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Chinese Journal of Natural Medicines 2022, **20**(2): 81-101
doi: 10.1016/S1875-5364(21)60114-0
Chinese Journal of Natural Medicines

•Review•

Old fusidane-type antibiotics for new challenges: Chemistry and biology

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Available online 20 Feb., 2022

[ABSTRACT] The spread of antibiotic-resistant bacteria and exhausted drug leads render some infections untreatable now and in the future. To deal with these "new challenges", scientists tend to re-pick up "old antibiotics". Fusidane-type antibiotics have been known for nearly 80 years as potent antibacterial agents against gram-positive bacteria, especially *Staphylococci*, and represent the only triterpene-derived antibiotic class in clinical setting. These attractive characteristics have drawn renewed attention on fusidane-type antibiotics in recent decades. Isolation, characterization, biological evaluation, as well as chemical modifications of fusidane-type antibiotics are increasingly being reported. Combinatorial biosynthesis of this type of antibiotics has been successfully utilized not only for elucidating the biosynthetic pathways, but also for expanding their structural diversity. Some isolated and synthetic compounds exhibit comparable or even more potent biological activity than fusidic acid. This review provides an overview of progress on the studies of structure and biology of fusidane-type antibiotics from 1943 to April 2021. The informative structure-activity relationship is also highlighted.

[KEY WORDS] Fusidane-type antibiotics; Fusidic acid; Biosynthesis; Structural modification; Biological activity
[CLC Number] R284 **[Document code]** A **[Article ID]** 2095-6975(2022)02-0081-21

Introduction

Antibiotics (also called antibacterials) belong to a class of chemical agents that treat bacterial infections [1]. These chemicals either exert bactericidal effects through promoting bacterial death, or possess bacteriostatic properties by simply inhibiting the proliferation of bacteria [2-3]. The first antibiotic, salvarsan, was chemically synthesized by Paul Ehrlich and clinically introduced in 1910 [4]. The discovery of the best-known antibiotic penicillin from the fungus *Penicillium notatum* in 1928 by Scottish microbiologist Fleming, started the golden age of antibiotic discovery that flourished in the mid-1950s [5-6].

Most of marketable antibiotics, which can be separated into more than twenty classes, are derived from natural products, especially those isolated from microorganisms [7-8]. They have dramatically changed modern medicine and assured human health [8]. However, the antibiotic development was hindered by the increasing emergence of drug-resistant microbes [9-11] and rediscovery of known natural products in recent decades [12-13]. To cope with this urgent situation, scientists pay more attentions back to some "old antibiotics" with intriguing structural features and excellent biological activity [14-16].

Fusidane-type antibiotics, which belong to a small group of fungal 29-*nor* protostane triterpenoids, have been known for nearly 80 years (Fig. 1) [17]. They represent the only triterpene-derived antibiotic class [18]. In this antibiotic group, fusidic acid is the only one that has been applied for clinical treatment [19-21]. More importantly, fusidane-type antibiotics are the only known antibiotics that selectively target bacterial elongation factor G (EF-G) [22-23]. EF-G acts as an indispensable translocase during protein translation. Once fusidane-type antibiotics bind to EF-G, they will prevent tRNA from

[Received on] 12-Apr.-2021
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 81903494 and 81903483).
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These authors have no conflict of interest to declare.

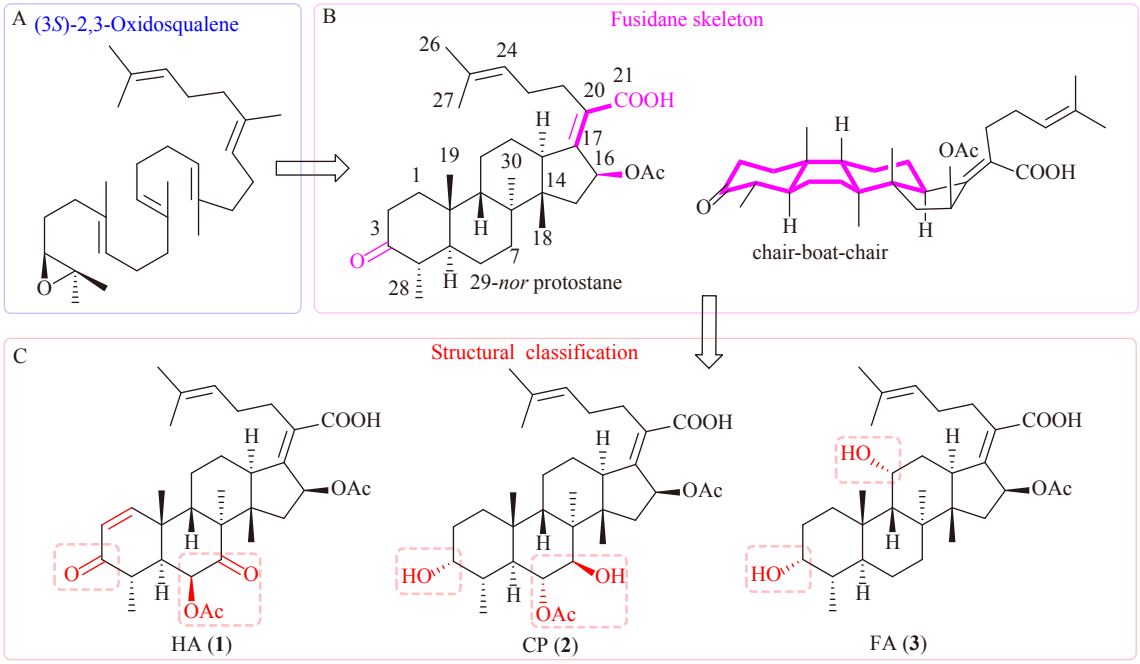


Fig. 1 Fusidane-type antibiotics. (A) The key biosynthetic precursor (3*S*)-2,3-oxidosqualene; (B) the fusidane skeleton related to 29-*nor* protostane structure; (C) structural classification based on representative compounds helvolic acid (HA, 1), cephalosporin P1 (CP, 2), and fusidic acid (FA, 3) and their key structural characteristics (in red rectangles)

translocating A site to P site on the ribosome and finally terminate elongation [24]. Therefore, fusidane-type antibiotics have little cross-resistance with other commonly used antibiotics [25].

The intriguing structures, remarkable antibacterial activity, and unique mode of action of fusidane-type antibiotics have aroused new interests of chemists and biologists in the increasing threat of antibiotic resistance. Natural occurrence, combinational biosynthesis, and chemical modifications of fusidane-type antibiotics and their biological activities were subsequently reported in recent years, facilitating the development of this "old antibiotic class". A number of research articles from June 1943 to April 2021 were collected in this review, which were further summarized to provide an overview of the chemical and biological properties of fusidane-type antibiotics, including their isolation, recent combinatorial biosynthesis progress, and structural modifications. The informative structure-activity relationship (SAR) was also highlighted at the end of this review.

Structural Classification

Fusidane-type antibiotics are fungal 29-*nor* protostane triterpenoids that originate from (3*S*)-2,3-oxidosqualene (Fig. 1) [26]. Their skeletons containing a special chair-boat-chair ABC-ring system, are typically featured by the presence of an acetoxy group at C-16, a *Z*-configured double bond between C-17 and C-20, a carboxylic acid at C-20, and a ketone at C-3 (Fig. 1) [17]. Furthermore, oxidation, substitution, dehydrogenation, or even cyclization greatly contributes to their structural diversity [17]. Helvolic acid (HA, **1**), cephalosporin P_1 (CP, **2**), and fusidic acid (FA, **3**) are the representative fusidane-type antibiotics and well-known as potent antibacterial agents against gram-positive bacteria, especially *Staphylococci* [27]. In this review, those secondary metabolites are mainly classified according to the structural characteristics of compounds **1**–**3** (Fig. 1). Specifically, they are broadly divided into three groups: (i) HA and its derivatives with a key carbonyl group at C-3 and oxidation at C-6 and C-7; (ii) CP and its derivatives with a key hydroxyl group at C-3 and oxidation at C-6 and C-7; and (iii) FA and its derivatives with a key hydroxyl group at C-3 and an important oxidation at C-11.

Naturally Occurring Fusidane-type Antibiotics

Many natural product chemists work on the isolation and identification of fusidane-type antibiotics to discover more biologically active compounds. Until now, many HA (**1**), CP (**2**), and FA (**3**) derivatives with diverse chemical structures have been reported from diverse fungi (Table 1). Some of them displayed comparable or even more potent activities than compound **1**, **2**, or **3** (Table 1).

HA and its derivatives

HA (**1**), the first fusidane-type antibiotic, was isolated in 1943 from the fungus *Aspergillus fumigatus* [28]. The correct structure of HA had not been determined until 1970 [59-63]. Ratnaweera *et al*. isolated HA from an endophytic fungus *Xylaria* sp., and reported its antibacterial activities against gram-positive bacteria *Bacillus subtilis* with an MIC of 2.0 $\mu g \cdot mL^{-1}$ and methicillin-resistant *Staphylococcus aureus* (MRSA) with an MIC of 4.0 $\mu g \cdot mL^{-1}$ [33]. Zhou [31] and Gao [32] groups

Table 1 Fungi-derived and bio-transformed fusidane-type antibiotics and their activities

Compound	Source	Reported activities	Reference
1	*Aspergillus fumigatus* *Aspergillus sydowi* *Melia azedarach* *Metarhizium anisopliae* *Pichia guilliermondii* *Xylaria* sp.	1. MIC = 2.0 μg·mL^{-1} (*S. aureus* 209P) 2. MIC = 4.0 μg·mL^{-1} (MRSA) 3. MIC = 2.0 μg·mL^{-1} (*B. subtilis*) 4. MIC = 49.94 μg·mL^{-1} (*E. coli*) 5. MIC = 6.24 μg·mL^{-1} (*M. lysoleikticus*) 6. MIC = 8.0 μg·mL^{-1} (*S. agalactiae*) 7. MIC = 10.0 μg·mL^{-1} (fungi) 8. IC_{50} = 3.69 μg·mL^{-1} (NF-κB)	[28-36]
2	*Cephalosporium* sp. *Cladosporium* sp. *Hapsidospora irregularis*	1. MIC = 0.06 μg·mL^{-1} (*S. aureus* 209P) 2. MIC = 8.0 μg·mL^{-1} (MRSA) 3. Chlorosis-inducing activities	[27,37-40]
3	*Fusidium coccineum* *Mucor ramannianus* *Cephalosporium lamellaecula* *Paecilomyces fusidioides* *Epidermophyton floccosum* *Acremonium pilosum*	1. MIC = 0.004 μg·mL^{-1} (*S. aureus* 209P)	[41-45]
4	*Metarhizium anisopliae*	1. MIC = 16 μg·mL^{-1} (*S. aureus* 209P) 2. IC_{50} = 1.54 μg·mL^{-1} (NF-κB)	[29, 35-36]
5	*Aspergillus sydowi*	1. MIC = 6.24 μg·mL^{-1} (*E. coli*) 2. MIC = 3.12 μg·mL^{-1} (*B. subtilis*) 3. MIC = 6.24 μg·mL^{-1} (*M. lysoleikticus*)	[30]
6−**12**	*Aspergillus fumigatus*	1. MIC = 16 μg·mL^{-1} for **6** (*S. agalactiae* and *S. aureus*) 2. MIC ≥ 2 μg·mL^{-1} for **7** (*S. agalactiae* and *S. aureus*) 3. MIC ≥ 64 μg·mL^{-1} for others (*S. agalactiae*)	[34]
13	*Aspergillus* sp.	No observed antibacterial activity	[46]
14−**16**	*Aspergillus terreus*	1. MIC = 6.25 μg·mL^{-1} for **14** (*S. aureus*) 2. MIC = 6.25 μg·mL^{-1} for **15** (*S. aureus*)	[35]
17−**19**	*Cladosporium* sp.	Chlorosis-inducing activity	[39, 47-48]
20−**23**	*Hapsidospora irregularis* Biotransformation by *Microbacterium oxydans*	1. MIC = 2 μg·mL^{-1} for **20** (*S. aureus* 209P) 2. MIC = 16 μg·mL^{-1} for **21** (*S. aureus* 209P) 3. MIC = 2 μg·mL^{-1} for **22** (*S. aureus* 209P) 4. MIC = 32 μg·mL^{-1} for **23** (*S. aureus* 209P) 5. MIC = 4.0 μg·mL^{-1} for **22** (*S. aureus*) 6. MIC = 8.0 μg·mL^{-1} for **22** (MRSA)	[27, 40]
24−**31**	*Fusidium coccineum*	1. MIC = 0.25 μg·mL^{-1} for **24** (*S. aureus* 209P) 2. MIC = 8 μg·mL^{-1} for **26** (*S. aureus* 209P)	[45, 49]
32	*Epidermophyton floccosum*	Not reported	[43]
33	*Acremonium crotocinigenum*	MIC = 16 μg·mL^{-1} (MRSA)	[50]
34	*Acremonium pilosum*	No antibacterial activity	[51]
35−**39**	Biotransformation by *Cunninghamella echinulata*	1. MIC = 2.5 μg·mL^{-1} for **35** (*S. aureus*) 2. MIC = 2.5 μg·mL^{-1} for **37** (*S. aureus*) 3. MIC ≥ 100 μg·mL^{-1} for **38** (*S. aureus*) 4. MIC = 1000 μg·mL^{-1} for **39** (MRSA)	[52-53]
40, **41**	Biotransformation by *Cunninghamella elegans*	1. MIC = 2.5 μg·mL^{-1} for **40** (*S. aureus*) 2. MIC = 2.5 μg·mL^{-1} for **41** (*S. aureus*)	[54]
42−**44**	Biotransformation by *Acrocylindrium oryzae*	Not reported	[55]
45	In man	No antibacterial activity	[56]
46	Biotransformation by *Streptomyces lividans*	MIC = 5.0 μg·mL^{-1} (*Micrococcus luteus* IFM 2066) MIC = 2.5 μg·mL^{-1} (*B. subtilis* PCI 189)	[57-58]
47	Biotransformation by *Nocardia brasiliensis*	MIC = 660 μg·mL^{-1} (*M. luteus* IFM 2066) MIC ≥ 660 μg·mL^{-1} (*B. subtilis* PCI 189)	[58]

