THE CHEMISTRY AND BIOLOGICAL ACTIVITY OF PLATENSIMYCIN

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INTRODUCTION

Hospital mortalities from multidrug-resistant bacteria have grown in recent years. Multidrug-resistant bacteria are becoming a major public health crisis because the antibiotics that exist today are no longer effective in all cases. Drugs, like vancomycin, which were once last-resort treatments are quickly becoming a first-line defense because the over-prescription of antibiotics is allowing for resistant bacteria to develop rapidly.[1] Additionally, the discovery of new effective classes of pharmaceuticals has diminished dramatically since the 1960s.[2] Inspired by this need for a new pharmaceutical that disrupts the function of a novel target, a Merck research group began high-throughput screening of secondary metabolites against FabF, an enzyme involved in bacterial fatty acid biosynthesis, which led to the isolation of platensimycin (**1**, Figure 1). Platensimycin, isolated from *Streptomyces platensis*, represents a new structural class of antibiotics with potent broad-spectrum antibacterial activity against Gram-positive bacterial strains.[3] To date, no cross-resistance has been observed, and this is thought to be due to platensimycin's unique mechanism of action. Given the magnitude of the threat multidrug-resistant bacteria poses, platensimycin offers a new weapon in the fight against bacterial infections. With a molecular-level understanding of its unique mode of action and the structure/function relationships involved, platensimycin stands to enable the development of new clinically viable antibiotics.

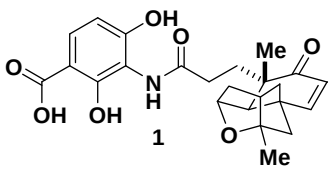


Figure 1. Structure of (-)-platensimycin (**1**).

DISCOVERY AND BIOLOGICAL ACTIVITY

There are two major types of fatty acid synthetic systems: type I (associated) and type II (dissociated).[4] Associated systems, found in higher organisms like mammals, consist of a large multidomain protein that carries out all of the steps in the biosynthetic pathway. Dissociated systems, found in plants and bacteria, consist of discrete enzymes the each carry out a single step in fatty acid synthesis (Figure 2).[5] These structural and organizational differences between type I and II make type II pathways ideal targets for antibiotics. Additionally, enzymes FabH and FabF/B in this pathway are

highly conserved across bacterial strains,[3] suggesting that broad-spectrum antibiotic activity is possible.

With type II fatty acid biosynthesis as an ideal antibacterial target, Wang and coworkers at Merck began a target-biased screen looking specifically at FabF, an enzyme involved in elongation of the fatty acid chain. To test antibacterial activity against *S. aureus*, antisense RNA was used to decrease expression of the targeted protein, FabF, sensitizing it to inhibition.[3,6] With a high-throughput screening method developed, Wang and coworkers tested over 250,000 microbial extracts for activity against FabF. From their efforts, the potent and selective antibiotic platensimycin was discovered in a soil sample from South Africa. The interesting tetracyclic enone core and absolute stereochemistry of this small molecule was then identified by Singh and coworkers using 2D NMR and X-ray crystallography.[7]

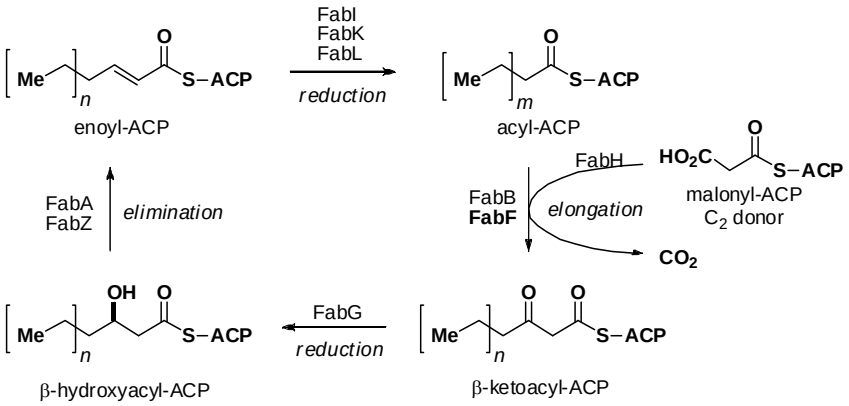


Figure 2. Type II (dissociated) fatty acid biosynthesis. ACP = acyl-carrier-protein.

