



Review

Chemical Elicitors of Antibiotic Biosynthesis in Actinomycetes

Anton P. Tyurin ^{1,*} , Vera A. Alferova ¹ and Vladimir A. Korshun ^{1,2}

¹ Gause Institute of New Antibiotics, Bolshaya Pirogovskaya 11, 119021 Moscow, Russia; alferovava@gmail.com (V.A.A.); v-korshun@yandex.ru (V.A.K.)

² Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Miklukho-Maklaya 16/10, 117997 Moscow, Russia

* Correspondence: ap2rin@gmail.com; Tel.: +7-499-246-6983

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Abstract: Whole genome sequencing of actinomycetes has uncovered a new immense realm of microbial chemistry and biology. Most biosynthetic gene clusters present in genomes were found to remain “silent” under standard cultivation conditions. Some small molecules—chemical elicitors—can be used to induce the biosynthesis of antibiotics in actinobacteria and to expand the chemical diversity of secondary metabolites. Here, we outline a brief account of the basic principles of the search for regulators of this type and their application.

Keywords: actinomycetes; antibiotic biosynthesis; silent biosynthetic pathways; γ -butyrolactones; HiTES; translation inhibitors

1. Introduction

Since the discovery of streptomycin by Selman A. Waksman, actinomycetes have become one of the most fruitful sources of new antibiotics. Most antibiotic classes in current clinical use were discovered during the “golden era”, 1940–1960s, by phenotypic screening of soil microorganisms. Moreover, since the 1960s, a significant number of approved drugs has been designed using chemical modifications of natural scaffolds. Due to an “innovation gap” in this area, society is now facing an emerging threat of microbial drug resistance. An urgent need for new effective antimicrobials has become an important social and political issue [1,2].

On the other hand, achievements in genome sequencing of actinomycetes has revealed a difference between their potential and observed biosynthetic gene expression. Biosynthetic gene clusters (BGCs) are several generally contiguous genes encoding enzymes responsible for a stepwise assembly of complex bioactive molecules. According to data from the majority of published genomes, most BGCs remain “silent” under standard cultivation conditions. These silent, or cryptic, BGCs represent a potential source of new scaffolds for the discovery of novel antimicrobials [3–5]. Several techniques for activation of silent BGCs have been developed in recent decades, e.g., direct identification of BGCs and their expression in heterologous hosts [6–10], and systematic alteration of cultivation parameters (the “one strain—many compounds” (OSMAC) approach) [11–13]. These strategies are extremely powerful although still remaining laborious and resource-intensive, especially for large (>40 kb) BGCs. Further techniques comprise co-cultivation [14,15], ribosome engineering [16–18], and the use of chemical elicitors—compounds that induce the synthesis of antibiotics in actinomycetes [14,19–21]. The last approach is accounted and discussed succinctly in this review, covering literature up to December 2017. Here, we focus on small organic molecules capable in nanomolar to micromolar minimum effective concentrations to induce biosynthesis of secondary metabolite in actinomycetes.

2. γ -Butyrolactones (GBL) and Related (auto)Regulators

Historically, A-factor (**1**, Chart 1) (A for “autoregulation”) was the first compound that revolutionized our views of the secondary metabolism and development cycle of actinomycetes. This γ -butyrolactone (GBL) derivative was discovered by Prof. Khokhlov and co-workers in 1967 [22].