obtained HA from two fungal endophytes *Pichia guilliermondii* and *Melia azedarach*. The antifungal bioassay revealed that HA strongly inhibited seven phytopathogenic fungi with MIC values around 10.0 μg·mL^{-1}, which were comparable to the positive control carbendazim.

Chemical investigation on an entomopathogenic fungus *Metarhizium anisopliae* HF293 led to the isolation of HA and its new derivative, 1,2-dihydrohelvolic acid (**4**) (Fig. 2). Both compounds showed inhibitory activity against *S. aureus* [29]. A new HA derivative, 6*β*,16*β*-diacetoxy-25-hydroxy-3,7-dioxy-29-nordammara-1,17(20)-dien-21-oic acid (**5**), along with HA was acquired from a marine-derived fungus *Aspergillus sydowi* [30]. New analog **5** exhibited antimicrobial activity against *Escherichia coli*, *B. subtilis*, and *Micrococcus lysoleikticus* (MIC = 10.65, 5.33, and 10.65 μmol·L^{-1}, respectively), which was more potent than HA (MIC = 87.92, 21.98, and 10.99 μmol·L^{-1}, respectively).

Seven new HA derivatives (**6**−**12**) (Fig. 2) were identified from a marine-derived fungus *Aspergillus fumigatus* by Kong *et al*. in 2018 [34]. Among them, 16-*O*-propionyl-16-*O*-deacetylhelvolic acid (**6**), 6-*O*-propionyl-6-*O*-deacetylhelvolic acid (**7**), as well as HA showed stronger antibacterial activity than a positive control tobramycin against a hazardous pathogen *Streptococcus agalactiae*. A new HA analog 6*β*,16*β*-diacetoxy-25-hydroxy-3,7-dioxo-29-nordammara-1,17(20)-dien-21,24-lactone (**13**) was also discovered from a marine *Aspergillus* species, without antibacterial effect [46].

Recently, four HA analogs maunakeanolic acids A and B (**14** and **15**) (Fig. 2), 6-deacetyl-1,2-dihydrohelvolic acid (**16**), and 1,2-dihydrohelvolic acid (**4**), along with HA, were isolated from a soil-derived *A. terreus* [35]. New compounds **14** and **15** were more potent against *S. aureus* (MICs of 6.25 and 6.25 μg·mL^{-1}) than **16** and HA. In addition, HA and **4** showed significant inhibitory activity against NF-*κ*B with IC_{50} values of 2.7 and 6.5 μmol·L^{-1}, respectively [35].

CP and its derivatives

CP (**2**) was first isolated in 1951 from a species of *Cephalosporium* [37-38]. Its name is a little confusing because its 29-nor protostane triterpenoid structure is not related to the well-known *β*-lactam antibiotics cephalosporins. Its correct structure was finally established in 1967 [60, 64].

In 1972, Kaise and Munakata reported the isolation, identification, and chlorosis-inducing activity of CP and three new derivatives viridominic acids A-C (**17**−**19**) from *Cladosporium* sp. 501-7Y (Fig. 3) [39, 47-48]. Compounds **17** and **18** showed ten-fold higher chlorosis-inducing activity against higher plants than CP, and around one hundred-fold higher than compound **19** [47]. CP and isocephalosporin P_1 (**20**) (Fig. 3) were produced by the fungus *Hapsidospora irregularis* FERM BP-2511 [40]. Further biotransformation of CP and compound **20** by *Microbacterium oxydans* CGMCC 1788 led to the discovery of three new derivatives 3-keto-isocephalosporin P_1 (**21**), 3-keto-cephalosporin P_1 (**22**), and 6-deacetyl-3-ketocephalosporin P_1 (**23**) (Fig. 2). Compound **22** showed comparable or even higher antibacterial activity against *S. aureus* with a MIC value of 4.0 μg·mL^{-1} than the parent natural products [40]. CP and **22** also exhibited antibacterial activity against MRSA with the same MIC value of 8.0

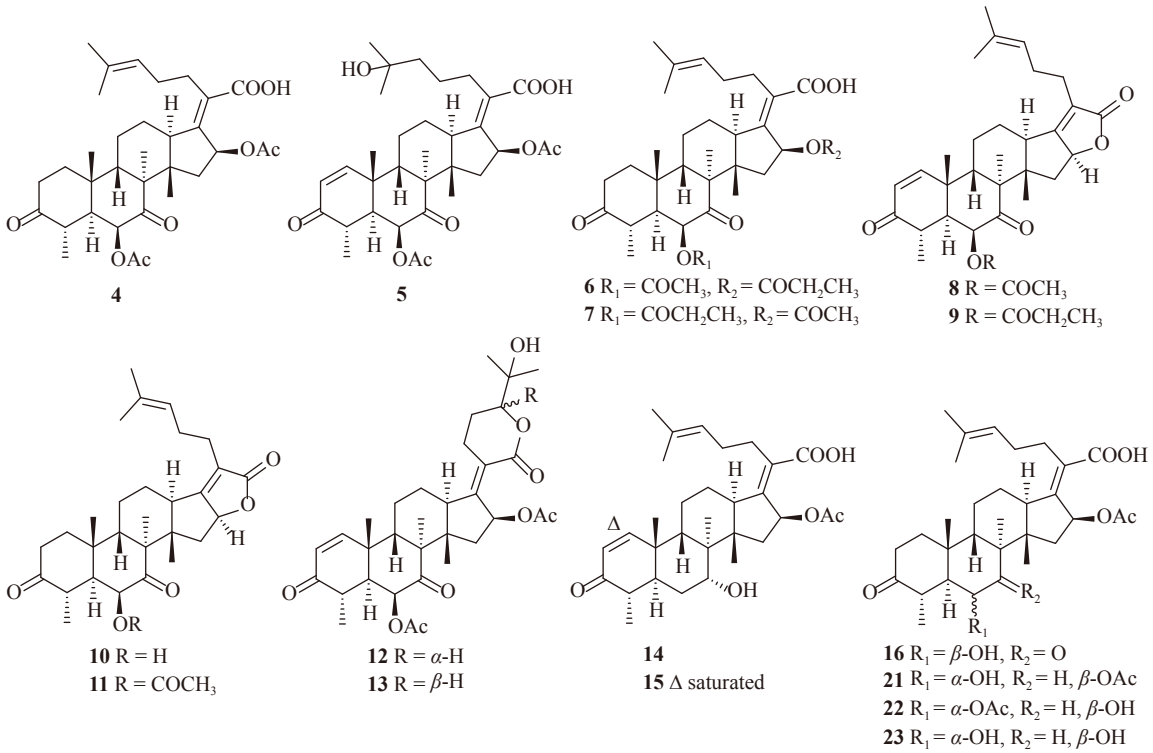


Fig. 2 HA and its derivatives

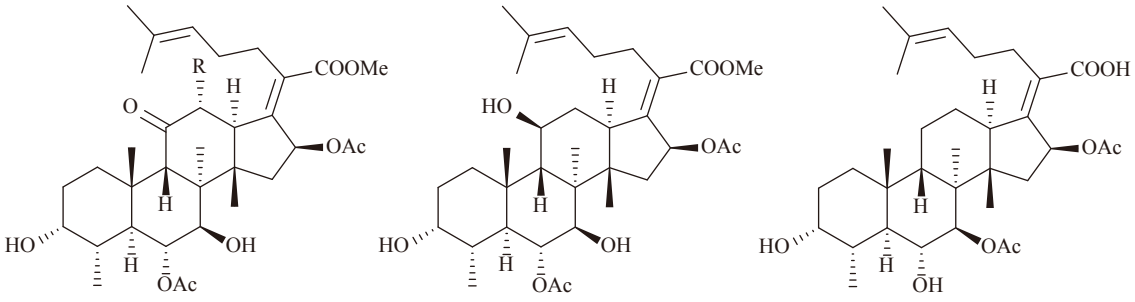


Fig. 3 CP and its derivatives

μg·mL^{-1}.

FA and its derivatives

FA (**3**) was characterized by Godtfredsen *et al*. from *Fusidium coccineum* in 1962 later than HA and CP [41]. This molecule was reported to be produced by *Mucor ramannianus*, *Cephalosporium lamellaecula*, *Paecilomyces fusidioides*, and *Epidermophyton floccosum* [42-43]. The planar structure and stereochemistry of FA were assigned in 1965 by Godtfredsen *et al*. [65], and then confirmed by X-ray diffraction by Cooper group in 1966 [66] and 1968 [67]. Rastrup-Andersen and Duvold reassigned the ^{1}H and ^{13}C NMR data of FA, and several derivatives [68]. Since it started to be used for clinical treatment in 1962, FA has been extensively used to deal with *Staphylococcal* infections in human [44].

Further chemical investigations on *F. coccineum* provided some derivatives of FA (Fig. 4) [49]: 3-ketofusidic acid (**24**), 11-ketofusidic acid (**25**), 3-epifusidic acid (3*β*-hydroxylfusidic acid, **26**), 11-epifusidic acid (**27**), 9,11-anhydrofusidic acid (**28**), 9,11-anhydro-9*α*,11*α*-epoxyfusidic acid (**29**), 7,8-dehydropseudofusidic acid (**30**), and 9,11-anhydro-12-hydroxy-fusidic acid (**31**). From *Epidermophyton floccosum*, 3,11-diketofusidic acid (**32**) was isolated and identified [43]. A new FA analog, 16-deacetoxy-7-*β*-hydroxy-fusidic acid (**33**) with a rare hydroxyl group at C-7 and without substitution at C-16, was isolated from a mitosporic fungus *Acremonium crotocinigenum* [50]. It exhibited inhibitory activity against MRSA with an MIC value of 16 μg·mL^{-1}. Recently, our research group reported the chemical investigation on an endophytic fungus, *Acremonium pilosum* F47 [51, 69]. FA and a new derivative, acremonidiol A (**34**) (Fig. 4), were isolated and characterized. Only FA showed strong inhibitory effect on gram-positive bacteria *S. aureus* and *B. subtilis*, suggesting the importance of motifs at C-11, C-16, and C-21.

