Review
Recent Developments in Bisintercalator Natural Products

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ABSTRACT:

The bisintercalator natural products are a family of nonribosomal peptides possessing a range of biological properties that include antiviral, antibiotic, and anticancer activities. The name bisintercalator is derived from the ability to directly bind to duplex DNA through two planar intercalating moieties. Although 19 members of this family of compounds have been identified over the past 50 years, the biosynthetic genes responsible for the formation of four of these molecules (thiocoraline, SW-163, triostin A, and echinomycin) were identified only recently. This recent progress opens an avenue towards understanding how Nature produces these bisintercalating products and provides the potential to develop and identify novel potent analogous lead compounds for clinical applications. This review discusses the mode of action of bisintercalators and summarizes recent genetic and biochemical insights into their biosynthetic production, analog formation, and possible mechanisms by which resistance to these compounds is achieved by their producing organisms. © *2010 Wiley Periodicals, Inc. Biopolymers 93: 777–790, 2010.*

Keywords: bisintercalators; nonribosomal peptides; resistance; biosynthesis

INTRODUCTION

Since the isolation of the first bisintercalator natural product, echinomycin,[1] in 1957 eighteen additional members have been added to this family of compounds (see Figure 1). These microbial secondary metabolites have been found in a variety of bacterial strains including several *Streptomyces*, some *Micromonospora*, *Actinomadura*, and *Nocardioides*. In some instances, the same compounds were isolated from a number of distinct bacterial species. For example, after being discovered in *Streptomyces echinatus* sp. 1,[1] echinomycin, also termed quinomycin A, was detected as a metabolite of *Streptomyces* sp. 732,[2] and later on as a product of *Streptomyces* sp. KN-0647[3] and *Streptomyces lasaliensis*.[4] For many years, their potent activity as antitumors, antivirals, and antibiotics, as well as a desire to understand how they target DNA contributed to a continued interest in these compounds. More recently, it is the aim to decipher how Nature produces these bisintercalating products and to identify novel potent lead compounds for clinical applications that has sustained the interest in this family of compounds.

Based on the structure of their nonribosomally biosynthesized peptidic core, these compounds can be divided into three classes: (i) cyclic, (ii) twofold symmetric bicyclic, and (iii) pseudosymmetric bicyclic. The main difference between

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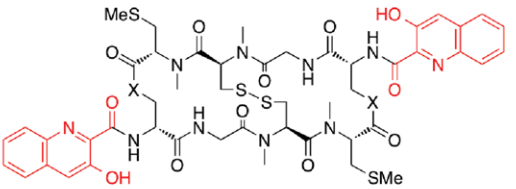
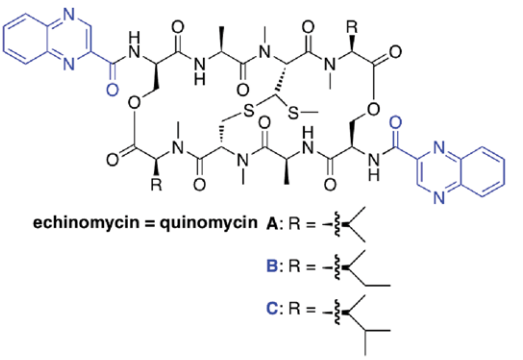
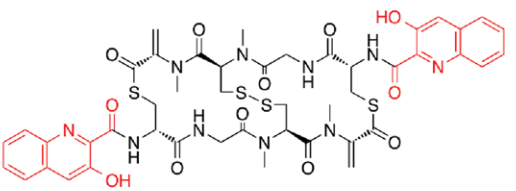


thiocoraline: X = S
(*Micromonospora* sp. ML1, *Micromonospora marina* L-13-ACM2-092)
Azathiocoraline: X = NH (synthetic derivative)
Oxathiocoraline: X = O (synthetic derivative)
NMe-Azathiocoraline: X = NMe (synthetic derivative)

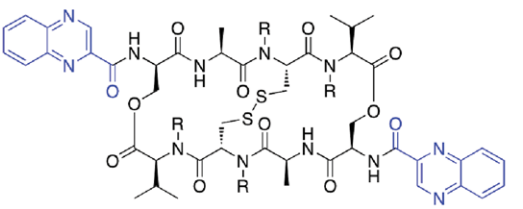


echinomycin = quinomycin **A**: R = –
B: R = –
C: R = –

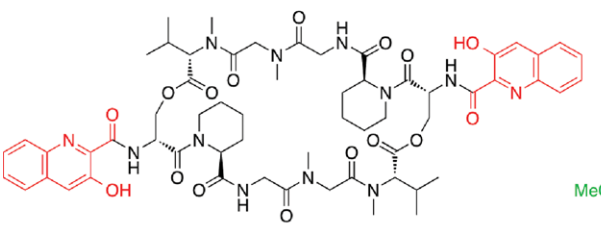
(*Streptomyces echinatus* sp. 1, *Streptomyces lasaliensis*, *Streptomyces* sp. KN-0647, *Streptomyces* sp. 732)



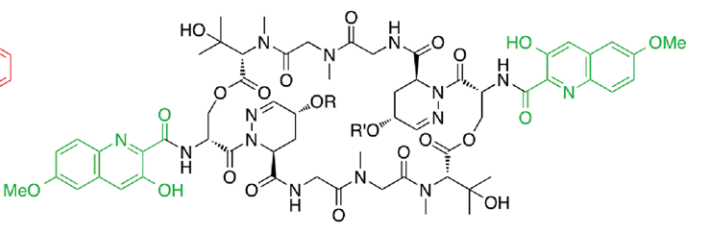
BE-22179
(*Streptomyces* sp. A22179)



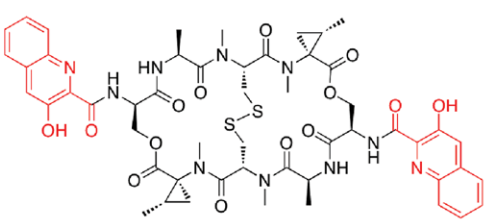
triostin A: R = Me
TANDEM: R = H (synthetic derivative)
(*Streptomyces aureus* s-2-210L, *Streptomyces triostinicus*, *Streptomyces lasaliensis*)



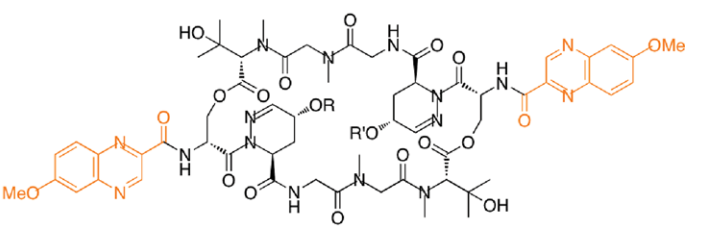
sandramycin
(*Nocardioides* sp. ATCC 39419)



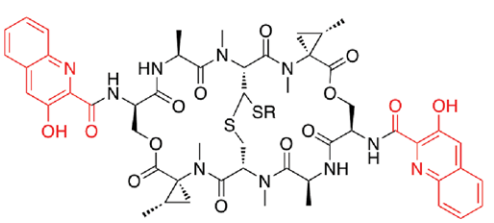
luzopeptin **A**: R = R' = COMe
B: R = H, R' = COMe
C: R = R' = H
(*Actinomadura luzonensis* nov. sp. G455-101)



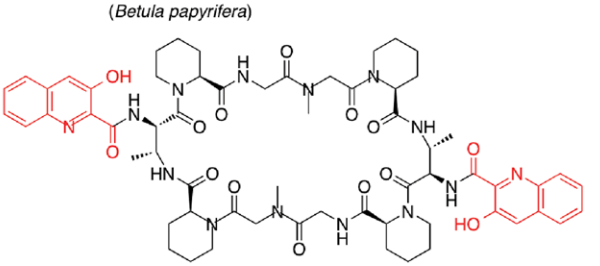
SW-163C
(*Streptomyces* sp. SNA15896)



quinoxapeptin **A**: R = R' = CO–
B: R = COMe, R' = CO–
C: R = R' = H
(*Betula papyrifera*)



SW-163D: R = Me
SW-163E: R = Et
SW-163F: R = i-Pr
SW-163G: R = sec-Bu
(*Streptomyces* sp. SNA15896)



quinaldopeptin
(*Streptoverticillium album* Q132-6)

FIGURE 1 Structures of bisintercalator natural products with their key pharmacophores for DNA intercalation colored in red (3-hydroxyquinaldic acid, 3HQA), blue (quinoxaline-2-carboxylic acid, QXC), green (6-methoxy-3-hydroxyquinaldic acid), and orange (6-methoxy-quinoxaline-2-carboxylic acid). Their producing organisms are presented into parentheses.

the symmetric and pseudosymmetric bicyclic molecules is the identity of their central linker, a disulfide bond, and a thioacetal bridge, respectively. Members of the cyclic bisintercalators include the luzopeptins,[5,6] the quinoxapeptins,[7] quinaldopeptin,[8] and sandramycin.[9,10] The twofold symmetric bicyclic class comprises BE-22179,[11] thiocoraline,[12,13] quinomycin B and C,[14] SW-163C,[15,16] and triostin A.[17,18] The pseudosymmetrical SW-163D-G[19] and echinomycin are derived from SW-163C and triostin A, respectively. The peptidic core of all of these compounds is further decorated with two planar heteroaromatic units [3-hydroxyquinaldic acid (3HQA) for thiocoraline, BE-22179, sandramycin, quinaldopeptin, and the SW-163s; quinoxaline-2-carboxylic acid (QXC) for the quinomycins and triostin A; 6-methoxy-3-hydroxyquinaldic acid for the luzopeptins; and 6-methoxyquinoxaline-2-carboxylic acid for the quinoxapeptins] essential for biological activity through binding to duplex DNA by bisintercalation.

