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Diverse DNA-Cleaving Capacities of the Jadomycins through Precursor-Directed Biosynthesis

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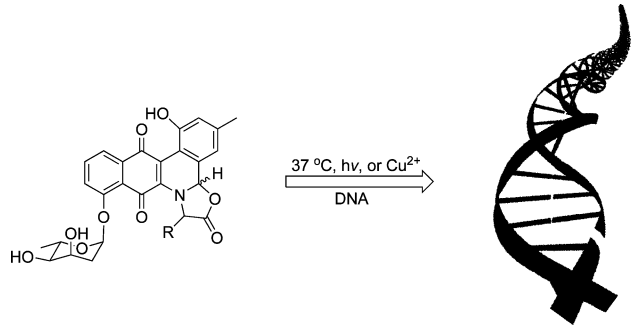
ABSTRACT



Gel mobility assays were used to establish that some members of the jadomycin family of natural products act as DNA cleaving agents. Moreover, it was found that subtle structural changes generated through the use of precursor-directed biosynthesis lead to marked effects on the DNA-damaging properties of these glycosylated polyketide-derived natural products.

Jadomycins belong to the angucycline group of polyketides, angular tetracyclines that are known to display a range of important biological activities, including antibacterial, antitumor, antiviral, and Aurora-B kinase inhibitory activities (Figure 1).[1−6] One strategy for generating structural diversity within the jadomycin family, as well as other natural product families, entails harnessing an organism's inherent biological machinery for synthetic means. The repertoire of jadomycins available for biological testing has been greatly expanded through the identification and exploitation of key steps in the jadomycin biosynthetic pathway of the Gram-positive soil bacteria *Streptomyces venezuelae* ISP5230.[6−10] System-

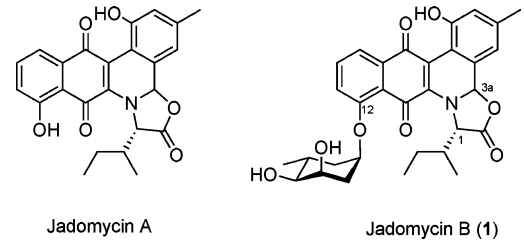


Figure 1. Structures of jadomycins A and B.

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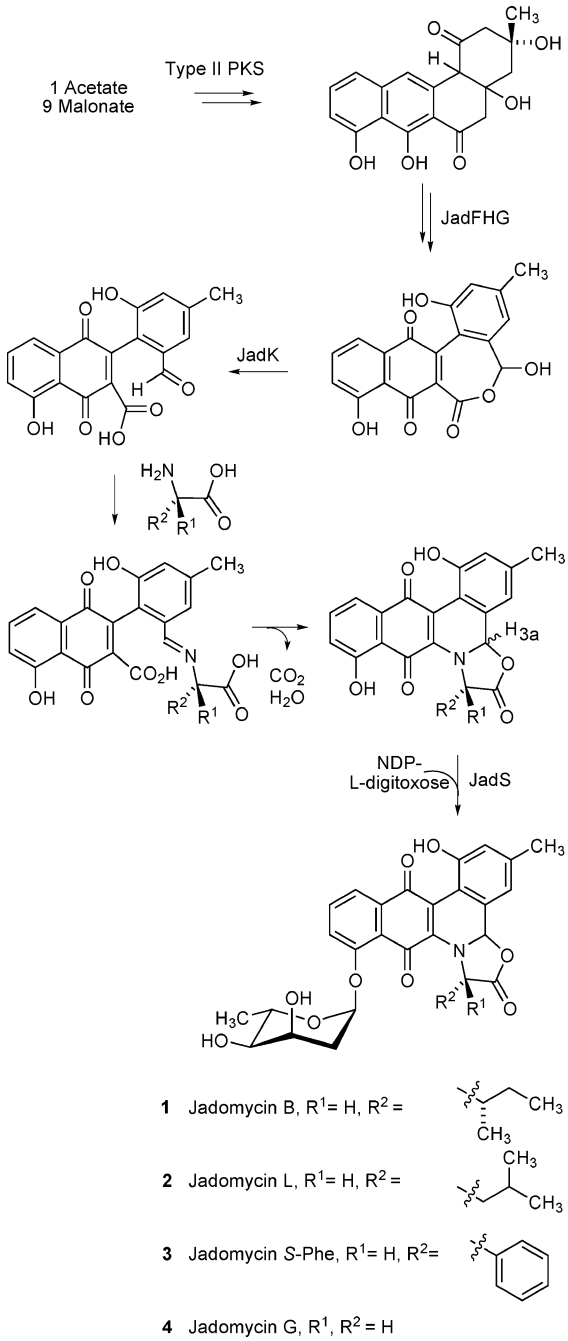
atic changes to the core oxazolone-fused angucyclinone skeleton have been achieved by judicious manipulation of a likely nonenzymatic step that incorporates an amino acid into the jadomycin framework (Scheme 1).[11] As previously

Scheme 1. Key Steps of the Jadomycin Biosynthetic Pathway



reported, replacement of L-isoleucine in the production medium with other amino acids has produced a variety of novel jadomycins as diastereomeric mixtures, with both natural and non-proteinogenic amino acids giving rise to structural congeners of the jadomycin oxazolone ring.[6,12] This use of precursor-directed biosynthesis is a proven strategy for generating structural diversity within the jadoymcins, and herein we demonstrate that this methodology provides access to jadomycin analogues that cleave DNA with mechanistic diversity.