During *in vitro* studies platensimycin demonstrated broad-spectrum activity against Gram-positive bacteria and compared favorably with other clinically used antibiotics such as linezolid, which has been in use since 2000 (Table 1).[3] Additionally, no cross-resistance to the life-threatening microbes methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), and vancomycin-resistant *enterococci* (VRE) was observed. In assays against Gram-negative bacteria, platensimycin was observed to only have activity against efflux-negative *E. coli* (*tolC*), a mutant without the ability to expel small molecules, and not wild-type *E. coli*. This suggests that an efflux mechanism is limiting activity against Gram-negative bacteria. Low mammalian cell toxicity (IC_{50} > 1,000 μg ml^{-1} in HeLa cells) revealed that platensimycin acts selectively on type II fatty acid biosynthesis and does not inhibit DNA, RNA, protein, or cell wall biosynthesis. This selective

Organism and genotype	Platensimycin	Linezolid
Antibacterial activity (MIC, μg ml^{-1})		
S. aureus (MRSA)	0.5	2
S. aureus (MRSA, macrolide^R)	0.5	2
S. aureus (MRSA, linezolid^R)	1	32
S. aureus (VISA, vancomycin^I)	0.5	2
Enterococcus faecium (VRE)	0.1	2
E. coli (wild-type)	>64	>64
E. coli (*tolC*)	16	32
Toxicity (μg ml^{-1})		
HeLa MTT (IC_{50})	>1,000	>100
Candida albicans (MIC)	>64	>64

Table 1. Microbiological profile and toxicity of platensimycin and linezolid. R = resistant, I = intermediate.

toxicity was demonstrated *in vivo* with *S. aureus* infections in a mouse model.

To demonstrate that platensimycin inhibits the FabF protein, Wang and coworkers performed a direct binding assay utilizing a radioactive derivative of platensimycin in the presence and absence of lauroyl-CoA, a mimic of the natural acyl-ACP substrate.[3] The observation that the binding signal increased by 19-fold in the presence of lauroyl-CoA suggested that platensimycin acts by binding to the covalent acyl-enzyme intermediate of FabF needed to proceed with fatty acid chain elongation. In order to further probe the chemical interactions leading to enzyme inhibition, Wang and coworkers attempted to crystallize platensimycin in the active site of FabF.[3] While they could not crystallize wild-type FabF with platensimycin bound, mutation of cysteine 163 to glutamine stabilized the acyl-enzyme intermediate and the binding of platensimycin allowing for a crystal structure to be obtained.

The 2.6Å structure of FabF(C163Q) showed that platensimycin binds in the malonyl subsite of FabF where it is positioned to interact with residues important for the chain elongation catalytic cycle. The key inhibitory interaction is observed to take place at histidines 303 and 340, which are part of the conserved His-His-Cys catalytic triad. The carboxylic acid of the aromatic ring in platensimycin is positioned ideally to engage in hydrogen bonding interactions with these two residues, preventing fatty acid precursors from interacting with the catalytic triad. Moreover, the aromatic ring itself can participate in edge-to-face π- stacking with phenylalanine 400 (Figure 3).[8] The tetracyclic enone lies at the mouth of the active site, linked to the aromatic ring via an amide bond. The enone participates in a hydrogen bonding interaction with the backbone amide bond of alanine 309 and the ether linkage hydrogen bonds to the side chain hydroxyl group of threonine 270. These hydrogen bonds effectively stabilize platensimycin in the active site, blocking access to fatty acid precursors such that chain elongation is halted.