The isolation, structure determination, and biological activity of most of the members of the family of bisintercalator natural products have been recently reviewed.[20] These topics will not be covered in this review, which is meant to focus primarily on the recent literature (2006–2010) related to the mode of action and the biosynthesis of these compounds, the production of their analogs, and the possible mechanisms by which resistance to them is conferred to their producing organisms.

MODE OF ACTION: DNA BISINTERCALATION

A large number of classes of synthetic and naturally occurring compounds exert their biological activity through one of three DNA-binding modes: (i) covalent binding, (ii) nonintercalative groove binding, and (iii) intercalation.[21] As their name implies, the bisintercalator natural products, with their cyclic peptidic backbone and two planar chromophores, possess the ability to bisintercalate tightly into DNA. Their interactions with their target DNA have been studied using a variety of techniques including DNase I footprinting, fluorescence quenching, surface plasmon resonance, NMR, X-ray crystallography, and, more recently, electrospray ionization tandem mass spectrometry.[22,23] In general, sandwiching of two bases between the two heteroaromatic units has been shown to ultimately lead to proper placement of the peptidic core, held by van der Waals interactions and mainly by hydrogen bonding with base pairs, into the DNA minor groove.[24] The amino acid composition of the peptidic backbone has been found to play an important role in dictating the binding sequence specificity of these molecules. The nature of the central cross-bridge (disulfide vs thioacetal) is also crucial in determining DNA sequence selectivity.[25] The effect of local DNA sequence on the interaction of the bisintercalators with their preferred binding sites has been studied.[26]

Bisintercalators with 5′-GC Selectivity

DNase I footprinting experiments showed that echinomycin preferentially binds at 5′-GC sites.[27] The molecular details of the interaction of echinomycin with DNA have been determined by a number of structural studies by X-ray crystallography and NMR, and confirmed a preference for AT base pairs at sites that flank the primary binding sites.[28–35] In echinomycin-DNA complexes, the base pairs next to the 5′-GC site are almost always exclusively in the Hoogsteen mode, with some rare Watson-Crick exceptions. Footprinting and NMR studies indicated that like echinomycin, SW-163G, previously termed UK-63052 or QN, mostly binds at 5′-GC sites.[36] However, in contrast to what is observed with echinomycin, only the usual Watson-Crick base pairs flank the bisintercalation site as revealed by a solution structure of a SW-163G-DNA oligomer complex. Binding of SW-163G to DNA is also more sensitive to the nature of the surrounding sequence when compared with echinomycin binding profiles. Until recently, even though a weak preference toward 5′-GC sites was observed, attempts to establish a clear sequence selectivity of DNA binding for thiocoraline were unsuccessful.[37] The binding preference for GC-rich sequences highly similar to those of echinomycin was unambiguously confirmed in 2007 by classical DNase I footprinting, fluorescence melting experiments, and X-ray crystallography.[38] The crystal structure of thiocoraline revealed a novel and unique arrangement of stacked arrays of docked pairs of staple-shaped molecules suggesting how the DNA bisintercalation occurs.

Bisintercalators with 5′-AT Selectivity

In contrast to echinomycin, SW-163G, and thiocoraline, which all display 5′-GC selectivity, luzopeptin A, sandramycin, triostin A, and TANDEM (a synthetic *N*-demethylated analog of triostin A, Figure 1) were found to bind with high affinity to AT-rich DNA. The luzopeptins do not display rigid sequence selectivity, and as for SW-163G, no evidence for Hoogsteen base-pairing was observed by NMR in a luzopeptin A-d(5′-GCATGC)$_2$ complex.[39] Studies by fluorescence quenching[40] and surface plasmon resonance[41] showed that, in many respects, the mode of interaction with DNA of sandramycin is very similar to that of luzopeptin A with the exception that sandramycin displays overall higher sequence selectivity. Interestingly, triostin A, a direct precursor of echinomycin, was shown to favor AT-rich DNA sequences over the GC-rich DNA species, illustrating the importance of the

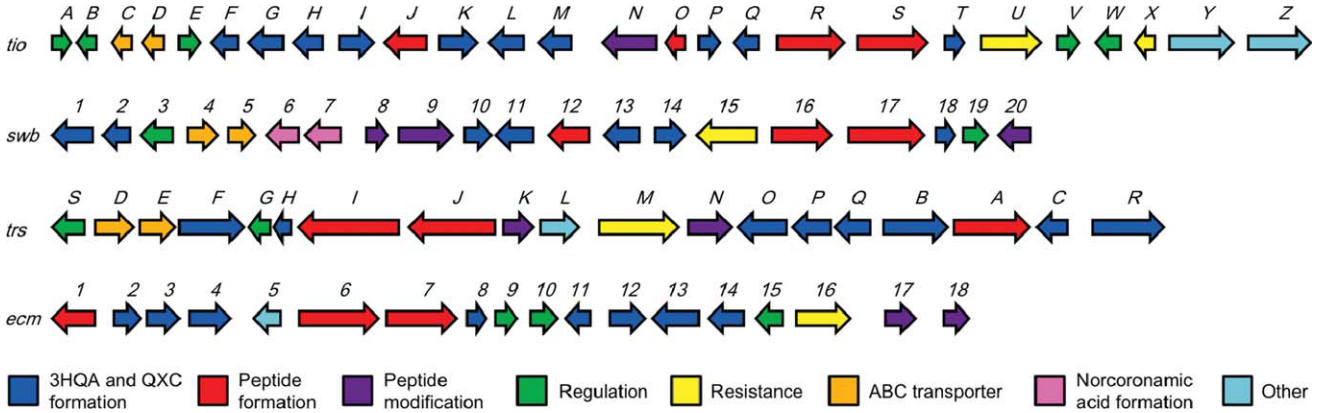


FIGURE 2 Genetic organization of the thiocoraline (*tio*), SW-163 (*swb*), triostin A (*trs*), and echinomycin (*ecm*) gene clusters.

nature of the central linker (disulfide vs thioacetal) of these molecules.[25]

BISINTERCALATORS BIOSYNTHESIS

The gene cluster sequences for four of the known bisintercalator natural products (thiocoraline,[42] SW-163,[43] triostin A,[44] and echinomycin[45]) have recently been determined (see Figure 2). Not surprisingly, considering the similar structural organization of these molecules, the reported gene clusters are remarkably similar in gene composition. All clusters comprise 7 to 10 proteins (highlighted in blue in Figure 2) that could be involved in the initial construction of the heteroaromatic chromophores 3HQA and QXC. They also include two nonribosomal peptide synthetases (NRPSs) (highlighted in red in Figure 2) from which the peptidic backbone of these compounds is proposed to arise after initiation by activation of the starter units 3HQA and QXC. Additional peptide modification enzymes (shown in purple and pink in Figure 2) are also suggested to be involved in peptidic core formation.

Generation of the 3HQA and QXC Appendages

Early efforts at determining the origin of the QXC chromophores of triostin A by ^{14}C-labeled tryptophan feeding studies established L-Trp as a likely precursor of QXC.[46] In a similar manner, using ^{15}N-labeled amino acids, the QXC of echinomycin was confirmed to be derived from L-Trp.[47] The formation of 6-fluoro-QXC during feeding studies with D,L-5-fluorotryptophan led to the proposal that the carbon at position 6 (C_6) of QXC corresponds to the C_5 of the indole ring of L-Trp.[48] More recent feeding, NMR, and mass spectrometry experiments using chemically synthesized (2*S*,3*S*)-5-deutero-β-hydroxy-L-Trp identified it as a key intermediate in echinomycin production. Based on these preliminary studies and the various genes found in the cluster for echinomycin and triostin A, a biosynthetic pathway involving eight proteins (TrsR, H, B, Q, C, F, O, and P for triostin A; Ecm13, 8, 12, 2, 11, 14, 4, and 3 for echinomycin) has been put forth for the biosynthesis of their QXC chromophore (Figure 3C).[44,45] The initial steps leading to the β-hydroxy-L-Trp intermediate are thought to commence with activation of L-Trp to L-Trp-AMP by the adenylation domain of the standalone A-T didomain TrsR/Ecm13 followed by its covalent attachment to the T domain of the enzyme. Hydroxylation of the β-carbon of L-Trp and release of the β-hydroxy-L-Trp by the type II thioesterase (TE) TrsQ/Ecm2 are the likely subsequent steps en route to QXC. The remaining stages of the QXC pathway require opening of the indole ring of β-hydroxy-L-Trp. Similarly to the first rate-limiting step during tryptophan catabolism in which L-Trp is converted to *N*-formylkynurenine,[49–51] oxidative cleaving of the C_2—C_3 bond of the pyrrole ring of β-hydroxy-L-Trp and incorporation of both atoms of molecular oxygen could be achieved by action of TrsC/Ecm11. Three additional enzymes are proposed to complete the QXC formation. TrsF/Ecm14 could produce β-hydroxykynurenine by deformylation, which could then be oxidatively cyclized and hydrolyzed by TrsO/Ecm4. TrsP/Ecm3 would be responsible for the final enzyme-catalyzed oxidation prior to spontaneous decarboxylation, imine formation, and oxidative aromatization to generate QXC. The MbtH-like protein TrsH/Ecm8 for which the exact biological function still remains to be determined could potentially also be involved in QXC formation. As of now, none of the steps involved in QXC production have been biochemically confirmed.