In order to obtain more FA derivatives, the biotransformation approach was frequently utilized, which provided various chemical modifications on original molecules, such as hydroxylation. Biotransformation of FA by *Cunninghamella echinulata* NRRL 1382 converted FA into four major secondary metabolites 27-hydroxyfusidic acid (**35**), 26-hydroxyfusidic acid (**36**), 26-formylfusidic acid (**37**), and 26-carboxyfusidic acid (**38**) (Fig. 5) [52], and a minor metabolite 3-*O*-formyl-27-hydroxyfusidic acid (**39**) [53]. Compared with FA, the obtained products showed significantly weaker antimicrobial activity against different gram-positive and gram-negative bacteria. These observations indicated that the methyl groups at C-26 and C-27 in the side chain of FA are crucial for antimicrobial activity [52-53]. Another *C. elegans* NRRL 1392 mediated the hydroxylation of ring B to yield 7-*β*-hy-

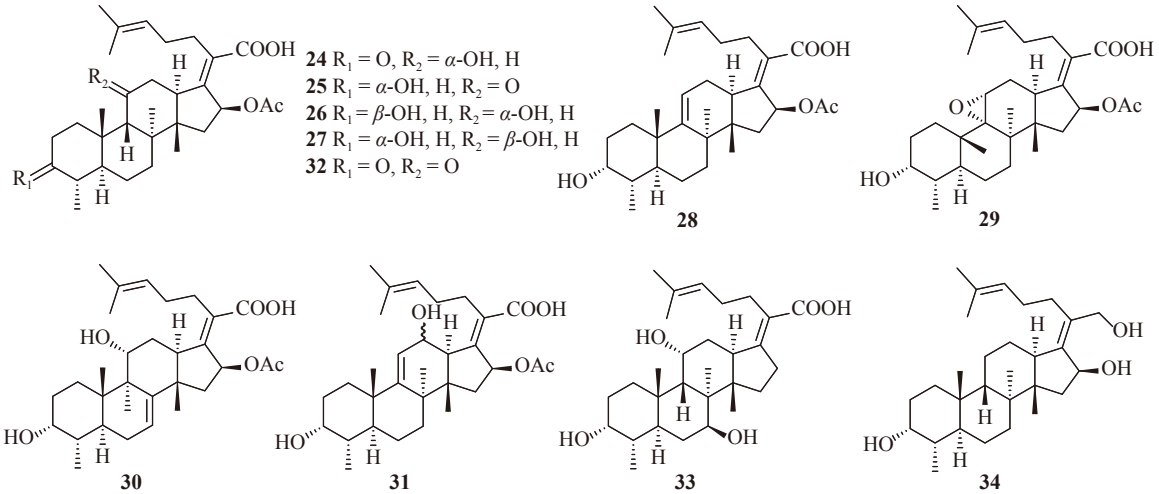


Fig. 4 FA and its derivatives

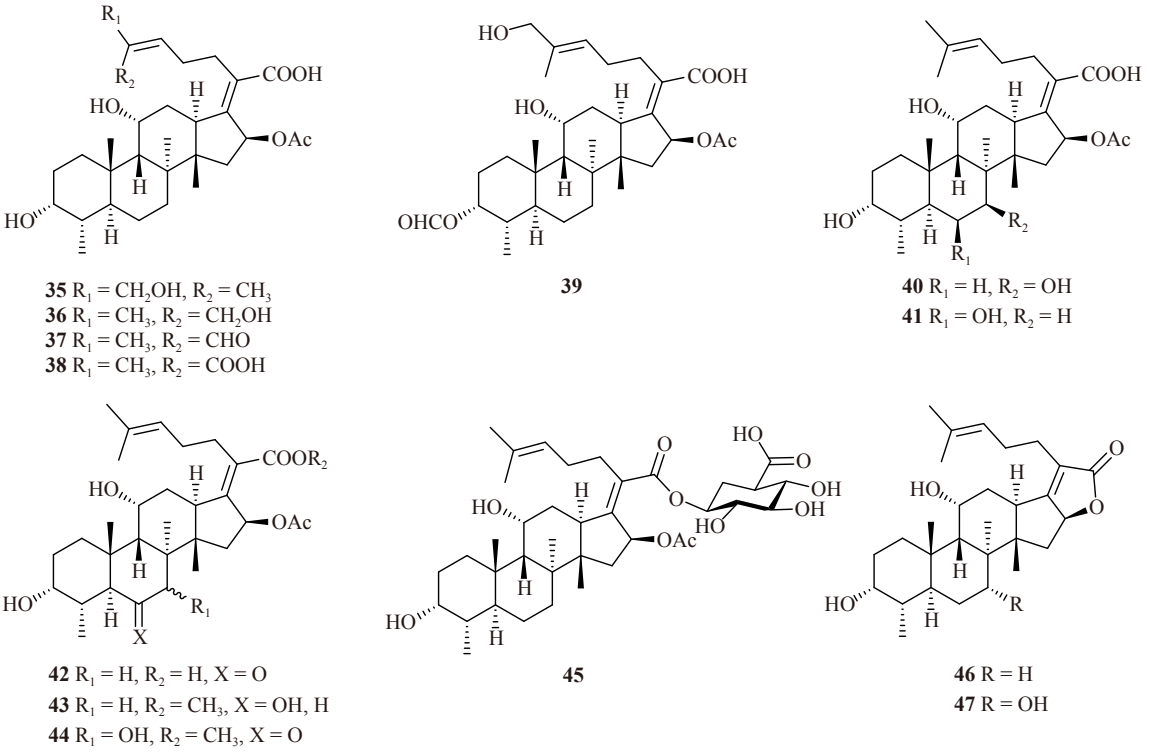


Fig. 5 FA derivatives from the biotransformation of FA

droxyfusidic acid (**40**) and 6-*β*-hydroxyfusidic acid (**41**) [54]. Similar to that of **35** and **37**, compounds **40** and **41** exhibited lower efficiency in antibacterial activity than FA, suggesting that the hydroxylation of ring B diminished the biological activity [54].

Greenspan and Alburn discovered that *Corynebacterium simplex* mediated the oxidation of C-3 of FA and identified a crystalline product 3-ketofusidic acid (**24**) [70]. Similarly, *Acrocylindrium oryzae* induced the oxidation of C-6 and/or C-7, affording 6-oxofusidic acid (**42**), 6-hydroxy-fusidic acid methyl ester (**43**), and 6-oxo-7-hydroxyl-fusidic acid methyl ester (**44**) (Fig. 5) [55]. FA was also observed to be metabolized into inactive dicarboxylic metabolite (**38**) [56], glucuronide derivative (**45**) [56], and 3-keto product (**24**) in human [71].

Antibiotic-resistant bacteria can inactivate FA by modifying its structure [72]. For example, Von der Haar and Schrempf isolated an extracellular enzyme from a wild-type strain *S. lividans* 66 [57]. This enzyme was found to inactivate FA through removing the acetyl group at C-16 and forming the lactone between C-16 and C-21 (**46**) (Fig. 5) [57]. Harada *et al*. described the inactivation of FA by *Nocardia brasiliensis*. Inactive FA lactone (**46**) and 7*α*-hydroxylatedfusidic acid lactone (**47**) were successfully isolated and identified [58].

Combinational Biosynthesis of Fusidane-Type Antibiotics

The development of microbial genomics, bioinformatics and analytical techniques helps understand natural product biosynthesis, which inspire us to rationally manipulate natural biosynthetic machinery to increase chemical diversity [73-74]. The biosynthetic studies of fusidane-type antibiotics flourished after the characterization of HA biosynthetic gene cluster (BGC) from *A. fumigatus* Af293 in 2009 [75-76]. So far, three biosynthetic pathways of representative fusidane-type antibiotics, HA (**1**) [36], CP (**2**) [27], and FA (**3**) [45] have been investigated and proposed.

Six conserved genes for yielding fusidane skeleton

The genomes of fusidane producing fungi were sequenced, which indicated the presence of biosynthetic gene clusters (BGCs) responsible for fusidane-type antibiotics (Table 2) [77]. Their BGCs had six conserved enzymes including an oxidosqualene cyclase (OSC), three cytochrome P450 enzymes (P450), a short-chain dehydrogenase/reductase (SDR), and an acyltransferase (AT) [77]. The core enzymes catalyzed an early stage of biosynthetic pathway which transformed the (3*S*)-2,3-oxidosqualene to fusidane skeleton (Table 2 and Fig. 6) [36]. OSC catalyzed the cyclization of (3*S*)-2,3-oxidosqualene to establish a common tetracyclic intermediate (17*Z*)-protosta-17(20),24-dien-3*β*-ol (protostadienol, **48**) (Table 2 and Fig. 6) [75-76]. Then, P450-1 oxidized the methyl at C-4*β* to carboxylic acid (**49**). P450-2 mediated the hydroxylation at C-16 (**50**). The acetylation of C-16*β* hydroxyl group (**51**) was further triggered by AT-1. P450-3 was responsible for converting methyl group at C-21 to carboxylic acid (**52**). The C-4*β* methyl group of intermediate **52** was finally removed by SDR-1 through a unique decarboxylation mechanism, constructing the fusidane skeleton (**53**) (Table 2 and Fig. 6) [78].

Table 2 The genes in BGCs responsible for the biosynthesis of fusidane-type antibiotics

Putative functions	Genes in BGCs		
	HA (1) (*A. fumigatus* Af293)	CP (2) (*A. chrysogenum* ATCC 11550)	FA (3) (*A. fusidioides* ATCC 14700)
Oxidosualene cyclase (OSC)	*helA*	*cepA*	*fusA*
Cytochrome P450 (P450-1)	*helB1*	*cepB1*	*fusB4*
Cytochrome P450 (P450-2)	*helB2*	*cepB3*	*fusB2*
Cytochrome P450 (P450-3)	*helB4*	*cepB2*	*fusB3*
Cytochrome P450 (P450-4)	*helB3*	*cepB4*	*fusB1*
Short-chain dehydrogenase/reductase (SDR-1)	*helC*	*cepC1*	*fusC2*
Short-chain dehydrogenase/reductase (SDR-2)	-	*cepC2*	*fusC1*
Acyltransferase (AT-1)	*helD2*	*cepD1*	*fusD*
Acyltransferase (AT-2)	*helD1*	*cepD2*	-
3-Ketosteroid-Δ^1-dedydrogenase (KSTD)	*helE*	-	-

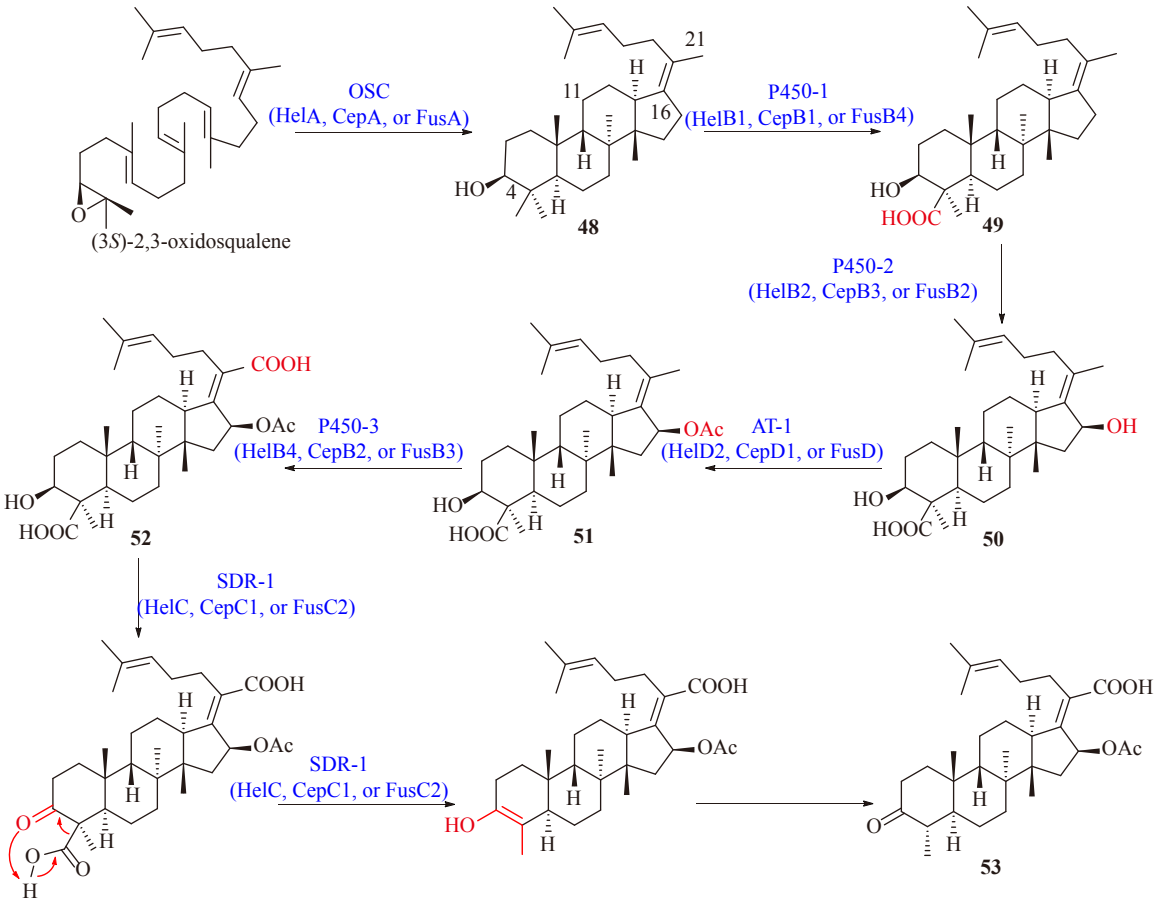


Fig. 6 Six conserved enzymes for yielding fusidane skeleton (53)

