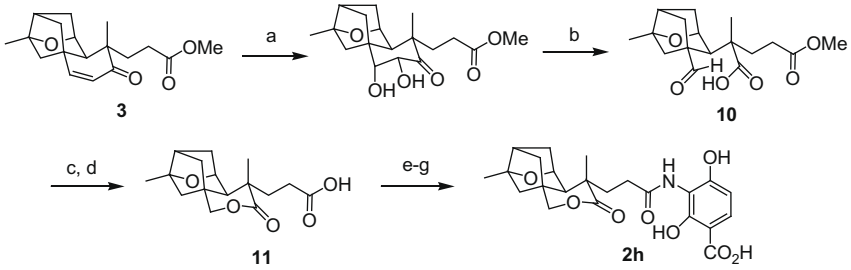
Scheme 3. Reagents and conditions: (a) NH_2NHMe, EtOH, reflux, 16 h, 54%; (b) LiOH, THF, MeOH, H_2O, rt, 2 h, 90%; (c) **6**, HATU, pyridine, DMF, rt, 24 h, 38%; (d) LiOH, THF, H_2O, rt, 16 h; (e) HCl, THF, 40 °C, 16 h, 65% over two steps.



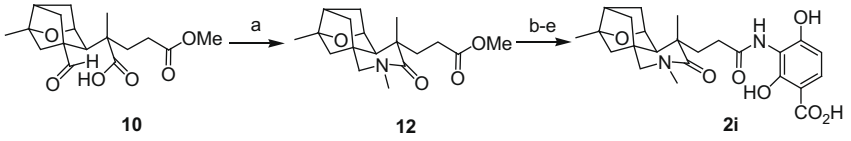
Scheme 4. Reagents and conditions: (a) $Me_3S^+I^-$, NaH, DMSO, rt, 16 h, 34%; (b) **6**, HATU, pyridine, DMF, rt, 16 h, 38%; (c) LiOH, THF, H_2O, rt, 16 h; (d) HCl, THF, 40 °C, 24 h, 50% over two steps.



Scheme 5. Reagents and conditions: (a) MeLi (3 equiv), CuI (6 equiv), THF, 0 °C to rt, 1.5 h, 78%; (b) HIO_3, cyclohexadiene, DMSO, 60 °C, 2 h, 20%; (c) LiOH, THF, H_2O, rt, 3 h, 95%; (d) **6**, HATU, pyridine, DMF, rt, 27%; (e) LiOH, THF, H_2O, rt, 24 h; (f) HCl, THF, 40 °C, 24 h, 34% over two steps.



Scheme 6. Reagents and conditions: (a) cat. OsO_4, NMO, CH_2Cl_2, rt, 3 days; (b) $NaIO_4$, acetone, water, rt, 4 days, 99% over two steps; (c) $NaBH_4$, MeOH, rt, 1 h, then HCl, 49%; (d) LiOH, THF, MeOH, H_2O, rt, 4 h, 90%; (e) **6**, HATU, pyridine, DMF, rt, 16 h, 19%; (f) LiOH, THF, H_2O, rt, 16 h; (g) HCl, THF, 40 °C, 16 h, 53% over two steps.



Scheme 7. Reagents and conditions: (a) $MeNH_2{\cdot}HCl$, $NaBH(OAc)_3$, Et_3N, THF/acetic acid (4:1), 16 h, rt, 37%; (b) LiOH, THF, MeOH, H_2O, rt, 1 h, 99%; (c) **6**, HATU, pyridine, DMF, rt, 16 h, 30%; (d) LiOH, THF, H_2O, rt, 16 h; (e) HCl, THF, 40 °C, 16 h, 12% over two steps.

The replacement of cyclohexenone of platensimycin with a lactone or lactam involved the oxidative degradation of the double bond that produced aldehyde acid **10** (Scheme 6). The reduction of aldehyde to alcohol, followed by an acid-mediated lactone formation allowed the formation of lactone intermediate **11**, which was subsequently converted to product **2h**. In a similar fashion, the sequential reductive amination of compound **10** and lactam formation yielded intermediate **12** during the synthesis of analog **2i** (Scheme 7).

In conclusion, several platensimycin analogs were prepared to test the hypothesis whether the enone moiety was essential for platensimycin's biological activity. The SAR strongly suggests that the conformation of the enone moiety is crucial in positioning the ketone carbonyl group to interact with A309 of FabF. Of all the analogs prepared, those bearing the ketone moiety and similar conformation, such as compound **2d** and **2g**, were generally quite active in the saFabF2 antisense assay but none were more active than platensimycin.