The production of the 3HQA chromophore of thiocoraline and SW-163C could proceed by one of two routes from the L-Trp precursor (Figures 3A and 3B). In the first route (Figure 3B), in a manner similar to that proposed for QXC formation, L-Trp is converted to β-hydroxykynurenine by consecutive action of the TioK/Swb11, TioI/Swb13, TioP or Q/Swb14, TioF/

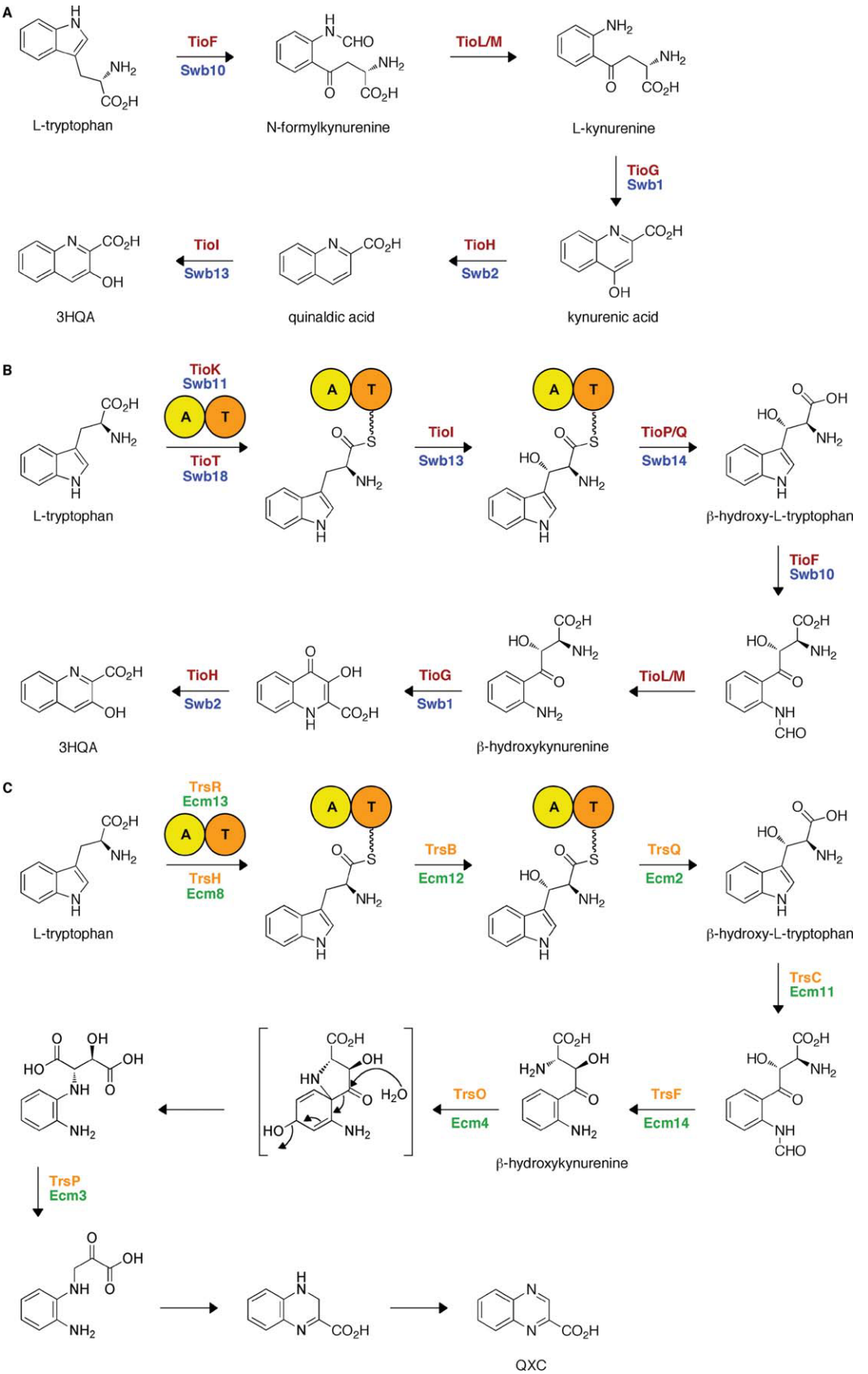


FIGURE 3 A and B: Two possible pathways for 3HQA biosynthesis during thiocoraline and SW-163 formation. The enzymes involved in thiocoraline and in SW-163 are indicated in red and blue, respectively. C: Proposed QXC biosynthesis during triostin A and echinomycin formation. The enzymes involved in triostin A and echinomycin are indicated in orange and green, respectively. The / indicates that either of the two enzymes proposed could perform the reaction.

Swb10, and TioL or TioM enzymes. The presence of two potential type II TE domains (TioP and TioQ) is unique to the thiocoraline gene cluster. Based on sequence alignment, TioQ is suggested to be the active unloading TE enzyme. No genes coding for enzymes with kynurenine formamidase activity have been identified in the thiocoraline or SW-163C clusters. TioL or TioM have been suggested as potential candidate for the deformylation reaction in thiocoraline biosynthesis. No homologs of TioL or TioM have been found in the SW-163C gene cluster. The transformation of β-hydroxykynurenine into 3HQA would then occur by cyclization by the kynurenine aminotransferase TioG and final elimination of the 4-hydroxy moiety by TioH. Alternatively, 3HQA could arise by a series of rearrangements that would utilize only five (TioF, TioL or M, TioG, TioH, and TioI) of the eight enzymes of the first pathway. In this route, the Trp 2,3-dioxygenase (TDO) TioF/Swb10 is the first enzyme to take action. TDOs are known to be highly specific for the L-Trp substrate.[52] For that reason, the latter strategy was originally favored (Figure 3A). However, it was recently demonstrated that TioF is a unique Trp 2,3-dioxygenase that is active against a variety of substrates including L-Trp, D-Trp, serotonin, and indole[53] as well as 5-fluoro-D,L-Trp, 6-fluoro-D,L-Trp, and 6-methyl-D,L-Trp (unpublished data). In conjunction with the preliminary data revealing (2*S*,3*S*)-5-deutero-β-hydroxy-L-Trp as a key intermediate during QXC formation, this result suggests the first pathway (Figure 3B) as a more plausible mechanism for 3HQA production. Further biochemical studies remain to be accomplished to confirm and gain a more advanced appreciation of the intriguing logic of molecular assembly of the 3HQA and QXC chromophores.

Peptidic Core Biosynthesis Initiated by Attachment of the 3HQA and QXC Chromophores

The bisintercalators' peptidic cores are biosynthesized by nonribosomal peptide synthetase (NRPS) assembly-lines (see Figure 4). NRPSs are multifunctional modular enzyme complexes, with each module responsible for adding a specific amino acid monomer to the growing peptide chain. The biosynthesis of nonribosomal peptides (NRPs) by NRPSs comprises several repeating steps based on the catalytic action of three essential components found in each NRPS elongation module: an adenylation (A), a thiolation (T), and a condensation (C) domain.[54–56] Adenylation (A) domains are involved in activation of the carboxylic group of the amino acid (or aryl acid) substrate through an ATP-dependent reaction that results in an aminoacyl-AMP intermediate,[57] which is subsequently readily transferred to the 4′-phosphopantetheinyl (Ppant) arm of the active (holo) downstream T domain partner. The successive amino acids are then joined by the formation of amide bonds catalyzed by C domains. The formation of NRP natural products proceeds in three distinct phases: initiation, elongation, and termination.

Early feeding studies in *S. triostinicus* and *S. echinatus* using several structural analogs of QXC revealed that QXC acted as a free intermediate during triostin A and echinomycin biosynthesis.[48,58–61] In line with this observation, Keller and coworkers isolated and characterized the enzyme that activates QXC to QXC-AMP in *S. triostinicus* and *S. echinatus*.[62] It was therefore proposed that during the biosynthesis of bisintercalator natural products the initiation occurs by activation of the starter units 3HQA and QXC by an adenosine monophosphate (AMP) ligase, followed by their covalent attachment to a standalone T domain. TioJ and Swb12 are proposed to activate 3HQA for initiation of thiocoraline and SW-163C biosynthesis, respectively, whereas TrsA and Ecm1 are suggested to convert QXC to QXC-AMP to initiate triostin A and echinomycin formation. Unique to the thiocoraline cluster is the presence of an independent T domain, TioO, proposed to be responsible for covalent tethering of the 3HQA prior to its condensation with the D-Cys attached to the T_1 domain of the TioR loading module. It was proposed that during the production of echinomycin, triostin A, and SW-163C, an acyl carrier protein from the fatty acid biosynthesis enzymatic complex, FabC, is recruited to play this role.[43–45]

Functional group and structural diversity is introduced into NRPs by two ways: (i) by utilizing a diverse monomer pools that includes not only the 20 naturally occurring amino acids, but also a large number (>300) of unnatural amino and aryl acid substrates,[63] and (ii) by the use of auxiliary domains strategically embedded into specific modules of the NRPS assembly lines. The primer unit 3HQA found in thiocoraline and SW-163 as well as the bisintercalating chromophore QXC of echinomycin and triostin A are representative examples of unnatural aryl acid substrates used during bisintercalators production. An additional nonproteinogenic amino acid, (+)-(1*S*,2*S*)-norcoronamic acid ((+)-NCA), is also found in the SW-163 family of compounds. Two proteins, the PLP-dependent aminotransferase Swb6 and the radical SAM protein Swb7, could potentially mediate the assembly of (+)-NCA from L-Val via radical cyclopropanation (see Figure 5). Even though the monomer composition of their peptidic core differs (D-Cys, Gly, *N*-Me-L-Cys, and N,S-diMe-L-Cys for thiocoraline; D-Ser, L-Ala, *N*-Me-L-Cys, and *N*-Me-L-Val for triostin A and echinomycin; and D-Ser, L-Ala, *N*-Me-L-Cys, and *N*-Me-NCA for SW-163C), the NRPSs' domain organizations of the elongation modules for thiocoraline, echinomycin, triostin A, and SW-163C are identical (see Figure 4). These bisintercalator natural products are biosynthesized on two NRPSs (TioR/S for thiocoraline; Swb16/17