Further tailoring genes for constructing HA, CP, and FA

Further tailoring steps increase the diversity of fusidane-type structures. For HA (Fig. 7), P450 enzyme HelB3-mediated dual oxidation at C-6 and C-7 (**54**), AT HelD1-dependent acetylation of 6-OH (**4**), and dedydrogenase HelE catalyzed dehydrogenation between C-1 and C-2 and provided the final product HA (**1**) [36-76]. During the biosynthesis of FA (Fig. 7), the carbonyl group at C-3 was stereoselectively reduced to 3α-OH (**55**) by SDR enzyme FusC1, and the hydroxylation at C-11 was achieved by P450 monooxygenase FusB1 [45]. It has to be noted that *helD1* and *helE* exhibited broad substrate specificities. This is also true for *fusB1* and

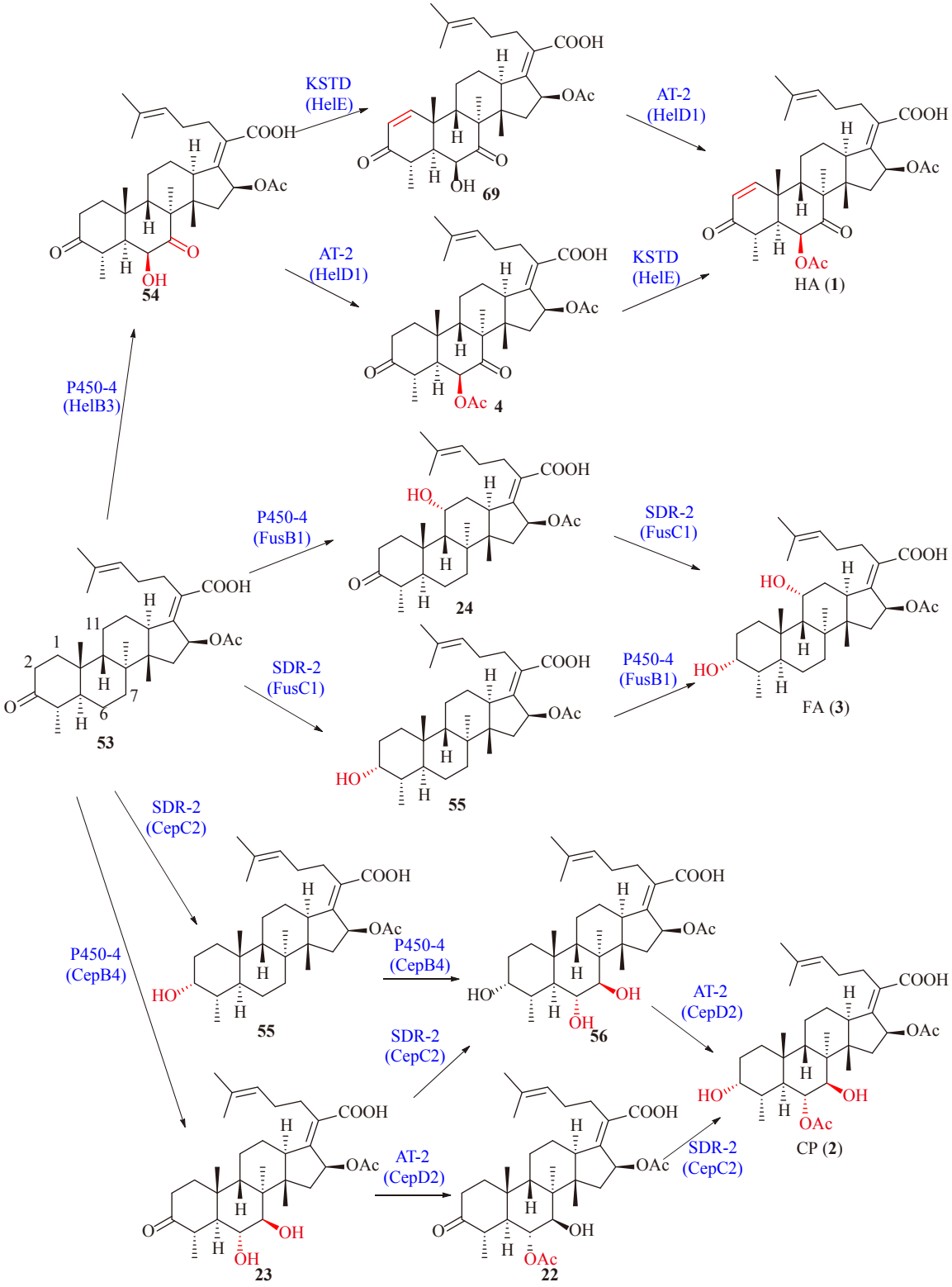


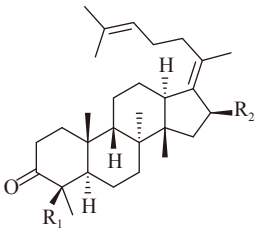
Fig. 7 The biosynthetic pathways of HA (1), CP (2), and FA (3)

fusC1 which worked independently without a strict reaction order. In the biosynthesis of CP (Fig. 7), additional three genes in a separate locus encoding P450 enzyme (CepB4), an AT protein (CepD2), and a SDR enzyme (CepC2), were required [27]. CepB4 worked on stereoselective dual oxidation of C-6 and C-7 (**23** or **56**), and CepD2 specifically catalyzed the acetylation of 6-OH (**22**). CepC2 showed high sequence identity to FusC1 and stereoselectively reduced 3-keto to 3α-OH [27].

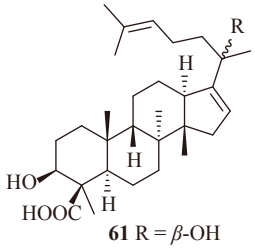
Combinational biosynthesis to expand the structural diversity

During stepwise reconstitution of HA biosynthesis in the

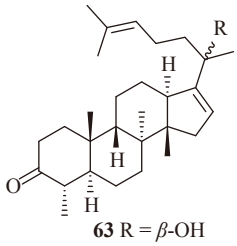
heterologous expression system *A. oryzae* NSAR1, 21 HA derivatives (**4**, **48**−**54**, **57**−**69**) were isolated by Yao group (Figs. 6−8) [36]. Three of them (**54**, **66**, and **69**) exhibited more potent inhibitory activity against *S. aureus* (0.5−1.0 μg·mL^{-1}) than orginal HA (**1**) (2.0 μg·mL^{-1}) (Tables 1 and 3). This work showed the potential of combinatorial biosynthesis to generate HA analogs with much better bioactivities. The investigation on combinatorial biosynthesis of CP provided 11 analogs (**20**−**23**, **55**, **56**, and **70**−**74**) (Figs. 3, 7, and 8) [27]. Most of them exhibited antibacterial activity against gram-positive *S. aureus*, and CP showed the most potent activity with a MIC value of 0.06 μg·mL^{-1} (Tables 1 and 3). Besides, the results of bioassay gave out that the antibacterial activity of CP related molecules was mainly dependent on acetyl groups at C-6 or C-7 [27]. FA and its three analogs **24**, **26**, and **55** (Fig. 4 and 7), were obtained during the heterologous expression of FA in *A. oryzae* NSAR1 [45]. FA (**3**) displayed the best antibacterial activity against *S. aureus* 209P with a MIC value of 0.004 μg·mL^{-1} compared with the positive control tobramycin (MIC = 0.06 μg·mL^{-1}) (Tables 1 and 3) [45]. Moreover, the MIC values of compounds **24**, **26**, and **55** were 0.25, 8, and 0.25 μg·mL^{-1}, respectively. These results suggested that 3α-OH and 11α-OH played an important role on the antibiotic activity of FA. More recently, Yao and colleagues introduced all the possible combinations of post-tailoring genes from HA, FA, and CP BGCs into the strain that had six



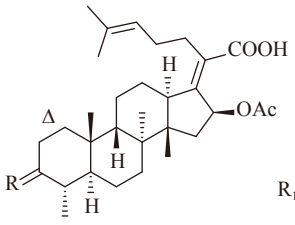
57 R_1 = CH_3, R_2 = H
58 R_1 = H, R_2 = H
59 R_1 = H, R_2 = OH
60 R_1 = H, R_2 = OAc



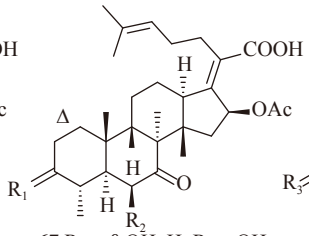
61 R = β-OH
62 R = α-OH



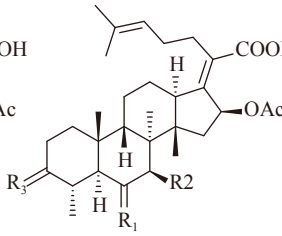
63 R = β-OH
64 R = α-OH



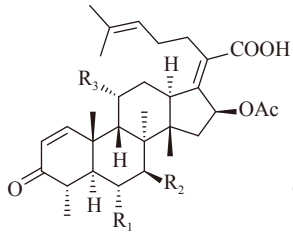
65 R = β-OH, H
66 R = O, Δ unsaturated



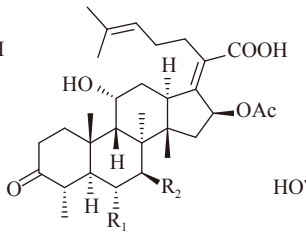
67 R_1 = β-OH, H, R_2 = OH
68 R_1 = β-OH, H, R_2 = OAc
69 R_1 = O, R_2 = OH, Δ unsaturated



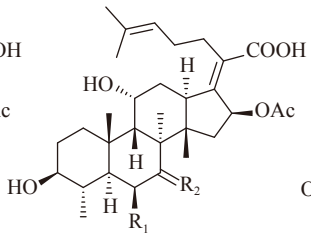
70 R_1 = O, R_2 = H, R_3 = α-OH, H
71 R_1 = α-OH, H, R_2 = H, R_3 = α-OH, H
72 R_1 = H, H, R_2 = OH, R_3 = α-OH, H
73 R_1 = α-OAC, H, R_2 = H, R_3 = O
74 R_1 = α-OAC, H, R_2 = H, R_3 = α-OH, H



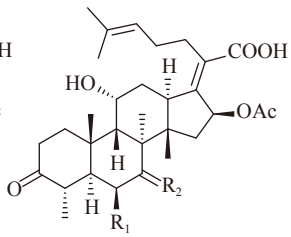
75 R_1 = H, R_2 = H, R_3 = OH
76 R_1 = OH, R_2 = OH, R_3 = H
77 R_1 = OH, R_2 = H, R_3 = H
78 R_1 = H, R_2 = OH, R_3 = H



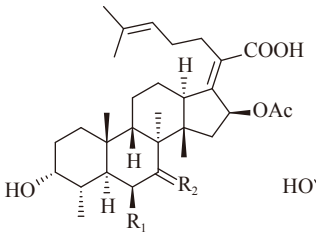
79 R_1 = OH, R_2 = OH
80 R_1 = OH, R_2 = H
81 R_1 = H, R_2 = OH



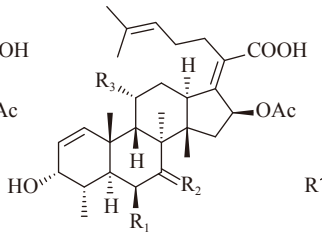
82 R_1 = OH, R_2 = O
83 R_1 = H, R_2 = α-OH, H
84 R_1 = H, R_2 = O