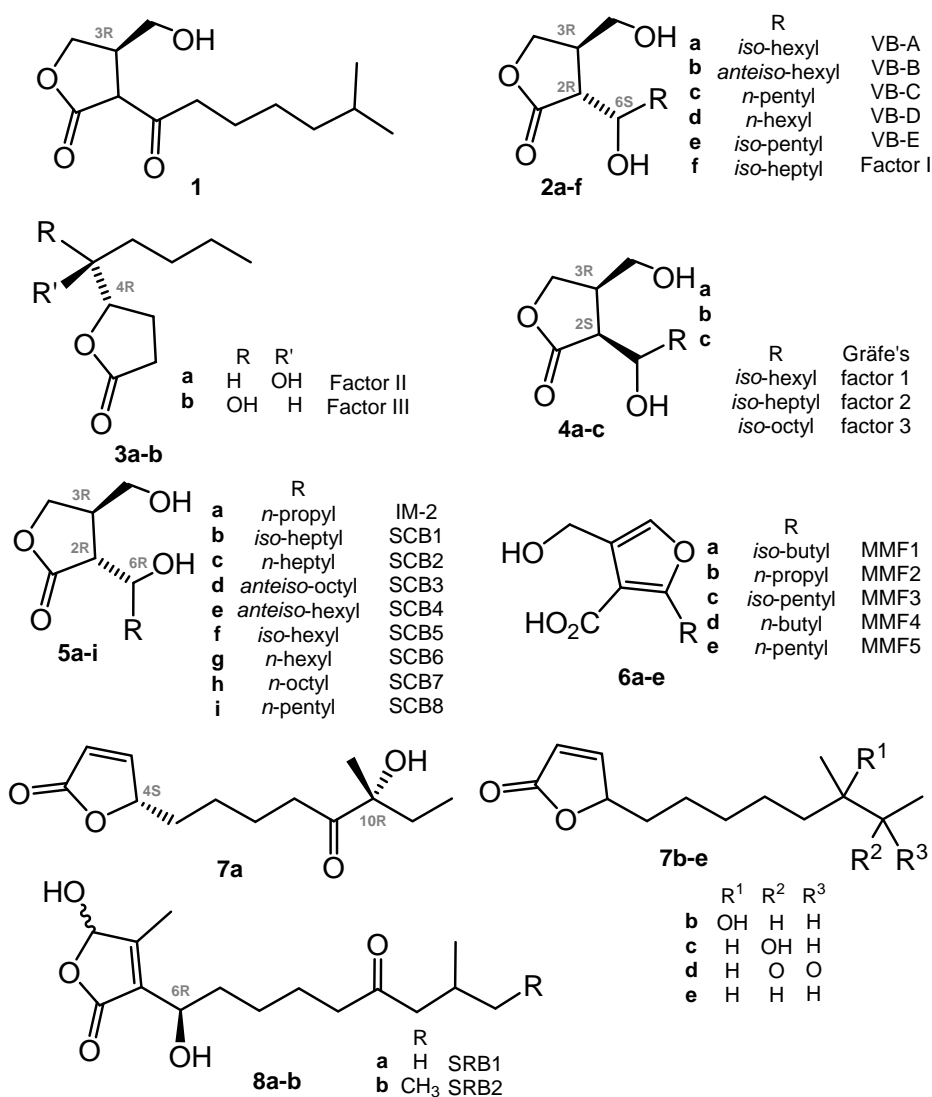


Chart 1. GBL and closely related regulators: butenolides (avenolide, *S. rochei* butenolides or SRB) and furans (methylenomycin furans or MMF).

A-factor acts as a pleiotropic regulator: it binds to the A-factor receptor protein (ArpA) and causes dissociation of this suppressor from DNA. This triggers the transcription of the *adpA* gene encoding the transcription activator AdpA, which in turn induces morphological differentiation, spore formation, and biosynthesis of secondary metabolites [23,24].

In further decades, many other closely related autoregulators were discovered, e.g., *Streptomyces virginiae* butenolides (VBs A-E, **2a–e**) [25–27], Factor I (**2f**) [28], Factors II and III (**3a,b**) [29], Gräfe's factors (**4a–c**) from *S. bikiniensis* and *S. cyaneofuscatus* [30], IM-2 (**5a**) [31,32], SCB1 (**5b**) [33,34], SCB2,3 (**5c,d**) [35] and SCB4–8 (**5e–i**) [36], methylenomycin furans (MMFs, **6**) [37], avenolide (**7a**) from *S. avermitilis* [38], related compounds (**7b–e**) from *S. albus* [39], and two *S. rochei* butenolides (SRBs, **8a,b**) [40]. The stereoconfiguration of avenolide analogues **7b–e** reported very recently has not been assigned [39]. All these compounds have a high degree of similarity in their chemical