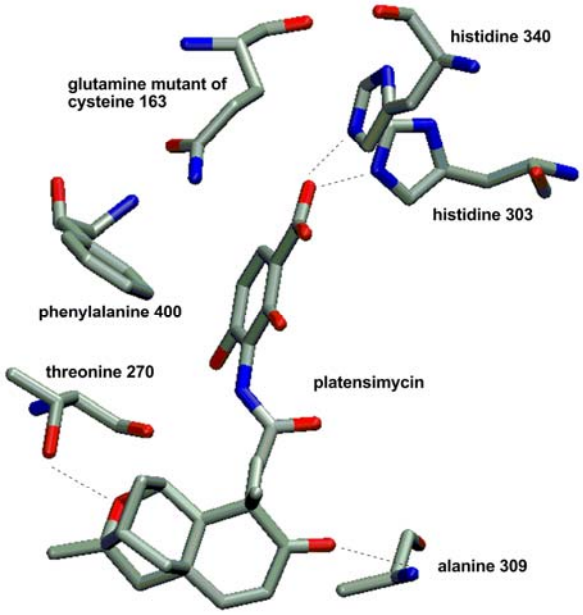
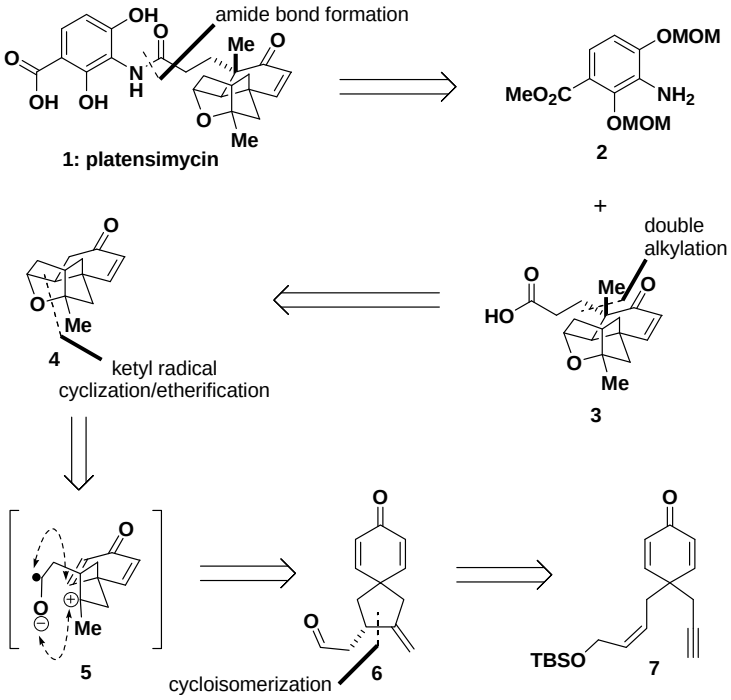


Figure 3. Crystal structure of platensimycin in the active site of FabF(C163Q).

TOTAL SYNTHESIS

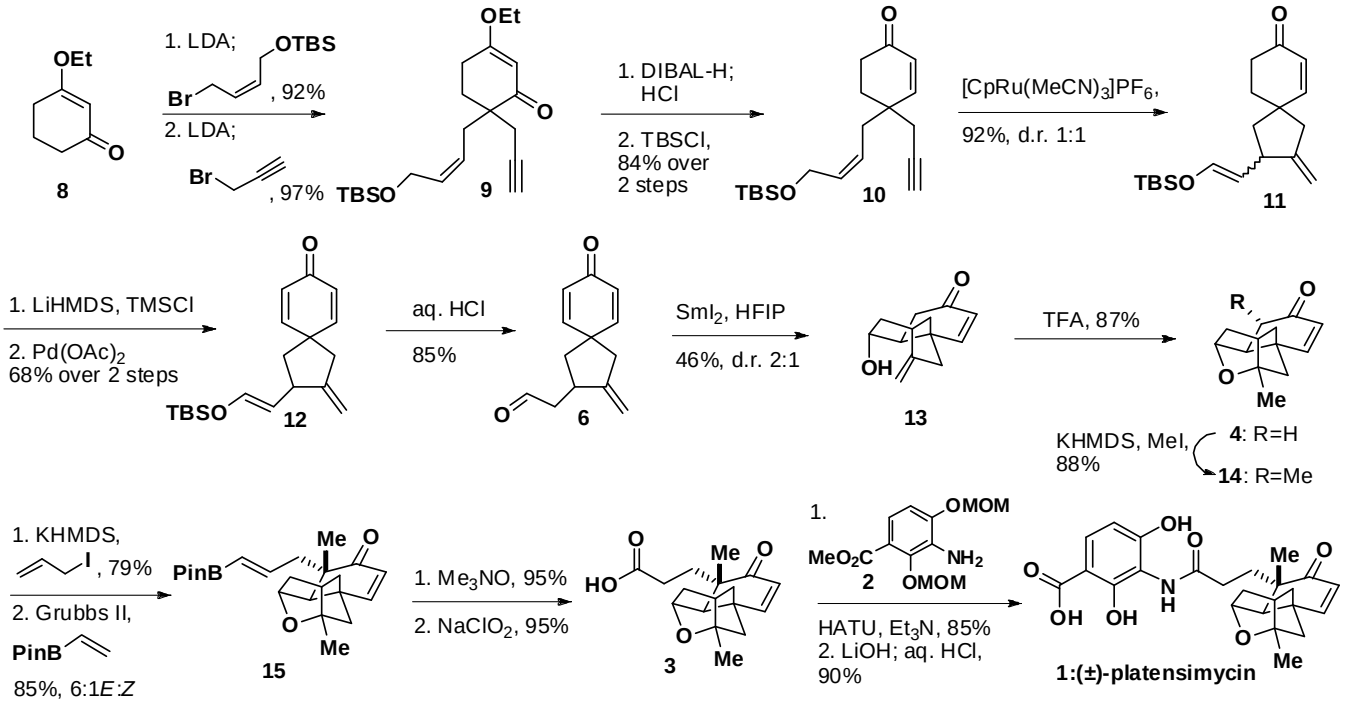
Despite platensimycin's promising biological activity, its pharmacological profile is not ideal. In mouse studies, high doses were required via continuous flow pump in order to achieve antibiotic activity. This is presumably because platensimycin is either unstable *in vivo* or is rapidly cleared from the body once administered.[5] Due to these challenges to clinical application, several synthetic groups have engaged in its total synthesis in the hopes of enabling future structure/function studies that may lead to the discovery of clinically useful derivatives. With this goal in mind, Nicolaou and coworkers completed the first racemic total synthesis of platensimycin in 2006.[9]