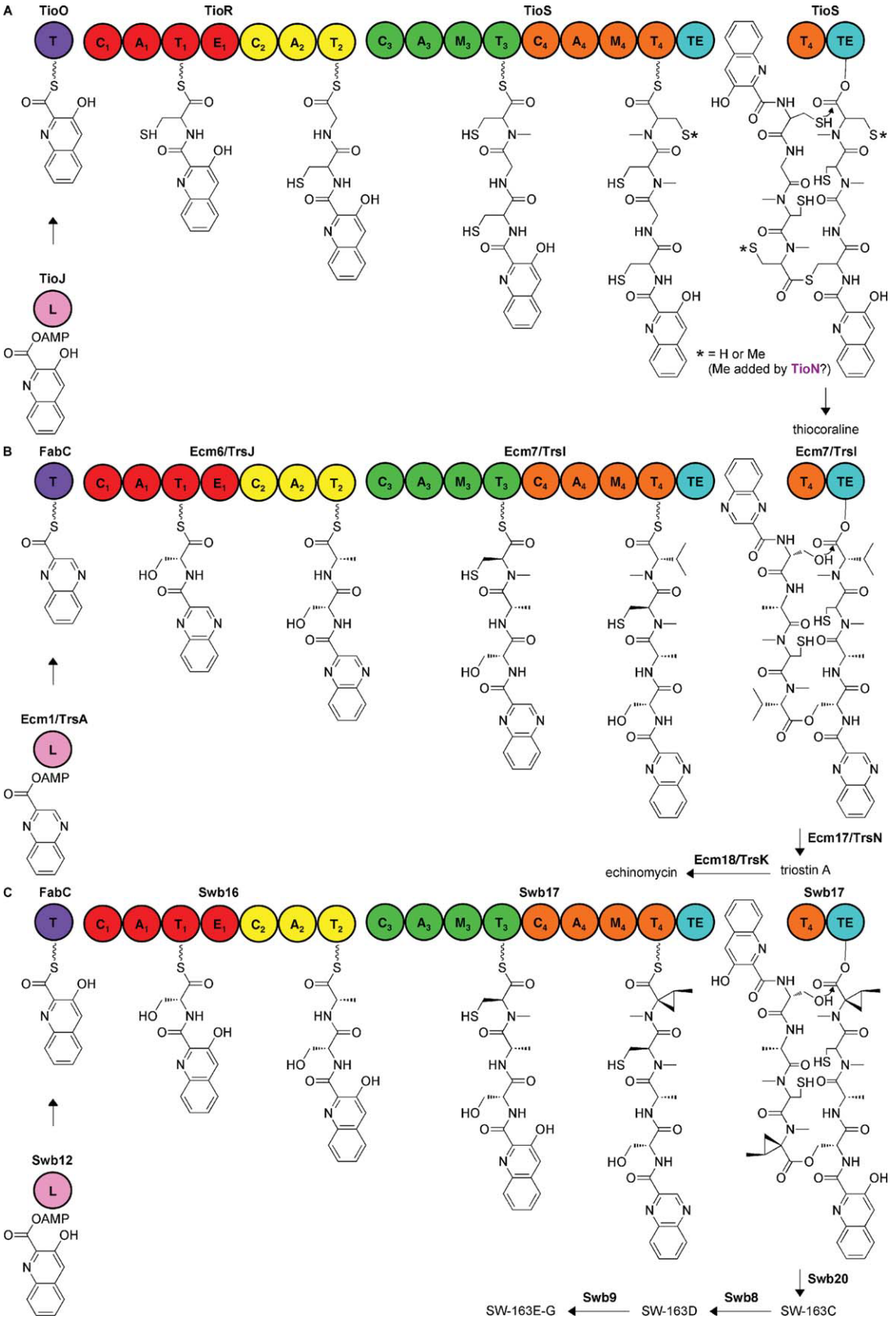


FIGURE 4 Structural organization of the (A) thiocoraline NRPSs, (B) echinomycin and triostin A NRPSs, and (C) SW-163C NRPSs. Each module of the NRPSs is represented by a different color. In panel B, the enzymes for echinomycin and triostin A biosynthesis are represented by Ecm and Trs, respectively. Abbreviations used: A, adenylation domain; C, condensation domain; E, epimerization domain; L, AMP-ligase; M, *N*-methyltransferase domain; T, thiolation domain (T domains have also been denoted in the literature as peptidyl carrier protein [abbreviated as PCP, PC, or P] and carrier protein [CP] domains); TE, thioesterase domain.

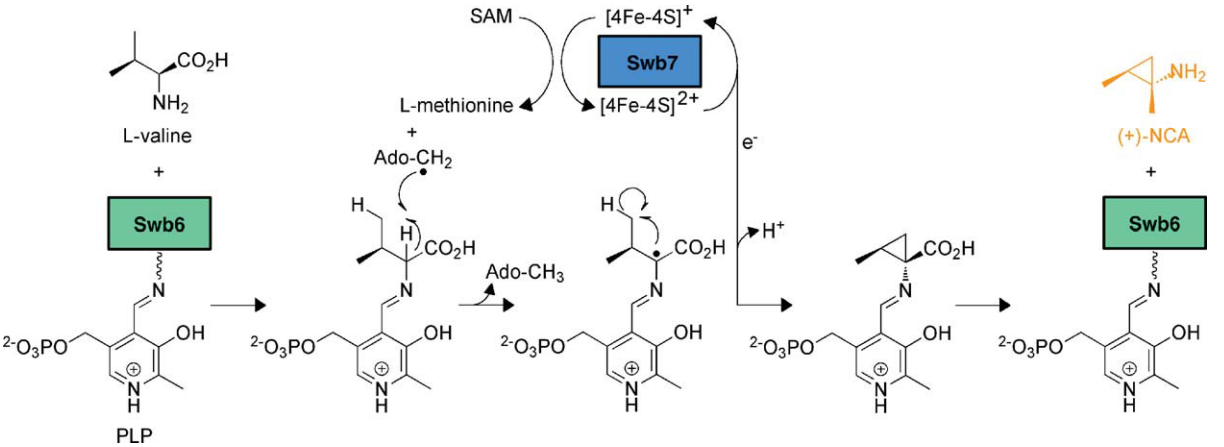


FIGURE 5 Possible biosynthetic pathway for (+)-NCA formation from L-Val. Abbreviations used: Ado-CH_3, 5′-deoxyadenosine; PLP, pyridoxal-5′-phosphate; SAM, *S*-adenosyl-L-methionine.

for SW-163; TrsJ/I for triostin A; and Ecm6/7 for echinomycin), each composed of two modules with C_1-A_1-T_1-E_1-C_2-A_2-T_2 and C_3-A_3-M_3-T_3-C_4-A_4-M_4-T_4-TE framework. The auxiliary epimerization (E) domain of the loading module is responsible for the L- to D-inversion of configuration after the covalent attachment of the L-amino acid to T_1 and prior to its condensation with the bisintercalating unit mediated by C_1. The methyltransferase (M) domains embedded into modules 3 and 4 are proposed to be involved in *N*-methylation of L-Cys, L-Val, and NCA during the biosynthesis of the four studied bisintercalators. TioN, an A domain interrupted between motifs A2 and A3 by the M1 core of an M domain, could be responsible for *S*-methylation during thiocoraline formation.

Finally, the terminal TE domain is proposed to homodimerize, cyclize, and release the peptidic chain prior to the disulfide bond formation potentially catalyzed by the oxidoreductases Ecm17, TrsN, and Swb20 during echinomycin, triostin A, and SW-163C biosynthesis, respectively. No corresponding oxidoreductase enzyme has been found in the thiocoraline cluster. Further conversion of triostin A into echinomycin and of SW-163C into SW-163D could be accomplished by disulfide bond rearrangement by the SAM-dependent methyltransferases TrsK, Ecm18, and Swb8. The activity of Ecm18 was confirmed in vitro,[45] but the activity of TrsK and Swb8 remains to be proven. Preliminary feeding studies using [methyl-D_3]-L-Met support subsequent stepwise additions of methyl groups for the formation of SW-163E-G from SW-163C by the radical SAM protein Swb9.[43]

BISINTERCALATOR ANALOGS

The desire to generate novel bisintercalators as novel pharmaceutically important compounds motivates our understanding of the details governing their mode of action. Since the isolation of the first bisintercalators, a number of researchers have investigated new ways to produce these compounds and their analogs. In the early days more traditional synthetic methods were used for the total synthesis of naturally occurring scaffolds and analogs production. Triostin A *N*-DEMethylated (TANDEM)[64,65] and triostin A[66] were the first to be synthesized. Boger et al. have chemically generated thiocoraline,[37,67] BE-22179,[37,67] sandramycin,[68] the luzopeptins,[69,70] the quinoxapeptins,[71] and their respective analogs.[40,41,72] A detailed review of the early syntheses and the insights gained from these studies has been published.[20] Here, we will focus on the latest analogs and the novel methodology used for their production. With echinomycin recently found to exhibit high activity against methicillin-resistant *Staphylococcus aureus* (MIC = 0.03 μM) and against biofilm-forming *Enterococcus faecalis* (MIC = 0.01 μM) the interest in the bisintercalators' biological properties has increased.[73,74] The access to the bisintercalator biosynthetic machineries has permitted the chemoenzymatic production and the biosynthetic engineering of novel analogs of this family of compounds.