nature (butanolides, butenolides, or furans), biosynthesis pathways, and in mechanisms of action on actinomycetes. The transduction of the chemical signal starts from binding with a specific receptor protein (like ArpA in the A-factor signaling cascade) belonging to the TetR family of transcriptional regulators; the binding of compounds probably causes conformational changes, thus inducing the relocation of the DNA-binding domain and DNA release [23].

TetR transcriptional repressors are well represented and widespread in bacteria [41,42]. The recent study on the distribution of the GBL (IM-2, VB)-type and butenolide (avenolide)-type signaling showed these hormones to be common in actinomycetes. Production of regulators was screened using *S. lavendulae* FRI-5 ($\Delta farX$), *S. virginiae* ($\Delta barX$), and *S. avermitilis* (Δaco) strains with disrupted essential biosynthetic genes for the corresponding hormone-like molecules. Twenty percent of the investigated strains produced **5a** (IM-2), another 20% produced VBs, and 24% of actinomycetes showed avenolide activity [43].

Thus, the potential for various applications of A-factor-like regulators is quite high: they induce biosynthesis of antibiotics of different classes, e.g., aminoglycosides (streptomycin), streptogramins (virginamycin), peptide ionophores (valinomycin) and others, including clinically significant antibiotics. For more detail see ref. [24].

3. Other Types of (auto)Regulators

However, GBLs and related compounds are not the only type of regulators found in actinomycetes. Hitherto, a few different types of autoregulators were reported (Chart 2), e.g., the pi-factor from *S. natalensis* (**9**) [44] and L-N-methylphenylalanyl-dehydrobutyryne diketopiperazine (**10**) from *S. globisporus* [45]. These molecules have no structural similarity to GBL-type compounds and their mechanisms of action remain unclear [46,47].

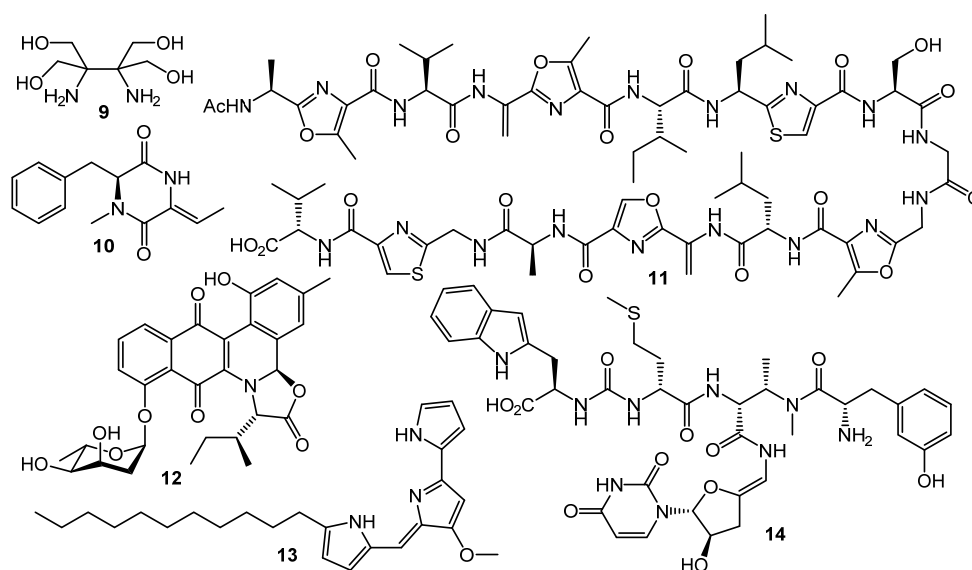


Chart 2. Other (non-GBL) types of regulators.

