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The Expansive Library of Jadomycins

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Draft

Abstract

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The jadomycin family of natural products were discovered from *Streptomyces venezuelae* ISP5230 in the 1990s. Subsequent identification of the biosynthetic gene cluster along with synthetic efforts established that incorporation of an amino acid into the polyaromatic angucycline core occurs non-enzymatically. Over two decades, the precursor-directed biosynthetic potential of the jadomycins has been heavily exploited, generating a library approaching seventy compounds. This review compiles the jadomycins isolated and characterized to date; these include jadomycins incorporating proteinogenic and non-proteinogenic amino acids, semi-synthetic derivatives, biosynthetic shunt products, compounds isolated in structural gene deletion studies, and deoxysugar sugar variant jadomycins produced by deletion or heterologous expression of sugar biosynthetic genes.

Key words: jadomycin, precursor-directed biosynthesis, semi-synthesis, biosynthetic gene cluster, gene inactivation, combinatorial biosynthesis, natural products, secondary metabolite

Introduction

The jadomycins (Jd) are a family of angucyclines, type-II polyketide-derived natural products,¹ which are produced by *Streptomyces venezuelae* ISP5230 (ATCC 10712).^{2,3} The jadomycins are distinct from the other natural product angucyclines by their inclusion of a nitrogenous heterocycle,¹ which is the result of a non-enzymatic incorporation of an amino acid into the polyaromatic core.^{4,5} This non-enzymatic process has permitted the generation of a large library of jadomycins, enabling the determination of structure-activity relationships with respect to the antibiotic⁶ and cytotoxic⁷⁻¹⁰ activity of the jadomycins. Jadomycin nomenclature often includes the incorporated amino acid, for instance, jadomycin S (Jd S) refers to the natural product isolated through incorporation of L-serine into the jadomycin. However, there are increasing numbers of exceptions to this naming convention, attributed to multiple compounds arising from incorporation of the same amino acid or the absence of an accepted single letter abbreviation for a particular non-proteinogenic amino acid.

Herein, the current library of jadomycins are compiled. Compounds include jadomycins produced through precursor-directed biosynthesis with proteinogenic and non-proteinogenic amino acids (Figures 1-2) or by subsequent synthetic derivatization (Figure 3). Compounds have also been isolated from gene deletion strains or as biosynthetic shunt products (Figure 4), or are glycoside variant jadomycins produced through deletion and/or heterologous expression of deoxysugar biosynthetic genes (Figure 5).

Precursor-Directed Biosynthesis

Oxazolone Ring Containing Jadomycins

Jd A (**1**, Figure 1) was the first jadomycin discovered, identified by Ayer *et al.* from a culture of *S. venezuelae* ISP5230 as a cryptic natural product induced by heat shock at 37°C.² The minimal galactose/isoleucine media, intended for production of chloramphenicol, a colourless natural product also produced by the same strain, became deeply pigmented, as a consequence of incubator temperature regulation failure.¹¹ Subsequent investigation by Doull *et al.* demonstrated that the major biosynthetic product was the L-digitoxosylated Jd B (**2**).³ As the structures of **1** and **2** incorporate the amino acid (isoleucine) used in the culture media, fused with the angucycline core into an oxazolone ring labelled the E-ring, a hypothesis was put forward that substitution of L-isoleucine in the production media would generate a variety of jadomycins, incorporating alternate amino acids. The hypothesis was proven correct; *S. venezuelae* cultures using each of the twenty proteogenic amino acids produced characteristically pigmented media.⁵ The isolation and preliminary characterization by Doull *et al.* of Jd V, F, and Ala (**3-5**) produced with L-valine, L-phenylalanine, and L-alanine, respectively, demonstrated the applicability of amino acid selection as a method of precursor-directed biosynthesis.⁴ The jadomycin repertoire was expanded by culturing with L-leucine, L-tyrosine, L-methionine, L-histidine, and L-asparagine (**6-10**).^{4,12,13} Low resolution MS/MS evidence supporting jadomycin incorporation of each of the remaining proteinogenic amino acids has been collected,¹⁴ although to date only the oxazolone-containing jadomycins incorporating L-tryptophan, L-lysine, and glycine (**11-13**) have been isolated and further characterized.^{13,15}

Figure 1. (A) Jadomycin A; (B) jadomycin biosynthesis i. type II polyketide synthesis (JadABCJMNDEI), oxidation (JadFGH); ii. ring cleavage and decarboxylation (JadGK); iii. amino acid incorporation; iv. intramolecular cyclization, oxidation; (C) oxazolone ring containing jadomycins produced by precursor-directed biosynthesis using proteogenic and non-proteogenic amino acids. Italicised names indicate jadomycins tentatively identified by HPLC/MS.