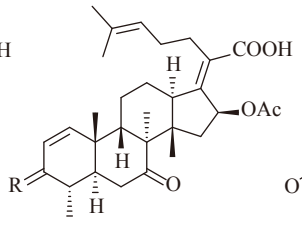
85 R_1 = OH, R_2 = O
86 R_1 = H, R_2 = α-OH, H
87 R_1 = H, R_2 = O



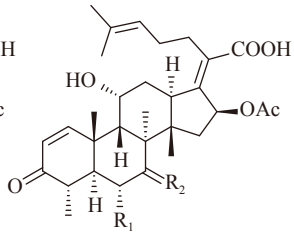
88 R_1 = OH, R_2 = O
89 R_1 = H, R_2 = α-OH, H
90 R_1 = H, R_2 = O



91 R_1 = H, R_2 = H, H, R_3 = OH
92 R_1 = OH, R_2 = O, R_3 = H
93 R_1 = H, R_2 = α-OH, H, R_3 = H



94 R = O
95 R = α-OH, H



96 R_1 = OH, R_2 = H, H
97 R_1 = H, R_2 = α-OH, H
98 R_1 = H, R_2 = O

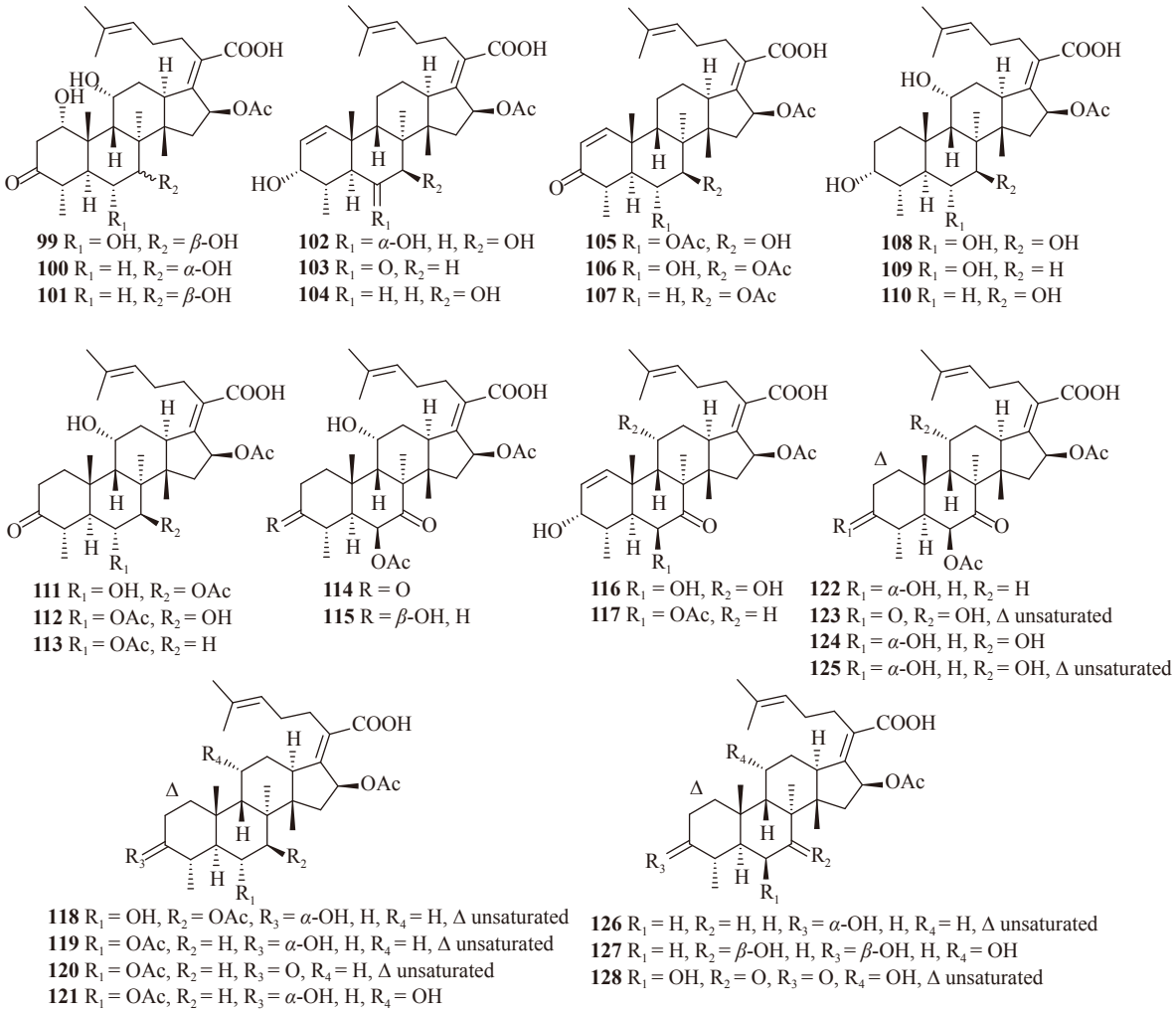


Fig. 8 Fusidane-type antibiotics from combinational biosynthesis

conserved genes [79]. This stochastic combinational strategy provided 24 combinations that produced 58 fusidane-type analogs, of which 54 were new compounds (**75**−**128**) [79]. Their antibacterial activity was evaluated as shown in Table 3, and SAR was further analyzed by the authors.

Chemical Modifications of Fusidane-Type Antibiotics

FA exhibits excellent antibiotic activity *in vitro* and *in vivo* with low degree of toxicity, and shows little cross-resistance with other clinically used antibiotics. Until now, only FA has been applied to clinical treatment. In order to explore more effective analogs, scientists try to acquire diverse fusidanes through chemical modification (Fig. 9). Hundreds of FA derivatives have been synthesized, and their antibacterial, cytotoxic, antiplasmodial, and antimycobacterial activities were evaluated *in vitro* or *in vivo*. The SAR of FA was extensively investigated.

Synthesis of the tricyclic or tetracyclic ring system

A number of synthetic strategies have been reported to construct the cyclic ring system of fusidane-type antibiotics. This ring system can be easily prepared from a bicyclictriene ether through an effective bicyclic transannular Diels-Alder reaction (Fig. 10A) [80]. An intermolecular/transannular Michael reaction cascade on a ten-membered carbocyclic ketone was also developed to stereoselectively prepare the tricyclic system (Fig. 10B) [81]. Ireland group reported the synthesis of several tetracyclic intermediates through a series of reactions on corresponding polycyclic precursors [82-83].

Dauben group first reported the total synthesis of a degradation tetracyclic product of FA (**129**) (Fig. 10C) [84]. Its tetracyclic skeleton was successfully constructed through a key Westphalen rearrangement reaction that involved an acid-catalyzed rearrangement of an angular methyl [84]. The synthetic tetracyclic structure and stereochemistry of **129** was confirmed by X-ray crystallography. Another $BF_3 \cdot Et_2O$-catalyzed rearrangement of 3-oxo-4β-demethyllanostane-9α,11α-epoxide also led to the formation of a chair-boat-chair skeleton [85]. The above tetracyclic skeleton was further utilized for the partial synthesis of FA (**3**) in 21 steps, affording a triacetate product [86]. The obtained triacetate product (Fig. 10C) was highly similar to that of FA, except for the side chain,

Table 3 Fusidane-type antibiotics from combinational biosynthesis and their anti-*Staphylococcus* activity[a]

Compd.	*S. aureus* 209P (MIC, μg·mL^{-1})	Compd.	*S. aureus* 209P (MIC, μg·mL^{-1})	Compd.	*S. aureus* 209P (MIC, μg·mL^{-1})	Compd.	*S. aureus* 209P (MIC, μg·mL^{-1})
48	> 128	**69**	0.5	90	16	**111**	16
49	> 128	**70**	32	**91**	1	**112**	8
50	32	**71**	1	**92**	128	**113**	8
51	64	**72**	8	**93**	8	**114**	128
52	64	**73**	4	**94**	32	**115**	> 128
53	2	**74**	0.5	**95**	32	**116**	128
54	1	**75**	16	**96**	> 128	**117**	128
55	0.25	**76**	32	**97**	> 128	**118**	16
56	8	**77**	128	**98**	> 128	**119**	4
57	128	**78**	16	**99**	> 128	**120**	32
58	> 128	**79**	> 128	**100**	> 128	**121**	2
59	> 128	**80**	64	**101**	> 128	**122**	64
60	> 128	**81**	32	**102**	32	**123**	128
61	16	**82**	> 128	**103**	128	**124**	64
62	32	**83**	128	**104**	16	**125**	128
63	> 128	**84**	> 128	**105**	16	**126**	4
64	> 128	**85**	32	**106**	64	**127**	> 128
65	8	**86**	8	**107**	16	**128**	128
66	1	**87**	128	**108**	64	Tobramycin	0.03
67	32	**88**	16	**109**	8	FA (**3**)	0.125
68	32	**89**	2	**110**	32	CP (**2**)	0.5

[a] Compounds **75**−**128**, tobramycin, FA (**3**), and CP (**2**) were evaluated for their antibacterial activity at the same time [79].

and served as a key intermediate for the final total synthesis of FA. Further introduction of the 6-methyl-5-heptenoic acid side chain to the C-17 ketone was achieved by Tanabe and co-workers, and several FA derivatives including the methyl ester of diacetoxyfusidic acid (**130**), were obtained (Fig. 10C) [87]. Even though these synthetic methods successfully constructed the chair-boat-chair ring system or core structure, existing reaction processes were relatively time-consuming (Fig. 10C). In addition, until now, there are no reports for the total synthesis of fusidane-type antibiotics, especially HA (**1**), CP (**2**), and FA (**3**). More convenient and environmentally friendly total synthetic approaches might be needed to obtain diverse fusidane-type antibiotics in the future.

Early SAR investigation based on diverse substitutions at C-3, C-11, C-16, and C-21

The SAR of FA has been extensively studied by Godtfredsen group [88-90]. A variety of FA analogs have been prepared and evaluated for their antibacterial activity against a number of bacteria, such as *S. aureus* CC 178B and *Corynebacterium xerosis* NCTC 9755 (Fig. 11 and Table 4). Certain modifications, such as saturation of the 24,25-double bond (**131**), replacement of the 16*β*-acetoxyl group by various other groups (**132**−**140**), and conversion of 11*α*-hydroxyl group into ketone (**25**), 11*β*-hydroxyl group (**141**), or 11*α*-acetoxyl motif (**142**), had limited effect on antibacterial activity. Other modifications at 3-OH (**143**−**146**) and 21-COOH (**147**−**150**) positions reduced the activity. It has to be noted that among these synthesized FA derivatives, only compounds **132** and **140** were more active than original FA (**3**) (Fig. 11 and Table 4).