Goadsporin (**11**) [48,49], an active 19-aa peptide found in the culture broth of *Streptomyces* sp. TP-A0584, shows extremely broad elicitation activity on actinobacteria. Induction of sporulation and growth inhibition were observed in about 80% of *Streptomyces* sp. and *Micromonospora* sp. isolates after goadsporin treatment [50]. Possible mechanism of the goadsporin action on susceptible cells was postulated by Prof. H. Onaka and co-workers. Bioinformatics analysis of *godI*—self-resistance gene from the goadsporin BGC—showed a high degree of homology with *ffh* encoding the Ffh protein. The Ffh and 4.5S RNA form the signal recognition particles (SRPs) in prokaryotic cells. The SRPs recognize the signal peptide of membrane or secretory proteins and facilitate their translocation to the

cytoplasmic membrane. Binding of Ffh protein with goadsporin causes the upregulation of protein translocation and maturation, growth inhibition, and alternation of secondary metabolism [51].

It was suggested that goadsporin and its analogs [51,52] could be useful in crop protection: *S. scabies*, which is known to cause potato scab, is effectively inhibited by the low concentrations of goadsporin.

Two “atypical” transcriptional regulators were discovered in *S. venezuelae*: JadR1 activates the transcription of jadomycin B (12) biosynthetic genes while repressing its own gene. Jadomycin B binds to the N-terminal receiver domain of JadR1, causing dissociation of JadR1 from target promoters. High expression of the biosynthetic enzyme genes leads to accumulation of the product in a concentration sufficient to inhibit the initial activation. Therefore, jadomycin acts as an autoregulator of its own synthesis. Similarly, the RedZ protein regulating production of undecylprodigiosine (13) is modulated by the end product. These findings demonstrate that end-product-mediated control of antibiotic biosynthesis may be widespread in nature, offering seminal basis for interspecies signaling and interaction [53,54]. Production of another class of antibiotics, uridyl peptides sansanmycins (sansanmycin A, 14), active against multidrug-resistant *Mycobacterium tuberculosis*, is also controlled at the transcriptional level by the end-product-mediated mechanism [55].

Polyether antibiotics at sub-inhibitory concentrations can elicit the synthesis of secondary metabolites. Promomycin (15, Chart 3) and closely related compounds (salinomycin, monensin, and nigericin) promoted the synthesis of cryptic isonitrile antibiotic SF2768 (16) by *S. griseorubiginosus* [56,57]. This was the first example of isolation of a novel original compound using the chemical elicitation approach. However, the action of polyether antibiotics has not yet been studied on the molecular level.

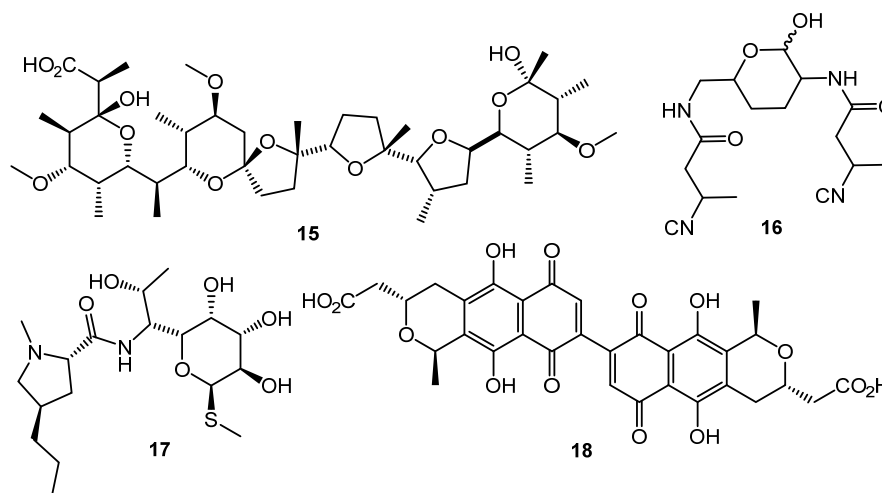


Chart 3. Antibiotic-antibiotic induction; elicitors and products.