References and notes

1. (a) Wang, J.; Soisson, S. M.; Young, K.; Shoop, W.; Kodali, S.; Galgoci, A.; Painter, R.; Parthasarathy, G.; Tang, Y. S.; Cummings, R.; Ha, S.; Dorso, K.; Motyl, M.; Jayasuriya, H.; Ondeyka, J.; Herath, K.; Zhang, C.; Hernandez, L.; Allocco, J.; Basilio, A.; 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. D.; Bartizal, K.; Barrett, J.; Schmatz, D.; Becker, J. W.; Cully, D.; Singh, S. B. *Nature* **2006**, *441*, 358; (b) Singh, S. B.; jayasuriya, H.; Ondeyka, J. G.; Herath, K. B.; Zhang, C.; Zink, D. L.; Tsou, N. N.; Ball, R. G.; Basilio, Á.; Genilloud, O.; Diez, M. T.; Vicente, F.; Palaez, F.; Young, K.; Wang, J. *J. Am. Chem. Soc.* **2006**, *128*, 11916; (c) Singh, S. B.; Herath, K. B.; Wang, J.; Tsou, N.; Ball, R. G. *Tetrahedron Lett.* **2007**, *48*, 5429; (d) Zhang, C.; Ondeyka, J.; Zink, D. L.; Burgess, B.; Wang, J.; Singh, S. B. *Chem. Commun.* **2008**, 5034; (e) Young, K. et al *Antimicrob. Agents Chemother.* **2006**, *50*, 519; (f) 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.
2. (a) Nicolaou, K. C.; Li, A.; Edmonds, D. J. *Angew. Chem., Int. Ed.* **2006**, *45*, 7086; (b) Nicolaou, K. C.; Edmonds, D. J.; Li, A.; Tria, G. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 3942; (c) Zou, Y.; Chen, C.-H.; Taylor, C. D.; Foxman, B. M.; Snyder, B. B. *Org. Lett.* **2007**, *9*, 1825; (d) Nicolaou, K. C.; Tang, Y.; Wang, J. *Chem. Commun.* **2007**, 1922; (e) Li, P.; Payette, J. N.; Yamamoto, H. *J. Am. Chem. Soc.* **2007**, *129*, 9534; (f) Lalic, G.; Corey, E. J. *Org. Lett.* **2007**, *9*, 4921; (g) Tiefenbacher, K.; Mulzer, J. *Angew. Chem., Int. Ed.* **2007**, *46*, 8074; (h) Nicolaou, K. C.; Pappo, D.; Tsang, K. Y.; Gibe, R.; Chen, D. Y.-K. *Angew. Chem., Int. Ed.* **2008**, *47*, 944; (i) Kim, C. H.; Jang, K. P.; Choi, S. Y.; Chung, Y. K.; Lee, E. *Angew. Chem., Int. Ed.* **2008**, *47*, 4009.
3. (a) Nicolaou, K. C.; Stepan, A. F.; Lister, T.; Li, A.; Montero, A.; Tria, G. S.; Turner, C. I.; Tang, Y.; Wang, J.; Denton, R. M.; Edmonds, D. J. *J. Am. Chem. Soc.* **2008**, *130*. ASAP; (b) Nicolaou, K. C.; Tang, Y.; Wang, J.; Stepan, A. F.; Li, A.; Montero, A. *J. Am. Chem. Soc.* **2007**, *129*, 14850; (c) Nicolaou, K. C.; Lister, T.; Denton, R. M.; Montero, A.; Edmonds, D. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 4712.
4. As shown in Ref. 3a, all-carbon cage analogs carbaplatensimycin and adamantaplatensimycin both gave about threefold less of activity with respect to platensimycin.
5. Mandai, T.; Yamakawa, T. *Synlett* **2000**, 862.
6. Corey, E. J.; Chaykovsky, M. *J. Am. Chem. Soc.* **1965**, *87*, 1353.
7. Nicolaou, K. C.; Montagnon, T.; Baran, P. S. *Angew. Chem., Int. Ed.* **2002**, *41*, 1386.
8. *For analog* **2g**: ^1H NMR (acetone-d_6, 500 MHz) δ 10.6 (1H, br s), 9.11 (1H, s), 7.65 (1H, d, J = 6.0 Hz), 6.48 (1H, d, J = 6.0 Hz), 5.79 (1H, s), 4.44 (1H, s), 2.66 (2H, m), 2.38 (4H, m), 2.10 (7H, m), 1.89 (3H, s), 1.70 (1H, m), 1.41 (3H, s), 1.24 (3H, s); LCMS m/z: 456.19 (M+1), 478.21 (M+Na). For analog **2h**: ^1H NMR (acetone-d_6, 500 MHz) δ 10.2 (1H, br s), 8.96 (1H, s), 7.68 (1H, br s), 6.31 (1H, bs), 4.50 (1H, d, J = 11.0 Hz), 4.38 (1H, s), 3.88 (1H, d, J = 11.0 Hz), 2.63 (2H, m), 2.32 (5H, m), 2.05 (5H, m), 1.99 (3H, s), 1.68 (1H, m), 1.53 (1H, m), 1.38 (3H, s); LCMS m/z: 446.52 (M+1).