Synthetic Analogs

In the last 5 years, the chemical syntheses of bicyclic depsipeptide analogs have mainly focused on the triostin A and thiocoraline scaffolds. The synthetic challenges encountered during triostin A synthesis are associated with the presence of consecutive *N*-methylated amino acids and two ester bonds that favor diketopiperazine formation. These difficulties were overcome by a new concept of protection referred to as conformationally restricted mobility as exemplified during the first solid-phase synthesis of triostin A (see Figure

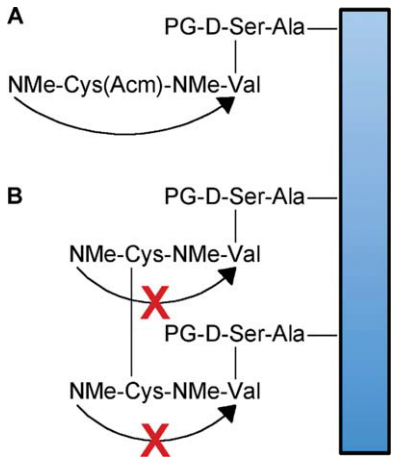


FIGURE 6 Schematic representation of (A) diketopiperazine formation, and (B) prevention of diketopiperazine formation by conformationally restricted mobility.

6).[75] The main idea is to form an inter-chain disulfide bridge that restricts the mobility of the peptide chain and prevents diketopiperazine formation. The thiocoraline analog oxathiocoraline (see Figure 1) with esters in place of the thioester moieties was also prepared using this strategy.[76] A manuscript on the lessons to be learned from the synthesis of complex *N*-methylated depsipeptides such as oxathiocoraline has been published last year.[77] In sum, the following factors should be taken into account: (i) the solid support (e.g., the choice of the resin is critical and CTC should be favored over Wang resin as it will minimize the risk of diketopiperazine formation), (ii) the protecting groups (e.g., Alloc and pNZ groups should be used instead of Fmoc as they also will also prevent diketopiperazine formation, however these groups can only be used at specific positions on the peptide chain), (iii) the identity of the C-terminal amino acid, (iv) the coupling reagents (e.g., HATU gives the best yields when coupling *N*-methylated amino acids), and (v) the cleavage cocktail.

Analogs of thiocoraline with amide (azathiocoraline) and *N*-methylated amide (NMe-azathiocoraline) moieties in place of the thioester groups have also been synthesized (see Figure 1). Two solid-phase strategies, a convergent [4 + 4] approach and a stepwise synthesis, were designed to generate azathiocoraline (see Figure 7).[78] The [4 + 4] fragment coupling method was also used to synthesize NMe-azathiocoraline, which was found to display an increased stability in human serum when compared to thiocoraline.[79] Using *N*-methyl amides as isosteres for ester or thioester bonds preserves hydrogen bonding properties; this idea could be used as a general strategy to prepare other bisintercalator

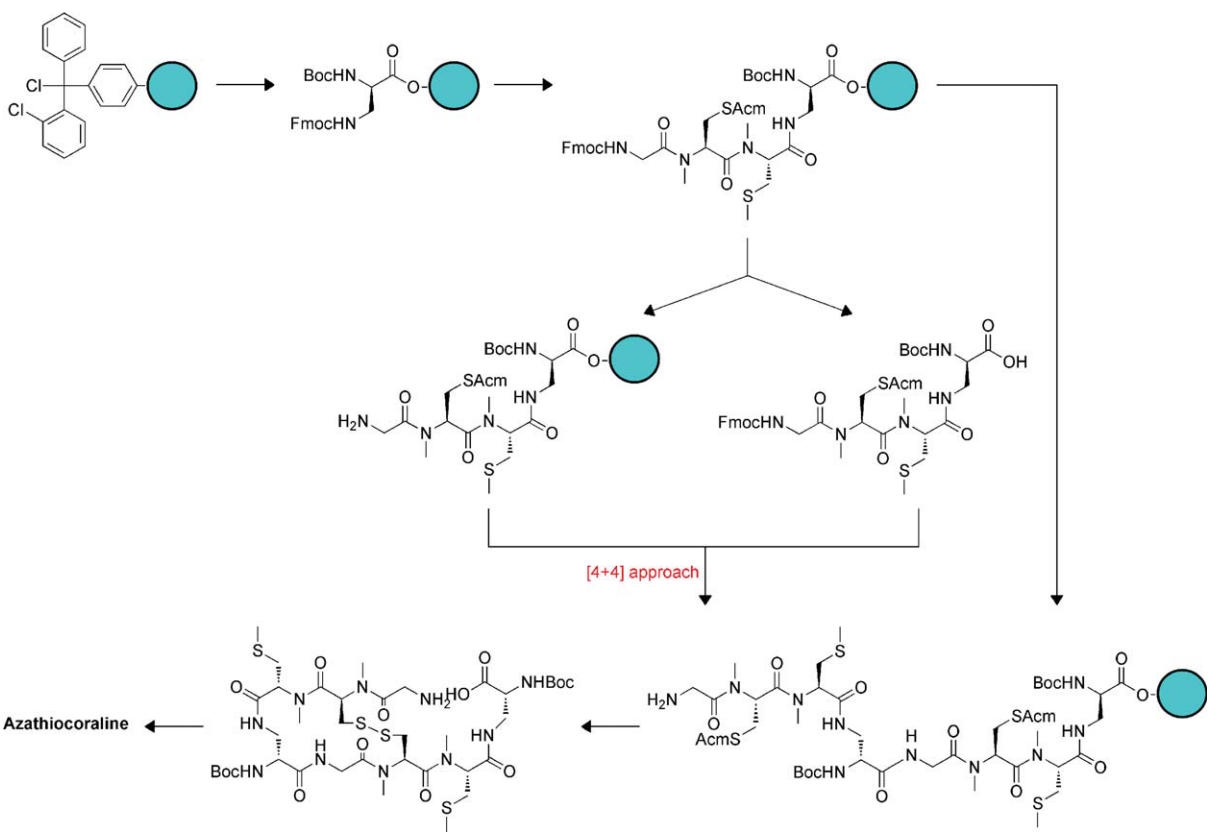


FIGURE 7 Two solid-phase strategies used for azathiocoraline production.

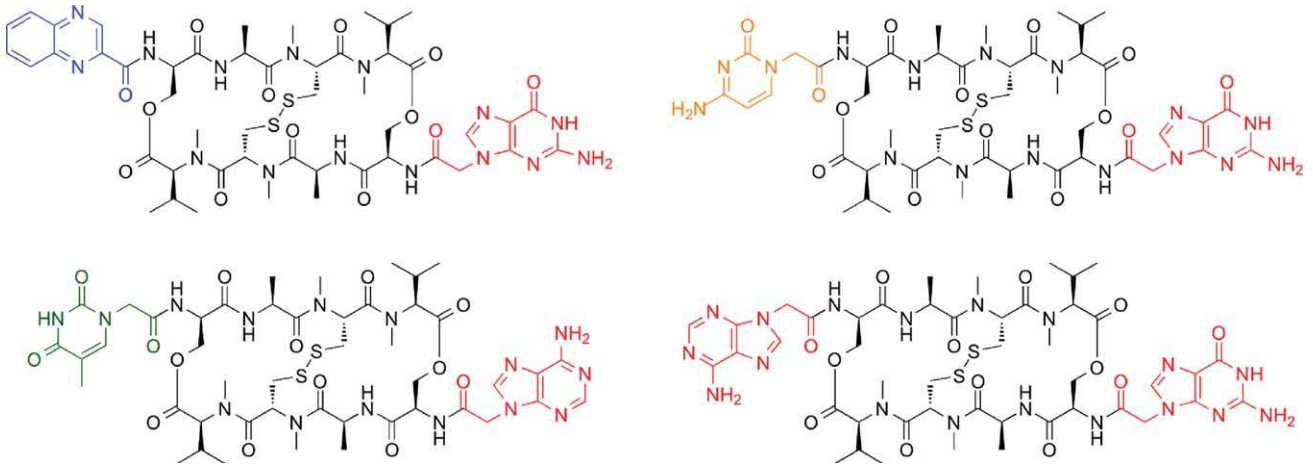


FIGURE 8 Structures of nucleobase-substituted triostin A analogs.

analogs. In the pursuit of synthesizing thiocoraline prodrugs, *N*,*N*,*N*′,*N*′-tetramethylchloroformamidinium hexafluorophosphate (TCFH) was discovered to be a powerful coupling reagent for bioconjugation that overcomes the problems associated with the lack of reactivity of the quinaldic alcohol.[80] This reagent could be useful for the development of prodrugs for other bisintercalators with similar limitations.

Symmetrical and pseudosymmetrical nucleobase-functionalized triostin A analogs were recently generated by solution-phase peptide chemistry and found to have the potential to recognize double-stranded DNA by hydrogen bonding (see Figure 8).[81,82] A series of novel TANDEM derivatives was also reported.[83] Replacement of L-Val at positions 4 and 8 with L-Lys residues was found to have no effect on selectivity for AT-rich sites whereas replacement of the QXCs by two naphthoyl chromophores completely abolished binding to DNA. Changing only one QXC for a naphthalene ring was found to decrease binding affinity. By combining structural features of the luzopeptins, triostin A, and thiocoraline, Albericio and coworkers developed a series of novel cyclic peptides, FAJANU 1-14 (see Figure 9).[84] From these studies, it has been concluded that (i) larger macrocyclic rings result in loss of cytotoxic activity, (ii) NMe-Gly is essential to maintain high cytotoxicity, but NMe-Leu can be replaced by Leu without considerable effect, (iii) the presence of ester moieties results in compounds that are less active than molecules with corresponding amide functionalities, and (iv) the presence of a heteroatom-containing bicyclic chromophore is required for activity.