Lincomycin (17) at one tenth of its MIC increased the expression of the pathway-specific regulatory gene actII-ORF4 in the enzyme gene cluster producing the blue-pigmented antibiotic actinorhodin (18) in *S. lividans*, thus resulting in actinorhodin overproduction [58]. Chloramphenicol activated biosynthetic genes at the transcriptional level and increased the amino acid pool 1.5- to 6-fold, enhancing the production of non-ribosomal peptide antibiotics [59]. Other ribosome-targeted antibiotics, especially thiostepone and spectinomycin, at sub-inhibitory concentrations were also capable of altering colony morphology and antibiotics (13 and 18) production in *S. coelicolor* M145 [60].

The HiTES (High-Throughput Elicitor Screening) approach [61–63] was developed to discover small molecule elicitors of silent biosynthetic gene clusters and novel secondary metabolites using a simple fluorescent assay format. Three copies of *eGFP* (enhanced green fluorescent protein encoding gene) inserted in the *S. albus* J1074 genome both at a neutral site (*attB*) and inside the biosynthetic

cluster of interest (*sur*, surugamide BGC). The difference in fluorescence between *attB::P_{sur}-eGFPx3* (the *P_{sur}* promoter region (~260 bp) upstream of *surE*) and *surE::eGFPx3* strains after testing compound treatment was used as an indicator of BGC's activation. Screening of a commercially-available natural products library (ca. 500 compounds) identified 6 potential elicitors, among these ivermectin b1a (**19**, Chart 4) and etoposide (**20**) had the maximum surugamide BGC activation effect and markedly changed the secondary metabolome profile of *S. albus* J1074—14 novel small molecules were isolated and characterized using these two inducers, e.g., surugamide J (**21**), albucyclone F (**22**), and surugamide F2 (**23**) [63].

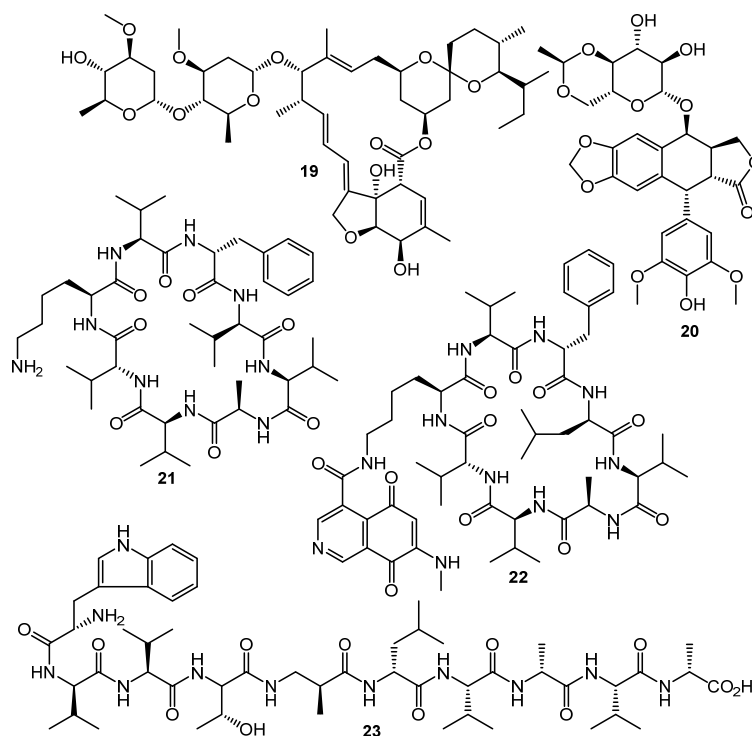


Chart 4. Natural products eliciting surugamide biosynthesis in *S. albus* J1074 (detected by HiTES).