Scheme 1. Nicolaou's retrosynthetic analysis of platensimycin.

Platensimycin consists of a polar 3-amino-2,4-dihydroxybenzoic acid domain linked to a non-polar tetracyclic enone core via an amide bond. Retrosynthetic analysis reveals the amide bond as a clear simplifying disconnect providing aromatic ring **2** and tetracyclic enone **3** as the key synthetic targets (Scheme 1).[9] While **2** can be readily accessed via protection and directed ortho metallation of 2-nitroresorcinol, the tetracyclic enone is a more complex target. Nicolaou and coworkers recognized the retron for a double alkylation, simplifying **3** to the cage-like structure **4**. From **4**, the ether bond can be cleaved providing a tertiary carbocation synthon. Further cleavage of the carbon-carbon bond adjacent to the retrosynthetically generated secondary alcohol brings cage-like structure **4** to spirocyclic cyclohexadienone **6** via a ketyl radical cyclization[10]/etherification transform. Finally, recognition of a cycloisomerization retron reveals enyne **7** as a readily accessible starting material for platensimycin.

Synthesis of carboxylic acid **3** began with the double alkylation of enone **8** providing **9** as a single constitutional isomer in excellent yields (Scheme 2). Subsequent reduction of **9** followed by acid hydrolysis and TBS reprotection gave cycloisomerization precursor **10** in 84% yield over two steps. Cycloisomerization was performed using the method developed by Trost.[11] Treatment of **10** with catalytic $[CpRu(MeCN)_3]PF_6$ gave **11** as a 1:1 mixture of diastereomers in 92% yield. Bis-enone **12** was



Scheme 2. Nicolaou's racemic total synthesis of platensimycin.

then generated by $Pd(OAc)_2$ oxidation of the TMS enol ether of **11**. TBS deprotection using aqueous HCl provided the aldehyde precursor (**6**) for the ketyl radical cyclization/etherification in 85% yield.

The key step, ketyl radical cyclization of **6**, was achieved by rapid addition of samarium(II) iodide,[10] a single-electron reductant, followed by immediate quenching with saturated aqueous NH_4Cl solution giving **13** as a 2:1 mixture of inseparable diastereomers in 46% yield. Subsequent etherification by treatment with TFA smoothly gave an 87% yield of **4** based on the desired diastereomer. The ease with which this reaction proceeded reflects the close proximity of the secondary alcohol to the olefin in the stereochemical configuration of the natural product. To further streamline the synthesis, a one-pot ketyl radical cyclization/etherification was used. Treatment of **6** with samarium(II) iodide followed by an acidic work-up with TFA provided **4** in 25% overall yield. The alkyl substituents were then installed by stereoselective alkylation. Addition of KHMDS and methyl iodide followed by treatment with KHMDS and allyl iodide gave the terminal olefin in good yield as a single diastereomer. This high selectivity can be rationalized by the fact that the rigid cage-like structure blocks approach of the electrophile from the top face of the enolate. Vinyl boronate ester **15** was then accessed via cross metathesis using Grubbs second-generation catalyst and vinyl pinacol boronate ester, giving an inconsequential mixture of *E* and *Z* olefins (6:1). Oxidation with trimethyl *N*-oxide to generate an aldehyde followed by a second oxidation using the Pinnick protocol[12] generated acid coupling partner **3** in excellent yields. Acid **3** was coupled under standard amide bond forming conditions (HATU, Et_3N) to

aromatic amine **2** in 85% yield. A one-pot deprotection of the methyl ester and the MOM ethers in 90% yield completed the total synthesis of (±)-platensimycin.

Since the completion of Nicolaou's racemic total synthesis, several enantioselective formal syntheses of platensimycin have been completed where cage-like structure **4** is accessed in enantioenriched form.[13] Some of the more interesting strategies to the tetracyclic core have included an enantioselective cycloisomerization,[14] a dearomatization,[15] an intramolecular Robinson annulation,[16] and an intramolecular Diels-Alder reaction (unfinished approach).[17] With an efficient synthetic route to platensimycin, recent efforts have been focused on structure/function studies.