Semisynthetic Analogs and Analogs Generated by Engineered Biosynthesis

The recent identification of the biosynthetic gene clusters of thiocoraline, echinomycin, triostin A, and SW-163 provided the opportunity to generate novel bisintercalator derivatives by chemoenzymatic synthesis and by engineered biosynthesis. The TE usually found as the C-terminal domain of modular NRPS catalyzes peptide release through hydrolysis or macrocyclization to yield linear or cyclic peptides, respectively. The TE domains of echinomycin, Ecm7-TE, and of thiocoraline in a TioS-T-TE construct were utilized to chemoenzymatically produce bisintercalator analogs from peptidyl-SNAC substrates (see Figure 10).[85,86] Using tetrapeptidic-SNACs as mimics of the tetrapeptides that would be covalently attached to the thiolation (T) domain of Ecm7 did not lead to the desired triostin A analogs. However, using similar tetrapeptidyl-SNAC precursors with the TioS-T-TE didomain led to the desired bisintercalators (Figure 10B). These observations might indicate that the presence of a partner T domain is important to achieve dimerization prior to macrocyclization. To obtain the triostin A bisintercalator derivatives, octapeptidyl-SNAC substrates needed to be used with Ecm7-TE (Figure 10A). It was shown that when using Ecm7-TE, coincubation with DNA allowed one to efficiently sequester the desired compounds possessing DNA-binding properties. In addition to the chemoenzymatic formation of novel bisintercalators by use of TE domains, an elegant method developed by Oikawa, Watanabe, Wang, and colleagues resulted in de novo production of heterologous bisintercalator antibiotics in *E. coli*.[87,88] In this approach multiple plasmids containing all the genes required for biosynthesis of the desired compound are transformed into *E. coli*. The method presents numerous advantages over chemical and chemoenzymatic syntheses: (i) expensive chemicals and time consuming reactions can be avoided, (ii) the low productivity from the original host can be overcome as *E. coli* is easily grown and production in this bacterial strain has been shown to be efficient upon optimization, and (iii) genetic manipulations are much simpler in *E. coli* than in

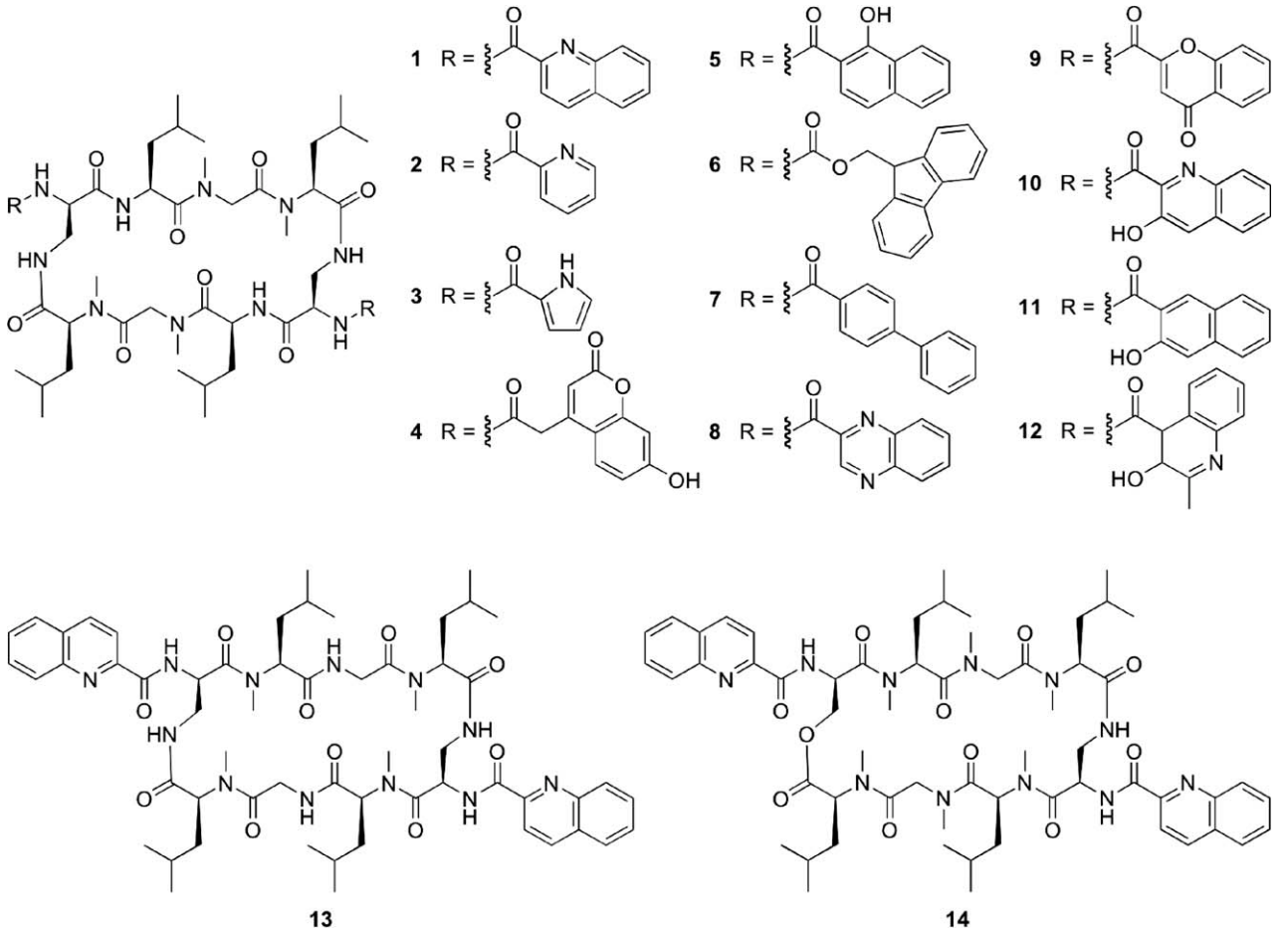


FIGURE 9 Structures of a set of representative first and second generation FAJANU peptides.

other bacterial systems, and (iv) the *E. coli* platform enables one to produce metabolites originating from bacteria that are not amenable to growth in artificial conditions.

RESISTANCE TO BISINTERCALATOR NATURAL PRODUCTS

The mechanisms by which microorganisms survive the toxic small molecules they produce can be many. For the bisintercalator natural products two self-resistance mechanisms have been proposed. First, a UvrA-like protein (TioU, Swb15, TrsM, and Ecm16), with high sequence homology with the daunorubicin-resistance-conferring factor DrrC[89] and the mithramycin resistance protein MtrX[90] could function in repair of chromosomal damage caused by cytoplasmic bisintercalators prior to their secretion through the cell membrane. By its introduction into *Escherichia coli* BL21 (DE3), Ecm16 was shown to confer echinomycin resistance to the *E. coli* host.[45] The role of TioU, Swb15, and TrsM as self-resistance proteins remains to be confirmed. An ABC transporter system composed of an ATPase (TioD, Swb4, and TrsD) and a permease subunit (TioC, Swb5, and TrsE) is also suggested to be involved in conferring self-resistance to the producing organism by acting through a transmembrane secretion mechanism.[42–44] Alternatively or additionally, the bisintercalator compound could be sequestered away by binding to a protein similar to bleomycin resistance protein. Crystal structure determination in conjunction with gene knockout and equilibrium titration studies suggested that TioX from the thiocoraline cluster, a twofold symmetric tetrameric protein with homology to a bleomycin resistance protein, but with an unusual organization of monomoers, could play this role.[91]

SUMMARY AND OUTLOOK

Since their discovery, bisintercalator natural products have intrigued scientists in many distinct, yet complementary, research areas. Biochemists, biologists, and chemists alike have been motivated to understand the mode of action of bisintercalators as DNA binding ligands and the ways in which Nature builds these unique structural scaffolds. Research has also been