4. Metabolism Remodeling and Epigenetic Control

Fully synthetic chemicals also can be utilized as elicitors. ARC2 (**24**, ARC from “Antibiotic-Remodeling-Compound”) and similar diphenyl ethers (ARC3–5, **25–27**) (Chart 5) have become the first inducer examples discovered by systematic screening of 30,569 small molecules [64,65]. Taking into account the structural similarity of ARC compounds with triclosan (**28**), a well-known biocide and inhibitor of fatty acid biosynthesis, the mechanism of ARCs' action (Chart 5A) has been proposed. Generally, ARCs act as metabolism remodeling compounds, changing the balance between primary and secondary metabolic pathways. The mode of action was confirmed by the experimental data: ARCs changed fatty acid pool by inhibition of key biosynthetic enzymes like FabI. Remarkably, triclosan also can stimulate polyketide antibiotic synthesis at sub-inhibitory concentrations [59]. Further investigation of the different ARCs' effects on the streptomycetes biochemistry revealed the targets for different molecules (even structurally close) as not being the same; however, all of them influenced fatty acid metabolism [66]. Chlorinated analog Cl-ARC (**29**) was used to enhance the expression of cryptic biosynthetic genes, and the observed difference between growth on control and elicitor-containing media was studied using a metabolomics approach. More than 100 induced secondary metabolites were detected, including rare antibiotics [67]. Several identified structures are represented in Chart 6: oxohygroolidin (**30**), germicidins A–E (**31a–e**) 9-methylstreptimidone (**32**), nactins (**33**, **34**, **35**), desferrioxamines B,E (**36**, **37**), and arylomycin A₄ (**38**).

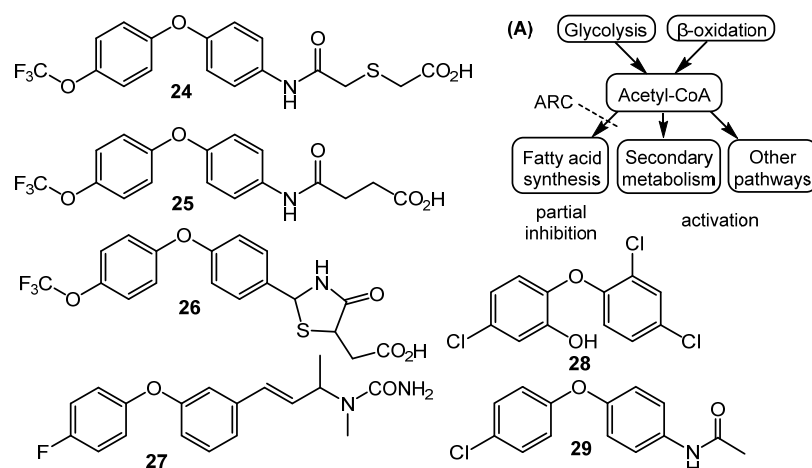


Chart 5. Synthetic diphenyl ethers (ARCs) as elicitors and their mode of action (A).