STRUCTURE/FUNCTION STUDIES ON DERIVATIVES

An understanding of the structural elements critical for the antibiotic activity of platensimycin could provide a basis for the development of derivatives with improved pharmacological profiles relative to the natural product. In an attempt to probes the interactions between the tetracyclic enone core and the FabF enzyme, Nicolaou and coworkers synthesized a short series of derivatives to determine how changing the structure of the core affects biological activity. In that vein, they synthesized adamantaplatensimycin (**16**)[18] and carbaplatensimycin (**17**, Figure 4).[19] Adamantaplatensimycin was synthesized in racemic form and was also resolved into its enantiomers. Using methods analogous to the asymmetric synthesis of platensimycin,[14] the total synthesis of (-)-carbaplatensimycin was completed. The structures of both (-)-**16** and (-)-**17** were unambiguously assigned through single crystal X-ray diffraction.

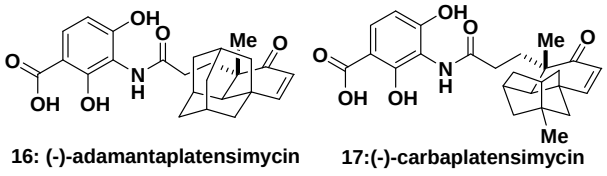


Figure 4. Structure of (-)-adamantaplatensimycin (**16**) and (-)-carbaplatensimycin (**17**).

The minimum inhibitory concentration (MIC) of carbaplatensimycin and the adamantaplatensimycins were determined *in vitro* against various strains of bacteria, including MRSA and VRE. Both (-)-**16** and (-)-**17** demonstrated antibacterial activity in the same order of magnitude as (-)-platensimycin (**1**) against Gram-positive bacteria (Table 2).[18,19] All compounds tested were inactive against the Gram-negative bacteria *E. coli*. Interestingly, (+)-adamantaplatensimycin ((+)-**16**) was inactive and the racemic adamantaplatensimycin ((±)-**16**) was half as active as the biologically active enantiomer (-)-**16**.

Organism and genotype	(-)-**1**	(±)-**1**	(+)-**16**	(-)-**16**	(±)-**16**	(-)-**17**
Antibacterial activity (MIC, μg ml^{-1})						
MRSA	0.2-0.4	0.4-0.8	>88	1.3-1.8	2.6-3.6	1.1-2.2
VRE	0.4-0.8	0.8-1.6	>88	1.3-1.8	2.6-3.6	1.1-2.2
Staphylococcus aureus	0.2-0.6	--	--	1.1-2.2	--	0.4-1.1
Staphylococcus epidermidis	<0.2	--	--	0.5-1.1	--	0.2-0.5
Bacillus cereus	2.2-4.4	--	--	8.8-11.1	--	17.6-22.0
Lysteria monocytogenes	<0.2	--	--	3.3-4.4	--	1.1-2.2
Escherichia coli	>88	>88	>88	>88	>88	>88

Table 2. Biological activity of platensimycin (**1**), adamantaplatensimycin (**16**), and carbaplatensimycin (**17**).

The biological assays of these derivatives provided some insight into the structure/function relationships involved in platensimycin antibiotic activity. It is clear that the absolute stereochemistry plays an important role in biological activity given that (+)-adamantaplatensimycin is inactive and that racemic platensimycin and adamantaplatensimycin show reduced activity compared to the active enantiomer. Additionally, while (-)-**16** and (-)-**17** show strong antibacterial activity, (-)-**1** is still the most potent. This supports the hypothesis that the ether oxygen makes a positive contribution towards stabilizing platensimycin in the active site of FabF, which is consistent with the crystal structure obtained by Wang and coworkers.[5]

CONCLUSION

The discovery of platensimycin has given new hope in the fight against antibiotic resistant bacteria. It has been shown to be very potent and highly selective, possessing a novel mechanism of action that targets only the bacterial type II fatty acid biosynthesis. While initial studies on *in vivo* activity have been promising, there are still challenges to be overcome with this structurally interesting small molecule such as the high dosage requirement. With the completion of a racemic total synthesis of platensimycin and several enantioselective formal syntheses, derivatives with an improved pharmacological profile may be readily accessed as evidenced by recent structure and function studies of the tetracyclic enone. The further study of platensimycin has the potential to open up a new class of clinically useful pharmaceuticals against resistant bacterial strains.

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