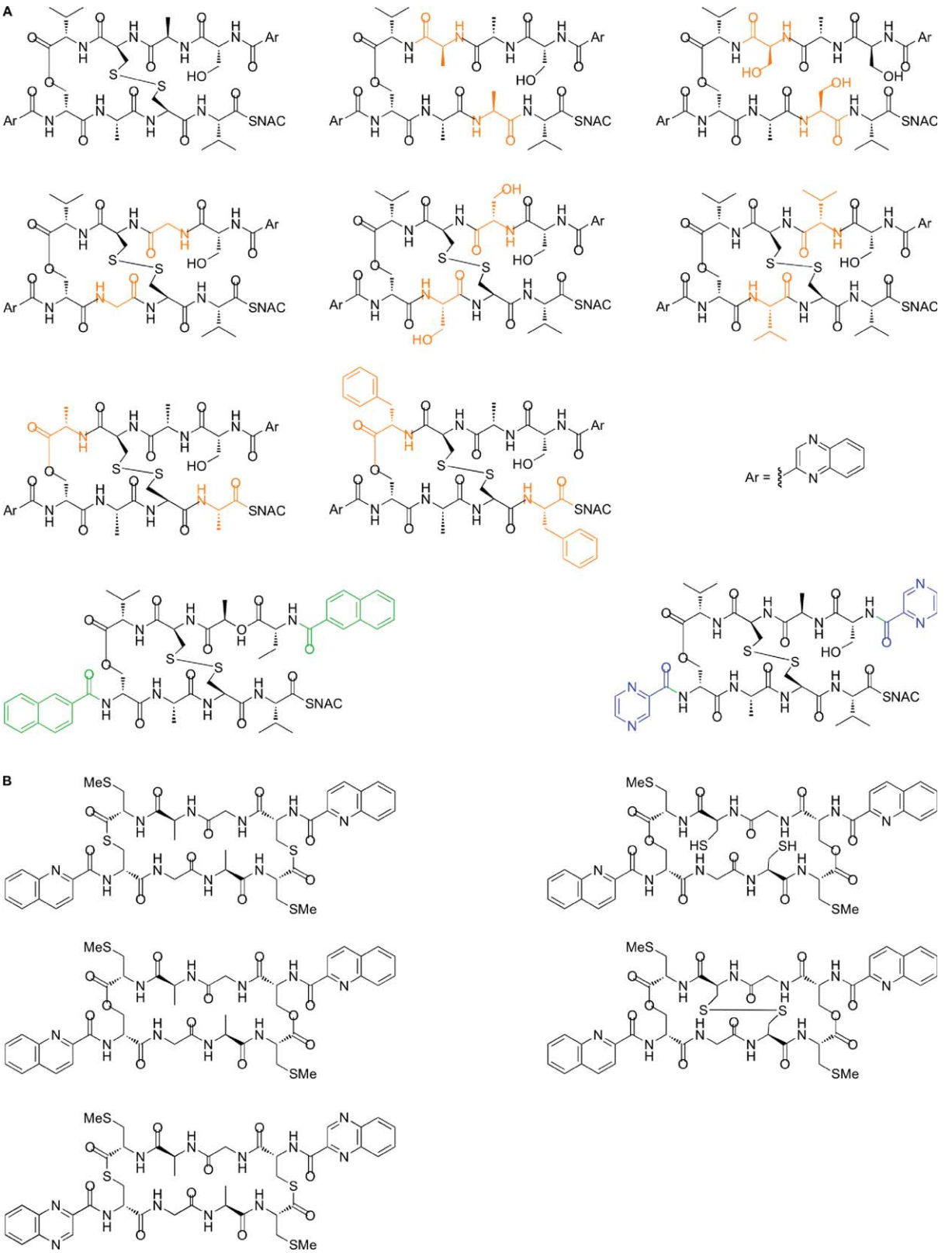


FIGURE 10 A: Structures of octapeptidyl-SNAC substrates used to generate triostin A analogs. B: Structures of some bisintercalators generated using the TioS-T-TE didomain.

directed at generating derivatives of these naturally produced compounds as novel therapeutics. Their poor solubility and the complexity associated with their chemical syntheses have been a cause of concern when considering the development of new bisintercalator derivatives. However, the production of novel compounds continues to progress at a fast pace as novel solid-phase synthesis strategies and reagents are being developed and discovered. The recent identification of four bisintercalator biosynthetic gene clusters has led to the development of novel chemoenzymatic strategies and methods at engineering natural product machineries for production of novel bisintercalators. It has also contributed significantly to our current understanding of these compounds and their biosyntheses. Many questions yet remain to be answered and further biochemical studies are needed to fully decipher the exact functions of the enzymes involved in bisintercalator biosynthesis. Future research should further investigate the pathway by which the 3HQA chromophore is biosynthesized. The exact mechanisms that confer resistance to these bisintercalating agents to their producing organisms also remain to be established. New members of this family of compounds have steadily continued to appear. With over a thousand of microbial genomes sequenced and with the availability of software that allows one to predict potential structures of molecules of nonribosomal peptide origin,[92] we can now envision discovering novel bisintercalators by genome mining. These compounds are predicted to continue to generate interest for some time to come in both the academic and industrial settings.

The authors would like to acknowledge the work on bisintercalator natural products of those not cited in this review due to the scope of the manuscript. The authors thank Oleg Tsodikov for his insightful comments.

REFERENCES

1. Corbaz, R.; Ettlinger, L.; Gäumann, E.; Keller-Schierlein, W.; Kradolfer, F.; Neipp, L.; Prelog, V.; Reusser, P.; Zahner, H. Helv Chim Acta 1957, 40, 199–204.
2. Yoshida, T.; Katagiri, K.; Yokozawa, S. J Antibiot (Tokyo) 1961, 14, 330–334.
3. Liu, H.; Qin, S.; Wang, Y.; Li, W.; Zhang, J. World J Microbiol Biotechnol 2008, 24, 2243–2248.
4. Steinerova, N.; Lipavska, H.; Stajner, K.; Caslavska, J.; Blumauerova, M.; Cudlin, J.; Vanek, Z. Folia Microbiol (Praha) 1987, 32, 1–5.
5. Tomita, K.; Hoshino, Y.; Sasahira, T.; Kawaguchi, H. J Antibiot 1980, 33, 1098–1102.
6. Ohkuma, H.; Sakai, F.; Nishiyama, Y.; Ohbayashi, M.; Imanishi, H.; Konishi, M.; Miyaki, T.; Koshiyama, H.; Kawaguchi, H. J Antibiot 1980, 33, 1087–1097.
7. Lingham, R. B.; Hsu, A. H.; O'Brien, J. A.; Sigmund, J. M.; Sanchez, M.; Gagliardi, M. M.; Heimbuch, B. K.; Genilloud, O.; Martin, I.; Diez, M. T.; Hirsch, C. F.; Zink, D. L.; Liesch, J. M.; Koch, G. E.; Gartner, S. E.; Garrity, G. M.; Tsou, N. N.; Salituro, G. M. J Antibiot (Tokyo) 1996, 49, 253–259.
8. Toda, S.; Sugawara, K.; Nishiyama, Y.; Ohbayashi, M.; Ohkusa, N.; Yamamoto, H.; Konishi, M.; Oki, T. J Antibiot (Tokyo) 1990, 43, 796–808.
9. Matson, J. A.; Bush, J. A. J Antibiot 1989, 42, 1763–1767.
10. Matson, J. A.; Colson, K. L.; Belofsky, G. N.; Bleiberg, B. B. J Antibiot 1993, 46, 162–166.
11. Okada, H.; Suzuki, H.; Yoshinari, T.; Arakawa, H.; Okura, A.; Suda, H.; Yamada, A.; Uemura, D. J Antibiot 1994, 47, 129–135.
12. Romero, F.; Espliego, F.; Perez Baz, J.; Garcia de Quesada, T.; Gravalos, D.; de la Calle, F.; Fernandez-Puentes, J. L. J Antibiot (Tokyo) 1997, 50, 734–737.
13. Baz, J. P.; Cañedo, L. M.; Fernández-Puentes, J. L.; Elipe, M. V. S. J Antibiot 1997, 50, 738–741.
14. Yoshida, T.; Katagiri, K. J Bacteriol 1967, 93, 1327–1331.
15. Takahashi, K.; Koshino, H.; Esumi, Y.; Tsuda, E.; Kurosawa, K. J Antibiot (Tokyo) 2001, 54, 622–627.
16. Kurosawa, K.; Takahashi, K.; Tsuda, E. J Antibiot (Tokyo) 2001, 54, 615–621.
17. Shoji, J.; Katagiri, K. J Antibiot 1961, A14, 335–339.
18. Kuroya, M.; Ishida, N.; Katagiri, K.; Shoji, T.; Yoshida, T.; Mayama, M.; Sato, K.; Matsura, S.; Niinome, Y.; Shiratori, O. J Antibiot 1961, 14, 324–329.
19. Nakaya, M.; Oguri, H.; Takahashi, K.; Fukushi, E.; Watanabe, K.; Oikawa, H. Biosci Biotechnol Biochem 2007, 71, 2969–2976.
20. Dawson, S.; Malkinson, J. P.; Paumier, D.; Searcey, M. Nat Prod Rep 2007, 24, 109–126.
21. Verma, R. P.; Hansch, C. J Pharm Sci 2008, 97, 88–110.
22. Mazzitelli, C. L.; Brodbelt, J. S. Anal Chem 2007, 79, 4636–4647.
23. Mazzitelli, C. L.; Chu, Y.; Reczek, J. J.; Iverson, B. L.; Brodbelt, J. S. J Am Soc Mass Spectrom 2007, 18, 311–321.
24. Wang, A. H.; Ughetto, G.; Quigley, G. J.; Hakoshima, T.; van der Marel, G. A.; van Boom, J. H.; Rich, A. Science 1984, 225, 1115–1121.
25. Lee, J. S.; Waring, M. J. Biochem J 1978, 173, 115–128.
26. Hampshire, A. J.; Fox, K. R. Biochimie 2008, 90, 988–998.
27. Low, C. M.; Drew, H. R.; Waring, M. J. Nucleic Acids Res 1984, 12, 4865–4879.
28. Ughetto, G.; Wang, A. H.; Quigley, G. J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. Nucleic Acids Res 1985, 13, 2305–2323.
29. Cuesta-Seijo, J. A.; Weiss, M. S.; Sheldrick, G. M. Acta Crystallogr D Biol Crystallogr 2006, 62, 417–424.
30. Cuesta-Seijo, J. A.; Sheldrick, G. M. Acta Crystallogr D Biol Crystallogr 2005, 61, 442–448.
31. Pfoh, R.; Cuesta-Seijo, J. A.; Sheldrick, G. M. Acta Crystallogr F Struct Biol Cryst Commun 2009, 65, 660–664.
32. Gao, X. L.; Patel, D. J. Biochemistry 1988, 27, 1744–1751.
33. Gao, X. L.; Patel, D. J. Q Rev Biophys 1989, 22, 93–138.
34. Gilbert, D. E.; Feigon, J. Nucleic Acids Res 1992, 20, 2411–2420.
35. Park, J. Y.; Choi, B. S. J Biochem 1995, 118, 989–995.
36. Chen, H.; Patel, D. J. J Mol Biol 1995, 246, 164–179.
37. Boger, D. L.; Ichikawa, S.; Tse, W. C.; Hedrick, M. P.; Jin, Q. J Am Chem Soc 2001, 123, 561–568.
38. Negri, A.; Marco, E.; Garcia-Hernandez, V.; Domingo, A.; Llamas-Saiz, A. L.; Porto-Sanda, S.; Riguera, R.; Laine, W.; David-