A variety of D-configured and other non-proteinogenic amino acids have been incorporated, demonstrating that *S. venezuelae* is tolerant of a variety of different nitrogen sources that are conducive to jadomycin derivatization by precursor directed biosynthesis. Jadomycins *S*- and *R*-phenylglycine (**14-15**),¹⁶ D-valine (**16**),¹⁴ D-methionine (**17**),¹³ D-norvaline (**18**), D-norleucine (**19**),¹⁷ L-norleucine (**20**), L-2-aminobutanoic acid (**21**), L-homoserine (**22**), L-2,4-diaminobutanoic acid (**23**), *O*-propargyl-L-serine (**24**),¹⁸ 4-amino-L-phenylalanine (**25**),¹⁹ L-ornithine (**26**),¹⁵ N_ε-trifluoroacetyl-L-lysine (**27**) have been isolated and fully characterized.²⁰ Additionally, HPLC-MS data supported the incorporation of D-isoleucine (**28**), *O*-methoxy-L-threonine (**29**), *O*-methoxy-methylene-L-threonine (**30**),¹⁴ DL-4-fluorophenylalanine (**31**), and 2-aminoisobutyric acid (**32**),¹⁶ however, these natural products have not been comprehensively characterized to date.

Non-Oxazolone and Extended E-ring Jadomycins

E-ring formation occurs as a result of amino acid condensation with an aldehyde to produce an imine intermediate (Figure 1B).²¹ A nucleophile then reacts with the imine, which is often the intramolecular α -carboxylic acid or side chain group (i.e. OH, NH₂) of the incorporated amino acid but not always, to produce a corresponding variant and generating an E-ring when an intramolecular reaction has occurred. When an intramolecular reaction does not occur, either a solvent or cellular nucleophile reacts with the imine to provide jadomycins with linear amino acid incorporation. The spontaneous nature of this reaction was established by total synthesis.²² Given the non-enzymatic process, the mode of incorporation is dependent on the properties of the amino acid.

Jd N (**10**, Figure 2A) was produced through the preferential cyclization of L-asparagine with the terminal amide over the carboxylic acid, giving a six-membered E-ring with 3a-*R* configuration.^{4,13,16} Likewise, a dominant stereoisomer (>95% 3a-*R* configuration) had been observed during characterization of Jd S (**33**) and T (**34**), produced with L-serine and L-threonine, respectively.⁴ Synthetic preparation of Jd S and Jd T aglycones by Akagi *et al.* prompted a structural revision of the initially proposed oxazolone-ring containing structures, as their characterization substantiated the formation of an oxazolidine ring arising from cyclization of the side-chain hydroxyl groups.²³ This was confirmed by Yang *et al.* during their total synthesis of **33** and **34**.²²

Dalomycin T (**35**) was isolated as an apparent shunt product in the isolation of **34**, where an oxazolidine E-ring is formed and the side chain carboxylic acid is proposed to be chemically decarboxylated and methylated.²⁴ Additional jadomycin congeners containing D-serine (**36**)⁶ and D-threonine (**37**)¹³ have also been characterized, each possessing the oxazolidine cyclization and a single dominant (>95%) 3a stereoisomer. Jadomycins **33-37** were produced from amino acids possessing primary or secondary alcohol sides chains. Jadomycins for which one diastereomer is predominant, (jadomycins S (**33**), T (**34**),⁴ N (**10**), H (**9**),¹³ K (**12**) and Orn (**26**)¹⁵), derive from amino acids with hydroxyl- or amino-containing side chains which cyclize as discussed above. Structures for jadomycins isolated from the incorporation of amino acids L-aspartic acid (D), L-glutamic acid (E), L-cysteine (C), L-glutamine (Q), L-proline (P) and L-arginine (R) are not proposed in this review; while the corresponding jadomycin analogues have been detected by low-resolution mass spectrometry, they have not been isolated and fully characterized and the available data does not provide insight into the structure of the E-ring.¹⁴

Figure 2. (A) Jadomycins produced by precursor-directed biosynthesis using proteogenic and non-proteogenic amino acids that do not incorporate a five-membered oxazolone ring. Italicised name indicates

jadomycin only identified by HPLC/MS. (B) Equilibration of jadomycin diastereomers through an aldehyde intermediate.²⁴