Modifications of double bonds at C-17 and/or C-24

In order to investigate the importance of the double bond between C-17 and C-20, Duvold and co-workers synthesized four 17,20-dihydrofusidic acid derivatives (Fig. 12), 17*R*, 20*S*-tetrahydrofusidic acid (**151**), 17*R*,20*R*-tetrahydrofusidic acid (**152**), 17*S*,20*R*-dihydrofusidic acid (**153**), and 17S,20S-dihydrofusidic acid (**154**). Their antibacterial activities were investigated with the aid of molecular modeling [91]. Only compound **154** with a similar stereo conformational space as that of FA, showed the same antibiotic activity as FA while others were virtually inactive [91]. These results indicated that the Δ17(20) double bond was not crucial for the antibacterial activity of FA, while the orientation of the carboxyl

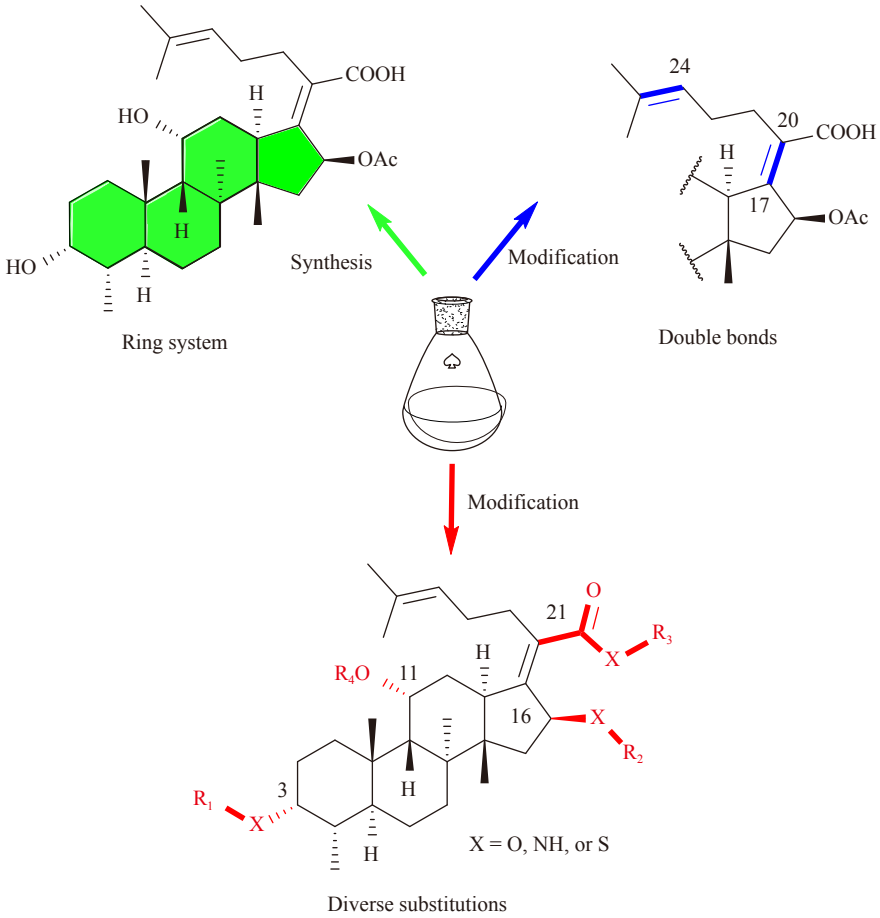


Fig. 9 The synthesis and structural modification of fusidane-type antibiotics

group and the lipophilic moiety on the side chain must be similar to that of FA. In order to reduce conformational freedom [91], Duvold and co-workers further constructed a spirocycloproane ring between C-17 and C-20 to afford two products (**155** and **156**) [92]. Only synthetic 17*S*,20*S*-methanofusidic acid (**155**) had a limited conformational orientation of side chain and carboxyl group [92]. As expected, it exhibited significant antibacterial activity which was equal to FA.

Duvold group also focused on the modification of lipophilic part of the side chain and prepared three photoaffinity labeled FA derivatives (Fig. 12), benzophenone FA (**157**), trifluoromethyldiazirine FA (**158**), and azide FA (**159**) [93]. These photoaffinity labeled compounds retained partial activity against six FA-sensitive *Staphylococcus* species with MIC values ranging from 1 to 4 μg·mL^{-1} compared with FA (MIC, around 0.063 μg·mL^{-1}), and were valuable tools to investigate the active sites interacted with FA in EF-G. Wu *et al*. prepared a hydrogenated derivative of FA, 24,25-dihydrofusidic acid (**131**) [94]. Both FA and **131** exhibited equal inhibitory activity against gram-positive bacteria, and showed strong anti-inflammatory effects *in vivo* [94]. These results suggested that the double bond between C-24 and C-25 had little effect on the improvement of FA activity.

Structural modifications at C-3 and/or C-21

Increasing the metabolic stability

The half-life of FA in blood is less than 2 hours, which dramatically reduce the drug effect of FA in clinical treatment [95]. The metabolism of FA and its C-3 or C-21 derivatives was investigated in rat liver microsomes, rat plasma, and mycobacterial cell culture [96]. FA (MIC_{99} < 0.15 μmol·L^{-1}) was metabolized to the corresponding 3-ketofusidic acid (**24**, MIC_{99} 1.25 μmol·L^{-1}) and 3-epifusidic acid (**26**, MIC_{99} 11.4 μmol·L^{-1}) with a relatively weak anti-*Mtb* activity. Meanwhile, FA was transformed into inactive FA lactone (**46**) in rat plasma. C-3 alkyl and aryl esters were hydrolyzed to FA, while C-3 silicate esters and C-21 aryl esters were stable [96]. Three stable C-3 silicate esters (Fig. 13), 3-triethoxysilyloxyfusidic acid (**160**), 3-triisopropoxysilyloxyfusidic acid (**161**), 3-trisoctyloxysilyloxyfusidic acid (**162**), showed comparable antimycobacterial activity as FA [96]. Representative C-21 aryl ester **163** with weak activity possessed longer half-life than FA. Interestingly, the FA C-3 alkyl ester prodrugs, FA butanoate(**164**)andFApentanoate(**165**),werefoundtohaveimproved bioavailability and tissue distribution compared with FA in pharmacokinetic and organ distribution experiments [97]. Based on these data, stable C-3 silicate esters and C-3 alkyl ester prodrugs may become potential drug leads for further

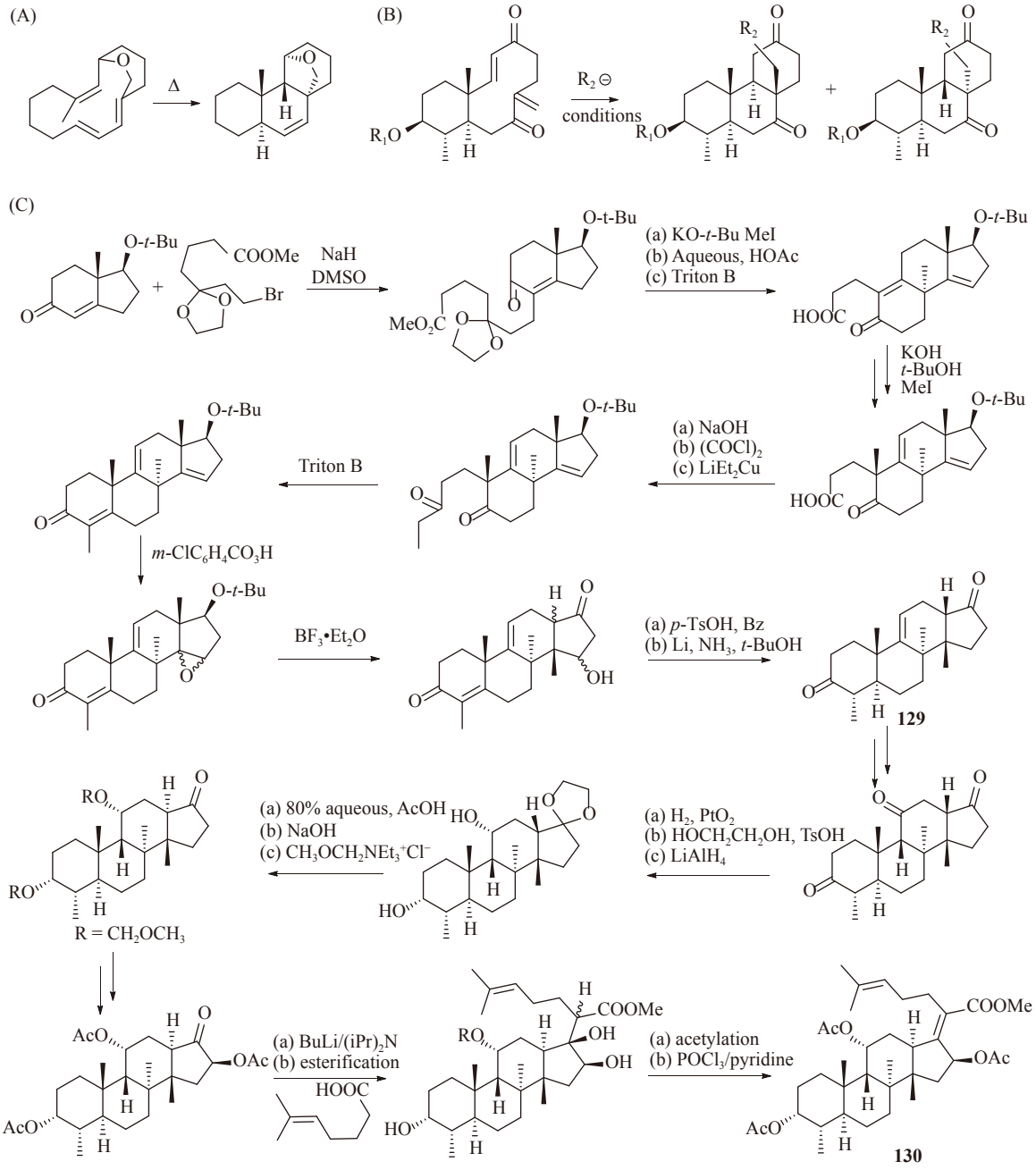


Fig. 10 Synthesis of the tricyclic or tetracyclic ring system. (A) The Diels-Alder reaction to construct the skeleton; (B) the intermolecular/transannular Michael reaction to form the cyclic system; and (C) total synthesis of a FA analog (130)

chemical and biological investigation.

In order to maintain antibacterial activity and prolong the half-life at the same time, Bi group blocked the metabolic sites of FA at C-3 and C-21 and obtained 14 related derivatives [95]. Among them, compounds **166**–**171** (Fig. 13) exhibited significant or moderate antibacterial activity against *S. aureus* and longer half-life. Particularly, two C-21 modified derivatives with the same MIC values (less than 0.25 $\mu g \cdot mL^{-1}$) as FA, 21-fusidic acid (6-chloro-benzotriazole-1) ester (**170**) and 21-fusidic acid (7-azabenzotriazole-1) ester (**171**), showed longer half-life than FA. Both of them were transformed into FA through hydrolysis *in vivo* [95]. These results indicated that blocking the metabolic sites of FA (21-COOH and/or 3-OH) can maintain all or partial antibacterial activity and prolong the half-life of FA.

Antitumor or antifungal derivatives

As C-21-fusidic acid benzyl esters exhibited improved antibacterial activity and half-life, Bi group further introduced different amino-terminal groups at C-3, and obtained a series of novel FA derivatives [98]. 3*β*-(4-Aminopropionyloxy)-21-fusidic acid (benzyl) ester (**172**) (Fig. 14) with a 4-aminopropionyloxy group at 3-OH showed cytotoxic activity

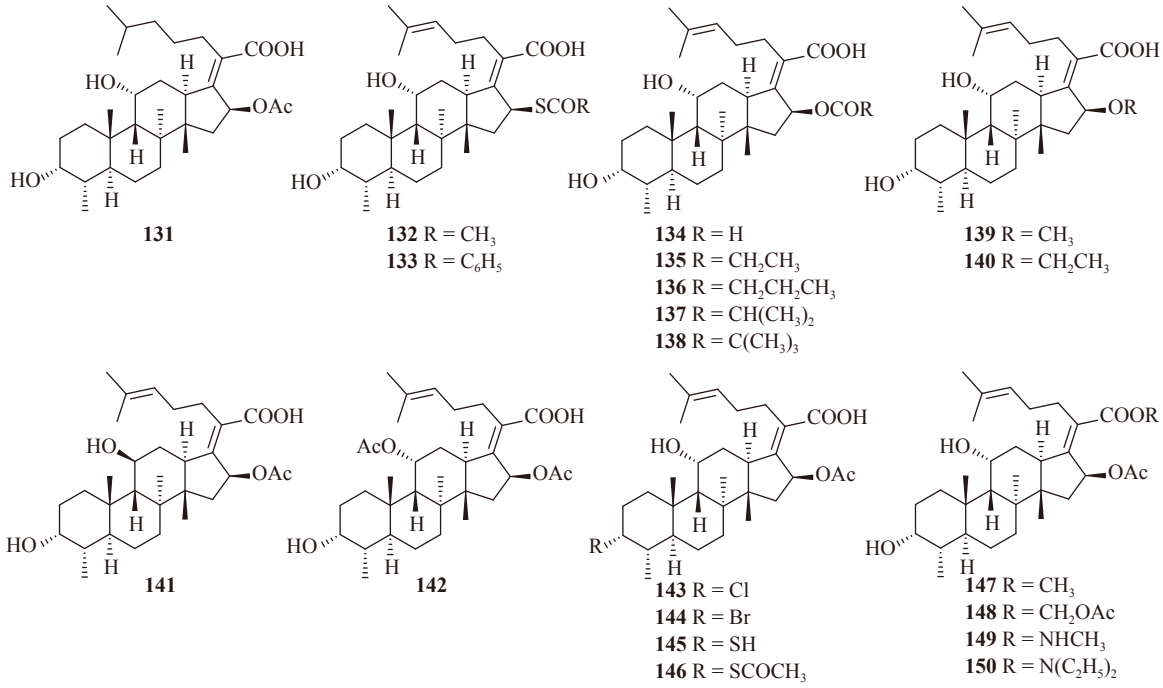


Fig. 11 The representative FA derivatives obtained by Godtfredsen group for SAR studies