Cordonnier, M. H.; Bailly, C.; Garcia-Fernandez, L. F.; Vaquero, J. J.; Gago, F. J Med Chem 2007, 50, 3322–3333.
39. Searle, M. S.; Hall, J. G.; Denny, W. A.; Wakelin, L. P. Biochem J 1989, 259, 433–441.
40. Boger, D. L.; Chen, J. H.; Saionz, K. W.; Jin, Q. Bioorg Med Chem 1998, 6, 85–102.
41. Boger, D. L.; Saionz, K. W. Bioorg Med Chem 1999, 7, 315–321.
42. Lombo, F.; Velasco, A.; Castro, A.; de la Calle, F.; Brana, A. F.; Sanchez-Puelles, J. M.; Mendez, C.; Salas, J. A. Chembiochem 2006, 7, 366–376.
43. Watanabe, K.; Hotta, K.; Nakaya, M.; Praseuth, A. P.; Wang, C. C.; Inada, D.; Takahashi, K.; Fukushi, E.; Oguri, H.; Oikawa, H. J Am Chem Soc 2009, 131, 9347–9353.
44. Praseuth, A. P.; Wang, C. C.; Watanabe, K.; Hotta, K.; Oguri, H.; Oikawa, H. Biotechnol Prog 2008, 24, 1226–1231.
45. Watanabe, K.; Hotta, K.; Praseuth, A. P.; Koketsu, K.; Migita, A.; Boddy, C. N.; Wang, C. C.; Oguri, H.; Oikawa, H. Nat Chem Biol 2006, 2, 423–428.
46. Yoshida, T.; Katagiri, K. Biochemistry 1969, 8, 2645–2651.
47. Reid, D. G.; Doddrell, D. M.; Williams, D. H.; Fox, K. R. Biochim Biophys Acta 1984, 798, 111–114.
48. Cornish, A.; Fox, K. R.; Santikarn, S.; Waring, M. J.; Williams, D. H. J Gen Microbiol 1985, 131, 561–570.
49. Mellor, A. L.; Munn, D.; Chandler, P.; Keskin, D.; Johnson, T.; Marshall, B.; Jhaver, K.; Baban, B. Adv Exp Med Biol 2003, 527, 27–35.
50. Kotake, Y.; Masayama, I. Z Physiol Chem 1936, 243, 237–244.
51. Kurnasov, O.; Jablonski, L.; Polanuyer, B.; Dorrestein, P.; Begley, T.; Osterman, A. FEMS Microbiol Lett 2003, 227, 219–227.
52. Sono, M.; Roach, M. P.; Coulter, E. D.; Dawson, J. H. Chem Rev 1996, 96, 2841–2887.
53. Sheoran, A.; King, A.; Velasco, A.; Pero, J. M.; Garneau-Tsodikova, S. Mol Biosyst 2008, 4, 622–628.
54. Tanovic, A.; Samel, S. A.; Essen, L. O.; Marahiel, M. A. Science 2008, 321, 659–663.
55. Meier, J. L.; Burkart, M. D. Chem Soc Rev 2009, 38, 2012–2045.
56. Felnagle, E. A.; Jackson, E. E.; Chan, Y. A.; Podevels, A. M.; Berti, A. D.; McMahon, M. D.; Thomas, M. G. Mol Pharm 2008, 5, 191–211.
57. McQuade, T. J.; Shallop, A. D.; Sheoran, A.; Delproposto, J. E.; Tsodikov, O. V.; Garneau-Tsodikova, S. Anal Biochem 2009, 386, 244–250.
58. Gauvreau, D.; Waring, M. J. Can J Microbiol 1984, 30, 730–738.
59. Gauvreau, D.; Waring, M. J. Can J Microbiol 1984, 30, 439–450.
60. Santikarn, S.; Hammond, S. J.; Williams, D. H.; Cornish, A.; Waring, M. J. J Antibiot (Tokyo) 1983, 36, 362–364.
61. Cornish, A.; Fox, K. R.; Waring, M. J. Antimicrob Agents Chemother 1983, 23, 221–231.
62. Glund, K.; Schlumbohm, W.; Bapat, M.; Keller, U. Biochemistry 1990, 29, 3522–3527.
63. Sieber, S. A.; Marahiel, M. A. Chem Rev 2005, 105, 715–738.
64. Ciardelli, T. L.; Olsen, R. K. J Am Chem Soc 1977, 99, 2806–2807.
65. Viswamitra, M. A.; Kennard, O.; Cruse, W. B.; Egert, E.; Sheldrick, G. M.; Jones, P. G.; Waring, M. J.; Wakelin, L. P.; Olsen, R. K. Nature 1981, 289, 817–819.
66. Chakravarty, P. K.; Olsen, R. K. Tetrahedron Lett 1978, 19, 1613.
67. Boger, D. L.; Ichikawa, S. J Am Chem Soc 2000, 122, 2956–2957.
68. Boger, D. L.; Chen, J.-H.; Saionz, K. W. J Am Chem Soc 1996, 118, 1629–1644.
69. Boger, D. L.; Schule, G. J Org Chem 1998, 63, 6421–6424.
70. Boger, D. L.; Ledeboer, M. W.; Kume, M. J Am Chem Soc 1999, 121, 1098–1099.
71. Boger, D. L.; Ledeboer, M. W.; Kume, M.; Jin, Q. Angew Chem Int Ed Engl 1999, 38, 2424–2426.
72. Boger, D. L.; Chen, J.-H. Bioorg Med Chem Lett 1997, 7, 919–922.
73. Park, Y. S.; Shin, W. S.; Kim, S. K. J Antimicrob Chemother 2008, 61, 163–168.
74. Socha, A. M.; Laplante, K. L.; Russell, D. J.; Rowley, D. C. Bioorg Med Chem Lett 2009, 19, 1504–1507.
75. Tulla-Puche, J.; Marcucci, E.; Fermin, M.; Bayo-Puxan, N.; Albericio, F. Chemistry 2008, 14, 4475–4478.
76. Tulla-Puche, J.; Bayo-Puxan, N.; Moreno, J. A.; Francesch, A. M.; Cuevas, C.; Alvarez, M.; Albericio, F. J Am Chem Soc 2007, 129, 5322–5323.
77. Bayo-Puxan, N.; Tulla-Puche, J.; Albericio, F. Eur J Org Chem 2009, 2957–2974.
78. Bayo-Puxan, N.; Fernandez, A.; Tulla-Puche, J.; Riego, E.; Cuevas, C.; Alvarez, M.; Albericio, F. Chemistry 2006, 12, 9001–9009.
79. Tulla-Puche, J.; Marcucci, E.; Prats-Alfonso, E.; Bayo-Puxan, N.; Albericio, F. J Med Chem 2009, 52, 834–839.
80. Tulla-Puche, J.; Torres, A.; Calvo, P.; Royo, M.; Albericio, F. Bioconjug Chem 2008, 19, 1968–1971.
81. Dietrich, B.; Diederichsen, U. Eur J Org Chem 2005, 1, 147–153.
82. Ray, A. K.; Diederichsen, U. Eur J Org Chem 2009, 28, 4801–4809.
83. Hampshire, A. J.; Rusling, D. A.; Bryan, S.; Paumier, D.; Dawson, S. J.; Malkinson, J. P.; Searcey, M.; Fox, K. R. Biochemistry 2008, 47, 7900–7906.
84. Garcia-Martin, F.; Cruz, L. J.; Rodriguez-Mias, R. A.; Giralt, E.; Albericio, F. J Med Chem 2008, 51, 3194–3202.
85. Koketsu, K.; Oguri, H.; Watanabe, K.; Oikawa, H. Chem Biol 2008, 15, 818–828.
86. Robbel, L.; Hoyer, K. M.; Marahiel, M. A. FEBS J 2009, 276: 1641–1653.
87. Watanabe, K.; Hotta, K.; Praseuth, A. P.; Searcey, M.; Wang, C. C.; Oguri, H.; Oikawa, H. Chembiochem 2009, 10, 1965–1968.
88. Praseuth, A. P.; Praseuth, M. B.; Oguri, H.; Oikawa, H.; Watanabe, K.; Wang, C. C. Biotechnol Prog 2008, 24, 134–139.
89. Furuya, K.; Hutchinson, C. R. FEMS Microbiol Lett 1998, 168, 243–249.
90. Garcia-Bernardo, J.; Brana, A. F.; Mendez, C.; Salas, J. A. FEMS Microbiol Lett 2000, 186, 61–65.
91. Biswas, T.; Zolova, O. E.; Lombo, F.; de la Calle, F.; Salas, J. A.; Tsodikov, O. V.; Garneau-Tsodikova, S. J Mol Biol 2010, 397, 495–507.
92. Li, M. H.; Ung, P. M.; Zajkowski, J.; Garneau-Tsodikova, S.; Sherman, D. H. BMC Bioinformatics 2009, 10, 185–202.

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