A series of amino acids that produce larger E-ring jadomycin variants have been studied. These include Jd N (**10**), containing an 6-membered oxohexahydropyrimidine ring.¹³ Other six-membered ring jadomycins were produced with β -alanine (**38**) and DL-3-aminoisobutyric acid (**39**) and initially identified by LCMS.¹⁶ **38** was later isolated and characterized.^{13,16} The incorporation of L-ornithine produced a jadomycin with an eight-membered E-ring, Jd Oct (**40**), the product of cyclization from the amino acid side chain terminal -NH₂.²⁵ The eight-membered ring within Jd Oct was consistent with observed HMBC and ROESY correlations; the α -amino side chain of **40** was subsequently synthetically decorated, as detailed in the next section.²⁵ Fan and co-workers have reported an L-ornithine analogue with a five-membered oxazolone E-ring jadomycin (Jd Orn, **26**).¹⁵ A series of linear alkyl amino acids produced jadomycins with seven- to nine-membered E-rings (Figure 2A), Jd Hep, Jd AVA,²⁵ and Jd Non²⁶ (**41-43**) has been reported. Isolation of a ten-membered E-ring analogue was reported, although later revised to structure **53u** in a corrigendum.²⁷ To date, Jadomycin Non (**43**) contained the largest ring size with nine-members. A kinetic explanation has been put forth as the limiting factor in producing larger ring sizes using extended alkyl amino acids.²⁸

Semi-synthetic jadomycin derivatives

Jadomycins incorporating amino acids with reactive handles have been used for further chemical derivatization. *O*-propargyl-L-serine was incorporated into a parent jadomycin, Jd OPS (**24**, Figure 1) and, on crude isolation, the terminal alkyne functionality was reacted with eight azides to produce a series of triazole-containing jadomycins (**24a-i**, Figure 3). In another study, jadomycins incorporating a free-amino group, 4-amino-L-phenylalanine (**25**)¹⁹ and L-ornithine (**40**)²⁵ were selectively acylated using a series of succinimidyl esters (**25j-l**, **40m-r**). In the case of L-ornithine, chemical derivatization served as a strategy to facilitate isolation of Jd Oct (**40**) due to significant breakdown of the jadomycin during purification; restoration of **40** for characterization was achieved by subsequent acyl cleavage from the semi-synthetic product **40n**.

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140

141 **Figure 3.** Jadomycins produced by semi-synthetic derivatization.

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Jadomycin Biosynthetic Shunt Products

Structures that preclude amino acid incorporation include L-digitoxosyl dehydrabelomycin (**46**), an intermediate in the jadomycin biosynthetic pathway which accumulates upon inactivation of the post-PKS gene *jadG*.²⁹ Several biosynthetic shunt products have been isolated by evaluating *jad* gene deletion/disruption strains but are not included in this manuscript because they are common to multiple biosynthetic pathways.^{30,31} Other isolated shunt products include two furan-containing structures, **47** and **48** (Figure 4) from a jadomycin production using *N*_ε-trifluoroacetyl-L-lysine (TFAL).²⁰ The furan ring was proposed to arise through an opening of the oxepinone ring (Figure 1B(ii)) and subsequent ring closure facilitated by the A-ring hydroxyl to generate **47**, proceeding to **48** by spontaneous or enzymatic reduction. It remains unclear whether the formation of these products is related directly to the presence of TFAL in the production media. L-Digitoxosylated phenanthroviridin (**49**) was isolated recently when Robertson and co-workers were investigating the incorporation of L-lysine.³² The authors proposed that apparent instability of the L-lysine jadomycin analogue, congruent with previous reports,¹⁵ led to a breakdown product **49**.

Jadomycin with Linear Amino Acid Incorporation and Post-PKS Modifications

A suite of jadomycin analogues have been isolated and characterized from cultures with amino acids where cyclization is unfavourable due to geometric constraints or intramolecular kinetics.^{20,27,33-35} Jd TFAL lactam (**50p**) was isolated with **27** in comparable yields. It was proposed that these compounds are produced through divergent mechanisms of B-ring cyclization, followed by oxidation at the C3a position to produce **50p**.

Figure 4. Jadomycin biosynthetic pathway precursors and shunt products.