Table 4 Antibacterial activities of the representative FA derivatives investigated by Godtfredsen group

No.	IC_{50} (μg·mL^{-1})		No.	IC_{50} (μg·mL^{-1})	
	S. aureus CC 178B	*C. xerosis* NCTC 9755		*S. aureus* CC 178B	*C. xerosis* NCTC 9755
1	1.5	0.047	**139**	1.6	0.16
2	0.19	0.068	**140**	0.032	0.005
3	0.058	0.008	**141**	25.0	4.0
25	0.05	0.004	**142**	6.3	0.13
131	0.071	0.009	**143**	0.79	0.01
132	0.013	0.002	**144**	2.0	0.05
133	0.79	0.04	**145**	6.3	0.1
134	1.6	0.079	**146**	13	1.3
135	0.063	0.006	**147**	250	50
136	1.6	0.1	**148**	5.0	1.6
137	1.6	0.05	**149**	>300	40
138	1.6	0.063	**150**	400	50

against a panel of cancer cell lines with IC_{50} values ranging from 1.25 to 3.57 μmol·L^{-1} *in vitro*, and exhibited anti-tumor activity against a xenograft tumor of Hela cells *in vivo* [98]. Further synthesis and antifungal evaluation of FA derivatives possessing a C-3 amino-terminal group and a C-21 benzyl moiety were also carried out by Bi group [99]. New FA derivatives had antifungal activity against *Cryptococcus neoformans*, which is a HIV-related opportunistic fungal pathogen. 3*β*-Lysine-21-fusidic acid (benzyl) ester (**173**) (Fig. 14) showed the strongest antifungal activity with a MIC value of 4.0 μg·mL^{-1} [99], while FA did not inhibit the growth of *C. neoformans* at a concentration of 32 μg·mL^{-1}. The benzyl substituent at 21-COOH and amino acyl substitution at 3-OH made the FA derivatives become cytotoxic and antifungal molecules. The data also highlighted the importance of medium-length amino-terminal groups at C-3.

Eighteen 3-amino substituted FA derivatives, containing linear, aromatic, heterocyclic, and polyamine substituents, were synthesized by Salimova and co-workers [100]. Derivatives substituted at C-3 by pyrrolidine (**174**), *n*-butylamine (**175**), and benzylamine (**176**) (Fig. 14) showed selective inhibitory effect against leukemia cell lines at 10 μmol·L^{-1}, highlighting the cytotoxic potential of 3-amino derivatives of FA [100].

Antiplasmodial derivatives

FA exhibited inhibitory activity against chloroquine-sensitive *Plasmodium falciparum* NF54 with an IC_{50} value of 59 μmol·L^{-1} and multidrug-resistant *P. falciparum* K1 with an IC_{50} value of 19 μmol·L^{-1} [101-103]. Chibale group synthesized 19 FA derivatives by substituting the 21-COOH group with three types of bioisosteres, obtaining compounds **177**, **178**, and **179** (Fig. 15) [102]. Most of them possessed antiplasmodial effect on chloroquine-sensitive *P. falciparum* NF54 with a 2-35 fold increase in activity as compared to FA (Table 5). Compound **179**, a 3-substituted-1,2,4-oxadiazole derivative, had a similar EF-G binding orientation as FA, and was the most active one with an IC_{50} value of 1.7 μmol·L^{-1} [102]. Further replacement of the carboxylic acid group at C-21 with various ester and amide moieties provided 25 FA derivatives [103]. Most of them exhibited stronger antiplasmodial activity against chloroquine-sensitive *P. falcipar-*

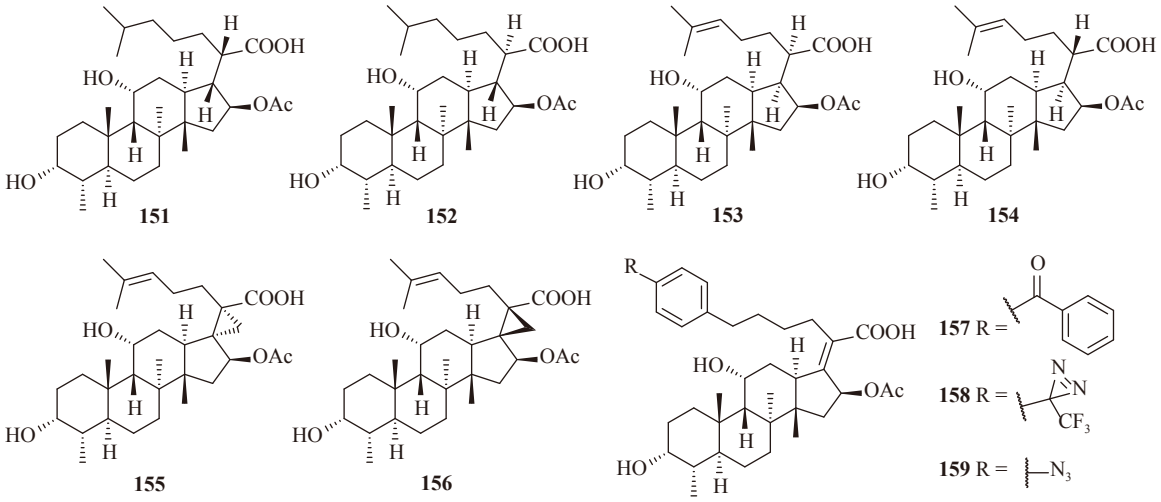


Fig. 12 FA derivatives from the structural modification of double bonds between C-17 and C-20 or between C-24 and C-25

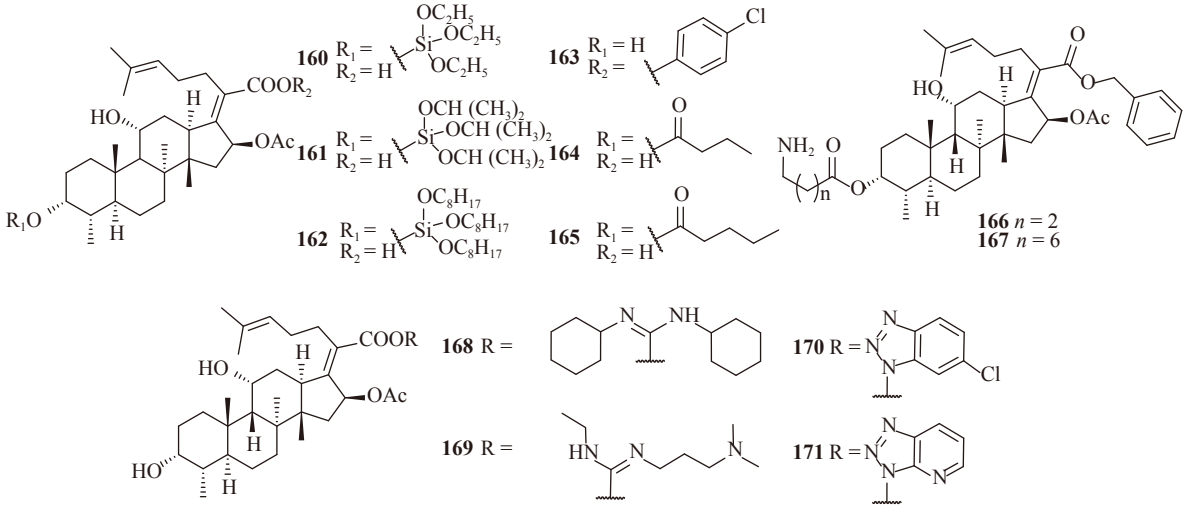


Fig. 13 FA derivatives with metabolic stability

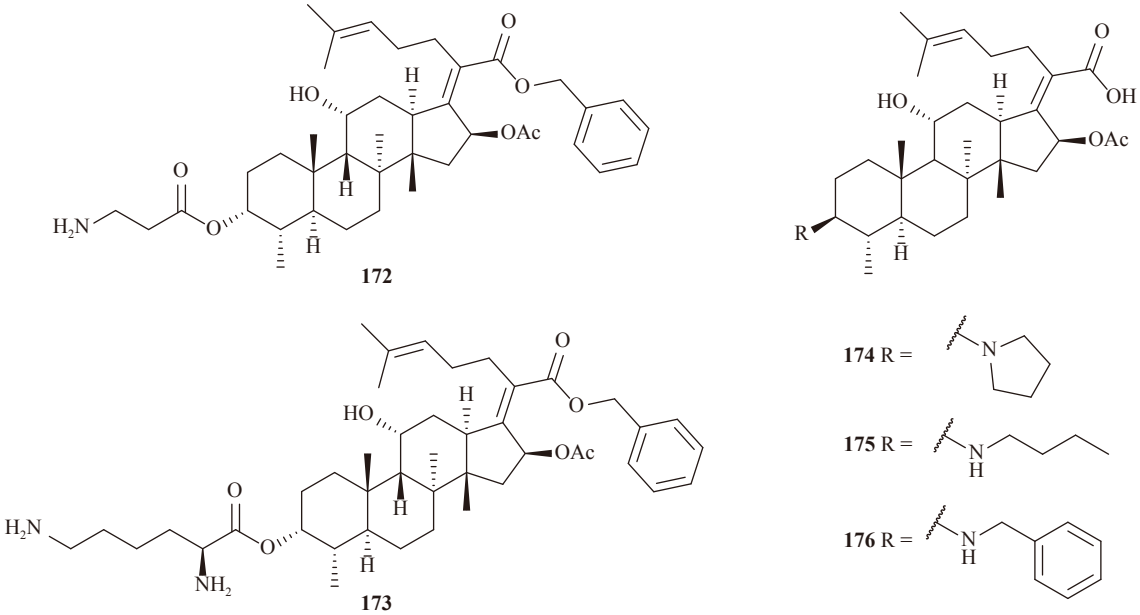


Fig. 14 Anti-tumor or antifungal FA derivatives

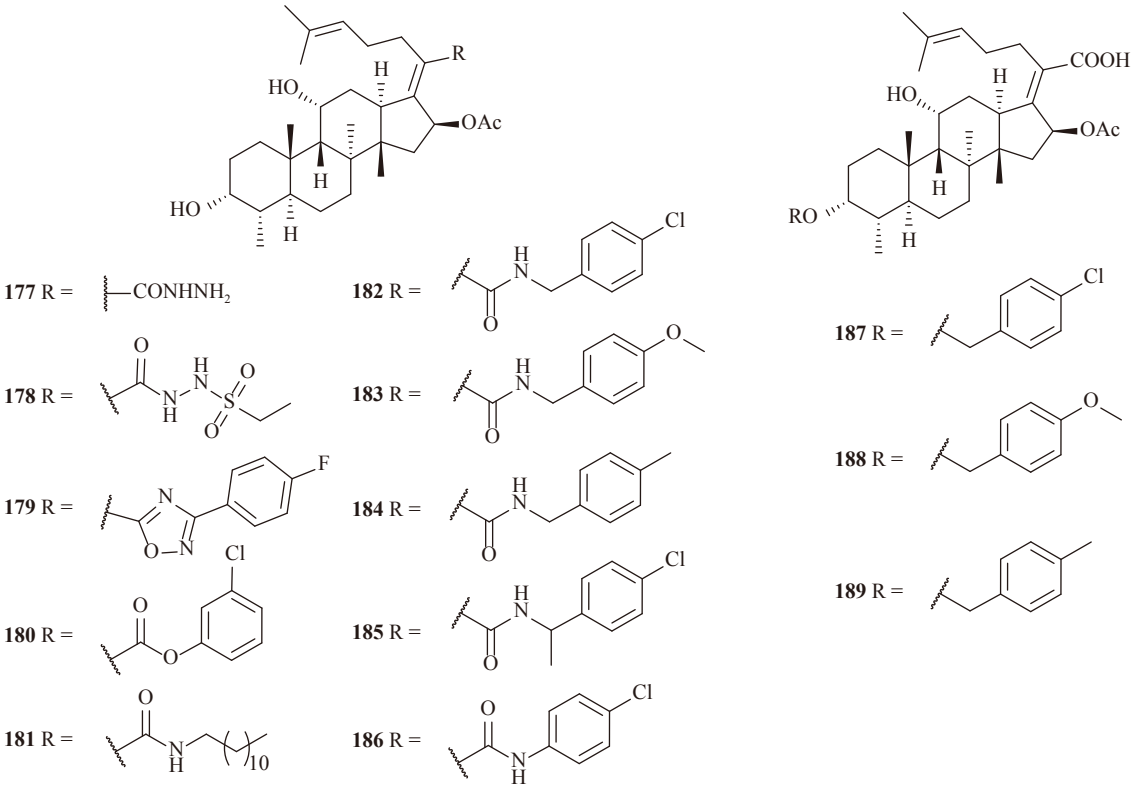


Fig. 15 C-3 and/or C-21 modified derivatives with antiplasmodial or antimycobacterial activity

Table 5 Antiplasmodial activities of C-3 and/or C-21 modified derivatives of FA (IC_{50}, μmol·L^{-1})

No.	*P. falciparum* NF54	No.	*P. falciparum* NF54
FA	59	**183**	5.9
177	21.3	**184**	3.6
178	12.4	**185**	0.3
179	1.7	**186**	0.7
180	1.4	**187**	6.4
181	1.2	**188**	7.6
182	2.7	**189**	9.2

um NF54 and multidrug-resistant *P. falciparum* K1, than FA [103]. Specifically, synthetic products **180** and **181** showed significant antiplasmodial activity against *P. falciparum* NF54 and *P. falciparum* K1 with IC_{50} values around 1.5 μmol·L^{-1}.