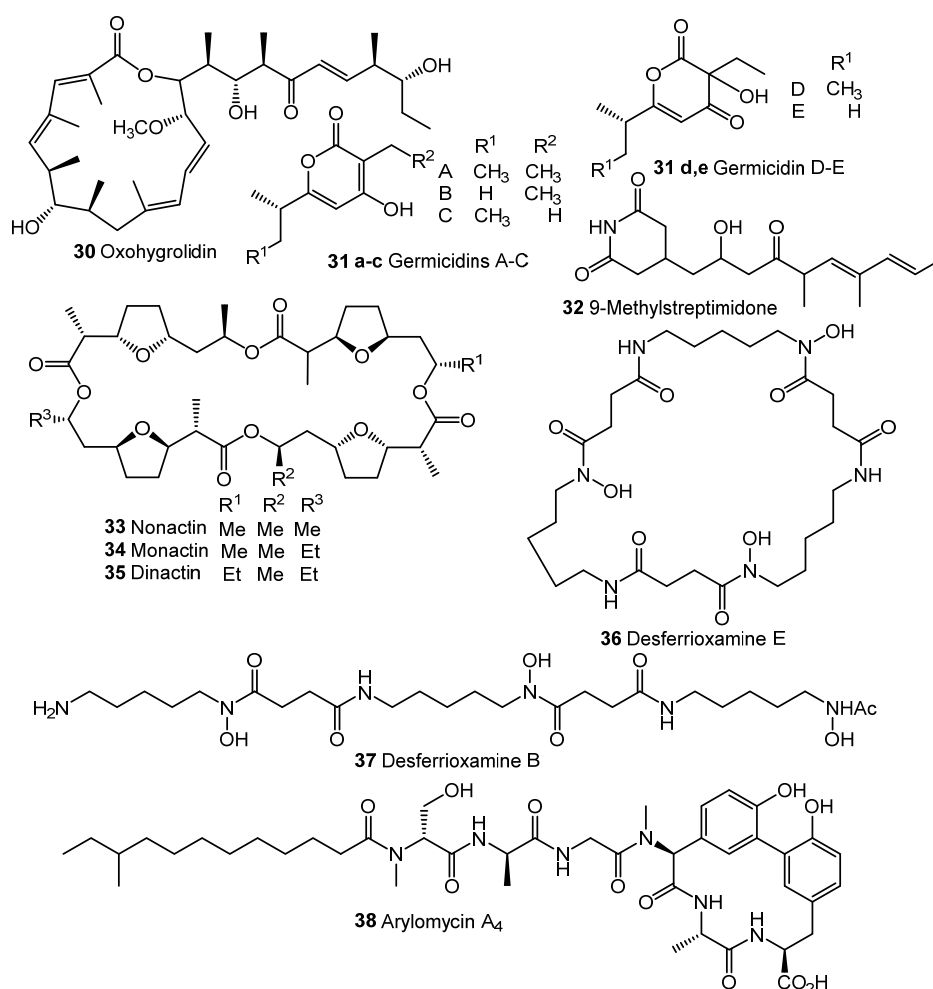


Chart 6. Cl-ARC-induced biosynthesis: examples of identified antibiotics [67].

Despite the significant difference in structural DNA organization between eukaryotes and actinomycetes, inhibitors of histone deacetylase (HDAC) like valproic acid (39) and suberanilohydroxamic acid (SAHA, 40) (Chart 7) showed effect on antibiotic production by several *Streptomyces* strains, especially on nutrient-poor media. The finding was explained by the homology

between several *S. coelicolor* enzymes (SCO0452, SCO6464, SCO3330) and human HDAC enzymes [68]. Epigenetic modification could be used for the control of antibiotic biosynthesis; the approach, however, is not well studied for actinobacteria.

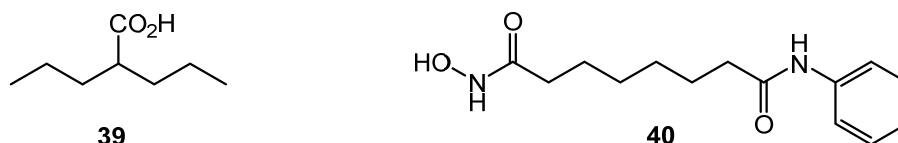


Chart 7. Histone deacetylase (HDAC) inhibitors.

5. Conclusions

Activation of antibiotics' biosynthesis in actinomycetes by using small-molecule elicitors seems to be a useful technique for drug discovery. The approach allows identification of new metabolite scaffolds and enhances synthesis of low yield secondary metabolites. Moreover, antibiotic-antibiotic induction relationships are believed to be the basis of chemical signaling between different actinobacterial strains and are, therefore, of interest for chemical ecology. Elicitation is an obvious cost-effective approach in biotechnology for increasing antibiotic yields and reducing fermentation time.

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Conflicts of Interest: The authors declare no conflict of interest.

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