A series of jadomycin analogues identified from productions using 4-aminomethylbenzoic acid (4-AMBA) varied by the functionality at the 3a position; these included a jadomycin with oxidation an C3 (**51p**), as

well as substitution to give methoxy (**51q**), hydroxy (**51r**), acetate (**51s**), and pyruvate (**51t**) substituents.³³ The hydroxy (**51r**) and methoxy (**51q**) analogues were shown to interchange through solvolysis. The acetate (**51s**) and pyruvate (**51t**) analogues represent post-PKS carbon-carbon bond formation products that are unusual in type II polyketide biosynthesis. These 3a-branched analogues are speculated to be produced by nucleophilic substitution of a reactive intermediate when intramolecular cyclization is prohibited or disfavoured, as determined by the properties of the amino acid. Robertson *et al.* proposed that these analogues were produced by trapping with primary metabolite cofactors, as there were no known post-PKS enzymes encoded in the jadomycin cluster predicted to catalyze this transformation.³³ Three corresponding 3-aminomethylbenzoic acid (3-AMBA) analogues (**52p**, **q**, **s**) were also reported in the study.³³ Similar carbon-branched analogues **53u** and **54u** were isolated from cultures using 7-aminoheptanoic or 8-aminooctanoic acid.^{27,35} Thus, when using linear alkyl amino acids, carbon-carbon bond formation at the 3a position became preferred as the chain length of the amino acids was extended since intramolecular cyclization of nine-membered and larger rings was found to be disfavoured.²⁸ The lactam **55q** was purified alongside **40** from jadomycin productions using L-ornithine and was proposed to be formed from the collapse of the five-membered oxazolone E-ring containing **26**.³⁴

Sugar modification of Jadomycins

The L-digitoxyl deoxy biosynthetic gene cluster was elucidated by Wang and co-workers through sequence homology and by evaluation of disruption mutants.³⁶ On entry into the pathway, α -D-glucose 1-phosphate is converted by a nucleotidyltransferase, JadQ, into an activated donor, NDP- α -D-glucose. Subsequently, the hexose ring is transformed by JadTOPUV to provide the final donor, NDP-L-digitoxose, that serves as the substrate for the pathway-specific glycosyltransferase JadS (Figure 5a). This L-digitoxyl biosynthetic pathway is conserved in the production of other natural products, including tetrocarcin A³⁷ and kijanimicin^{38,39} and is consistent with natural product deoxysugar biosynthetic pathways.^{40,41}

Jadomycins bearing sugars divergent from the usual L-digitoxose have been generated through genetic engineering of *S. venezuelae*, with the exception of the synthetically prepared carbasugar-containing jadomycin B (**56**).⁴² Such efforts have generated glycodiversified jadomycins with eight distinct sugar moieties (Figure 5B). Jakeman *et al.* identified a jadomycin B analogue (**57**) decorated by a sugar with the 2-hydroxy group intact isolated from a mutant (strain ILEVS1080³⁶) with disruption of the biosynthetic 2,3-dehydratase, *jadO*.⁴³ Identification of the sugar as 6-deoxy-L-altropyranoside implicated that downstream enzymes JadU and JadV, a 5-epimerase and 4-ketoreductase, respectively, were able to act on the JadT product, NDP-4-keto-6-deoxy-D-glucose. Forget *et al.* evaluated the strain *S. venezuelae* Δ *jadT*, possessing a deletion of the gene encoding a 4,6-dehydratase, which was found to produce a jadomycin D-serine congener with D-glucose appended (**58a**).⁴⁴ A double gene deletion mutant, *S. venezuelae* Δ *jadST*, was not found to produce the glucosylated jadomycin, indicating that JadS was responsible for catalysis. Both **57** and **58a** were isolated in milligram quantities and fully characterized.^{43,44} In another study, *S. venezuelae* Δ *jadUTV* was evaluated by Li *et al.* and was found to produce a D-glucosyl jadomycin B analogue (**58b**).⁴⁵ In that same study, complementation of *S. venezuelae* Δ *jadTUV* with an expression vector encoding JadT and UrdR, the 4-keto-reductase generating D-olivose in urdamycin biosynthesis,⁴⁶ was found to generate a D-olivosylated jadomycin B analogue (**60a**). *S. venezuelae* Δ *jadTUV* and *S. venezuelae* Δ *jadOPQSTUV* served as hosts for heterologous expression of various combinations of partially reconstituted L-digitoxose biosynthetic genes and/or urdR to produce jadomycin B analogues decorated with 6-deoxy-D-glucose (**59**), 4-keto-6-deoxy-D-glucose (**61**), 2,6-dideoxy-D-threo-4-hexulose (**62**) and 2,6-L-erythro-4-hexulose (**63**). Culturing the strain *S. venezuelae* Δ *jadUTV::jadTurDR*, which produces D-