Chibale and co-workers also applied 3D-QSAR (three-dimensional quantitative structure-activity relationship) modelling method to design antiplasmodial FA analogs [104]. Five compounds with C-21 amide groups (**182**−**186**) and three virtual hit molecules with C-3 ether groups (**187**−**189**) were synthesized. C-21 amide derivatives showed superior antiplasmodial activity compared with C-3 ether derivatives (Fig. 15 and Table 5) [104]. Among them, two non-cytotoxic C-21 amide products (**185** and **186**) displayed the highest activity against chloroquine-sensitive *P. falciparum* NF54 with IC_{50} values of 0.3 and 0.7 μmol·L^{-1}, respectively. They also significantly inhibited the chloroquine-resistant *P. falciparum* K1 strain with an IC_{50} value of 0.2 μmol·L^{-1} [104].

Antimycobacterial derivatives

FA has also showed antimycobacterial activity against *Mycobacteria tuberculosis* (*Mtb*) with an MIC_{90} value of 0.24 μmol·L^{-1} [105-106]. Chibale group synthesized a series of C-21 amide analogs of FA to investigate the function of C-21 carboxyl acid [106-107]. Some synthesized ethanamides of FA were potent against *Mtb* with an MIC_{90} less than 10 μmol·L^{-1}. Specially, *N*-(4-sulfamoylbenzyl)fusidic acid amide (**190**) (Fig. 16) had the highest activity with an MIC_{90} value of 2.71 μmol·L^{-1} [106]. Thus, the substitutions at C-21, especially aryl amide moieties, increased the antiplasmodial or antimycobacterial activity of FA. The cyano group is usually applied for chemical modifications due to its biocompatibility and complicated interaction with biological targets [108]. Salimova and co-workers also introduced cyanoethyl group into FA at C-2, C-3, and C-11 (**191**−**194**) (Fig. 16) [109]. However, none of them showed antibacterial activity, indicating that cyanoethyl fragment was not a good choice for the improvement of FA activity.

Summary and Conclusions

The SAR of FA

The structural and antibacterial data summarized in this review enables us to further discuss the SAR of FA (Fig. 17). Fundamentally understanding the SAR will facilitate the fu-

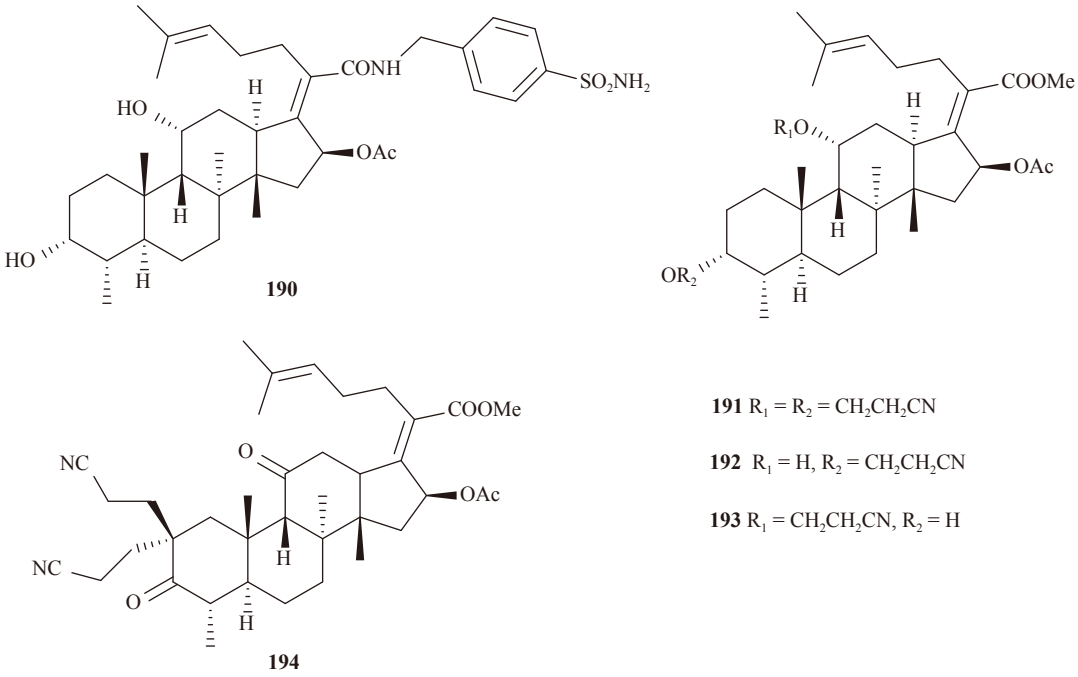


Fig. 16 C-3 and/or C-21 modified derivatives with antibacterial derivatives

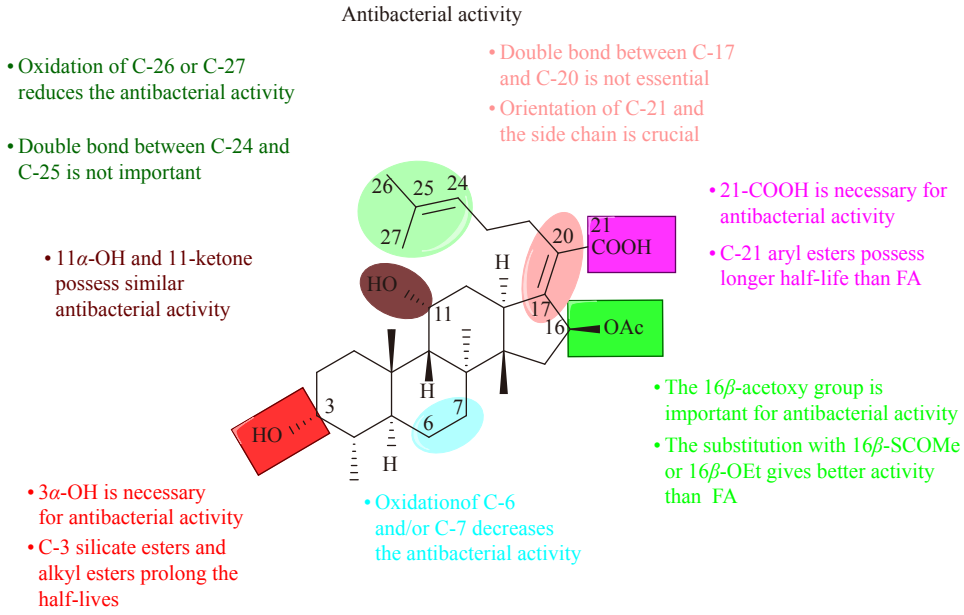


Fig. 17 The SAR of FA

ture discovery of new biologically active fusidane-type antibiotics. Compared with FA, compounds **35**−**39** with oxidation at C-26 or C-27 show significantly weaker activity against different gram-positive and gram-negative bacteria, indicating the importance of the methyls at C-26 and C-27. Δ17(20) and Δ24(25) double bonds are not crucial for FA activity, which is supported by the structures of compounds **131**, **154**, **155**, and **157**−**159**. They exhibited comparable inhibitory effect against gram-positive bacteria as FA. The orientation of the C-21 carboxyl group and the lipophilic moiety of the side chain must be similar to that of FA, with significant effects on antibacterial activities. The substitution at C-21 carboxyl group dramatically changes the antibacterial activity of FA. Its substitution by alkyl esters or amides (**147**−**150**) reduces the activity. It has to be noted that C-21 aryl esters with moderate antibacterial activity, such as compounds **163**, **170** and **171**, generally possess longer half-life than FA.

Replacement of the 16*β*-acetoxy unit by various other groups assures all or part of the activity of FA (Fig. 17). Compounds **132** with a 16*β*-SCOMe group and **140** with a 16*β*-OEt are proved to be more active than FA, providing

lead compounds for further chemical and biological investigations. By comparing compounds **3** and **55**, the 11α-OH in FA is important for antibacterial activity. Interestingly, a FA derivative (**25**) with an 11-ketone instead of 11α-OH shows the same antibiotic activity as FA. The oxidation of C-6 or C-7 (**40** and **41**) diminishes the biological activity, and 3α-OH is more important than 3-keto, 3β-OH, and other halogen or sulfur substitutions (**24**, **26**, and **143**−**146**). Moreover, C-3 silicate esters (**160**−**162**) and alkyl esters (**164** and **165**) prolong the half-life of FA, and represent attractive lead compounds for further investigation.

Conclusions and Future Perspectives

As summarized in this review, researches on characterization and biological evaluation of fusidane-type antibiotics from diverse fungal species are mainly reported before 1980. However, new naturally occurring fusidane-type antibiotics are increasingly being discovered since 2006. Chemical modifications and biosynthesis of fusidane-type antibiotics have been reported in recent years. It seems that scientists tend to re-pick up "old weapons" to deal with "new challenges" associated with exhausted drug lead resources and increasing resistant bacteria. Herein, we present a review on 47 fusidane-type antibiotics isolated from diverse fungi and biotransformation, and 81 analogs discovered from combinational biosynthesis, together with 66 synthetic or semi-synthetic fusidanes.

More importantly, the BGCs of fusidane-type antibiotics containing six conserved genes and several tailoring genes are well studied now. A number of structurally diverse derivatives have been obtained during combinatorial biosynthesis, illustrating the great power of biosynthetic approaches to expand the chemical diversity of fusidane-type antibiotics. Many synthetic methods have been reported to construct the chair-boat-chair ring system of fusidane-type antibiotics. Unfortunately, until now, there are no reports for total synthesis of their structures, especially HA (**1**), CP (**2**), and FA (**3**). Furthermore, FA is the main target of structural optimization for obtaining more biologically active derivatives. However, despite extensive semi-synthetic studies of FA, the modifications are mostly related to the introduction of different substitutions at C-3, C-16, and C-21. More modification strategies or sites might be needed for investigating the SAR. Fusidane-type antibiotics not only have obvious antibacterial activity, but also exhibit significant antiplasmodial, antimycobacterial, antifungal, and cytotoxic activity. Based on the references listed, only compounds **132** and **140** were more active than FA in antibacterial evaluation. More interestingly, nearly all synthetic C-21 amide analogs, such as compounds **181**, **185**, and **186**, show superior antiplasmodial activity compared with FA. Recent advances by isolation, biological synthetic, and synthetic chemistry approaches offer promising opportunities to access diverse fusidane-type antibiotics. Discovery of more fusidane-derived antibiotics, even antiplasmodial agents, will be expected.

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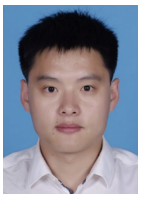
Cite this article as: TIAN Chuan, WANG Ke, ZHANG Xuan, LI Gang, LOU Hong-Xiang. Old fusidane-type antibiotics for new challenges: Chemistry and biology [J]. *Chin J Nat Med*, 2022, **20**(2): 81-101.



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