209 olivose, with different amino acids, allowed for the detection of eleven additional E-ring variant D-
210 olivosylated jadomycins (**60b-l**). Of the compounds described, **60a-d** were produced in sufficient yields for
211 isolation and full characterization; the authors cited poor yield and compound instability as limiting factors
212 in attempting to isolate the remaining analogues.⁴⁵

213 A survey of the literature demonstrated that jadomycin yields are highly variable depending on the amino
214 acid selected in the culture media. The studies highlighted here demonstrate that manipulation of the sugar
215 results in significant decreases in yield. Indeed, the reported yield for sugar modified **58a** suggests
216 production of 3 $\mu\text{g mL}^{-1}$ in comparison to 22 $\mu\text{g mL}^{-1}$ for its L-digitoxylated counterpart, Jd DS (**36**).^{44,47}
217 Likewise, for D-olivosylated jadomycin (**58a**) the reported yield suggested 0.3 $\mu\text{g mL}^{-1}$, in contrast to
218 standard Jd B (**2**) reported to reach up to 69 $\mu\text{g mL}^{-1}$.^{5,44,45} In all cases, these complemented systems were
219 reliant on the relaxed specificity of native JadS to catalyze glycosyltransfer and deliver the sugar-modified
220 jadomycin analogues. These studies reveal JadS as a glycosyltransferase with tolerance to variation about
221 the C-2, C-3, C-5, and C-6 positions of the donor hexose ring. Over the past decade or so, the
222 characterization of various bacterial glycosyltransferases as flexible with respect to sugar donor specificity,
223 including, UrdGT2,⁴⁸ DesI,⁴⁹ StagG,⁴⁰ EmlGT,⁵⁰ CalGI, CalG4, GftD, GfTE,⁵¹ RebG,⁵² and YijC,⁵³ have
224 challenged the dogma of stringency in secondary metabolite biosynthesis. It is evident that JadS can be
225 considered amongst the growing ranks of flexible glycosyltransferases involved in natural product
226 biosynthesis.

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Figure 5. Jadomycins containing alternate sugar moieties produced by gene deletion and heterologous expression of deoxysugar genes. (A) Biosynthesis of L-digitoxose and the corresponding (inset) dideoxy sugar gene cluster shown from *S. venezuelae*; (B) jadomycins decorated with non-native sugars.

Looking Forward

At the time of publication, over forty-five of the described jadomycins have been evaluated for biological activity, including various cancer cytotoxicity assays^{7-10,13,15,17-20,25,32-35} and antimicrobial panels.^{6,20,33,34} Jadomycins have been implicated in the inhibition of aurora kinase B^{10,54} and topoisomerases.^{8,55} Other studies have demonstrated DNA-cleavage by jadomycin through a generation of reactive oxygen species in the presence of Cu(II),^{9,17,18,25,56} although further studies have concluded that ROS-dependant mechanisms are not the primary contributor to bioactivity.⁸ In triple negative breast cancer cell lines, jadomycins have shown resistance of drug efflux mechanisms.^{10,33} While an extended evaluation of the structure-activity relationships was beyond the scope of this review, given the various techniques employed in the evaluations rendering direct comparisons challenging, there was ample evidence in the above studies to suggest that the incorporated amino acid effected the observed bioactivity.

A co-amino acid supplementation method for jadomycin production was recently reported for use with amino acids that were unable to support cell proliferation of *S. venezuelae* alone in liquid culture. This was used for the isolation of jadomycins incorporating 3- or 4-aminomethylbenzoic acids (**51** and **52**) and 8-aminooctanoic acid (**54**).^{33,35} Going forward, the use of this co-amino acid supplementation method to facilitate jadomycin production will broaden the repertoire of non-proteinogenic amino acids that can be incorporated through precursor-directed biosynthesis by limiting the reliance on the physiological compatibility of the amino acid. Additional limitations to amino acid selection are the physicochemical properties of the amino acids, such as the solubility in the production media at pH levels conducive to cell proliferation. With respect to heterologous expression as a method of jadomycin analogue production, non-native sugar biosynthetic genes in *S. venezuelae* have furnished corresponding sugar modified jadomycins, however, such engineering approaches have provided only limited quantities of the modified analogues, often less than 0.5 $\mu\text{g mL}^{-1}$.^{10,33,34} Improving the yields in these systems to enable isolation, characterization and subsequent biological evaluation of new analogues remains a crucial aim to broaden the scope of such approaches. Nevertheless, the observed substrate flexibility of the glycosyltransferase, JadS, offers opportunities for glycodiversification of jadomycin type natural products.

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