Monitoring cleavage of the DNA backbone in the presence of metal ions and natural products is a robust and proven strategy for documenting the DNA-damaging properties of a variety of natural products, including pluramycins, prodigiosin, and doxorubicin.[13−16] Employing these and other methods, we show that discrete mechanisms of action exist for four structurally related jadomycins, establishing an unprecedented breadth of biological tunability within a single natural product family.

Figure 2 highlights the DNA-damaging power exhibited by selected jadomycins in the presence of copper ions, a

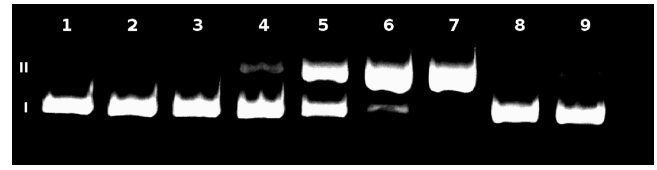


Figure 2. Copper-mediated DNA cleavage by **1**. Reaction mixtures (20 μL total volume) contained 400 ng of form I DNA in 10 mM MOPS buffer, pH 7.4, and were incubated at 37 °C for 4 h: lane 1, 0.1 μM **1**/Cu(II); lane 2, 0.2 μM **1**/Cu(II); lane 3, 0.5 μM **1**/Cu(II); lane 4, 1 μM **1**/Cu(II); lane 5, 2 μM **1**/Cu(II); lane 6, 5 μM **1**/Cu(II); lane 7, 10 μM **1**/Cu(II); lane 8, 10 μM **1**; lane 9, 10 μM Cu(II).

known oxidant of prodigiosin that is involved in DNA cleavage.[15,16] At 10 μM concentration, jadomycin B produced no detectable DNA damage. Addition of copper ions promoted single-strand cleavage of duplex DNA (form II) by jadomycin B in a concentration-dependent manner. Optimal cleavage occurred at jadomycin B/Cu(II) ratios between 0.5 and 1; no cleavage was observed with 10 μM copper only. Lowering the concentration of either reagent reduced the extent of cleavage, indicating that the reaction is not catalytic. The requirement for Cu(II) to elicit DNA damage is reminiscent of the metal-mediated mechanism of action exhibited by the bleomycins,[17] glycopeptide antibiotics that are used in the treatment of certain cancers. These drugs exert their cytotoxic effects through oxygen-dependent iron-mediated cleavage of the DNA backbone. Like the natural product prodigiosin,[15,16] jadomycin B may be capable of reducing Cu^{2+} ions to produce similar Fenton-type chemistry, wherein reactive oxygen species are responsible for DNA damage.

When cultured under conditions with leucine as the sole nitrogen source, *Streptomyces venezuelae* ISP5230 produces jadomycin L,[18] a structural isomer of jadomycin B

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(Scheme 1). This shift of a single methyl group completely eliminated the copper dependence documented for jadomycin B. Figure 3 shows that jadomycin L elicits single-

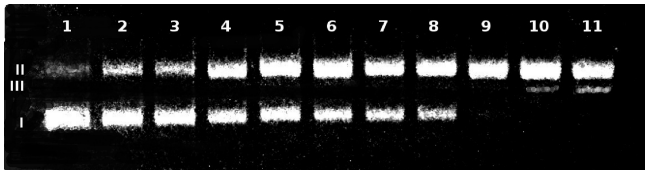

Figure 3. DNA cleavage by **2**. Reaction mixtures (20 μL total volume) contained 400 ng of form I DNA in 10 mM MOPS buffer, pH 7.4, and were incubated at 37 °C for 4 h: lane 1, 0 μM **2**; lane 2, 1.0 μM **2**; lane 3, 2.5 μM **2**; lane 4, 5.0 μM **2**; lane 5, 7.5 μM **2**; lane 6, 10 μM **2**; lane 7, 12.5 μM **2**; lane 8, 15 μM **2**; lane 9, 20 μM **2**; lane 10, 40 μM **2**; lane 11, 80 μM **2**.

strand breaks in the DNA backbone at concentrations well below 5 μM in the absence of exogenous copper ions. Interestingly, double-strand breaks (form III) are detected when the concentration of jadomycin L exceeds 20 μM; this behavior was not observed for other jadomycins evaluated. The DNA-cleavage profile for jadomycin L at elevated concentrations indicates that double-strand breaks arise from the accumulation of single-strand breaks within the 15-base pair limit[19] and not the ability of a single molecule to introduce simultaneous breaks in duplex DNA.[20,21]

Precursor-directed biosynthesis has also been used to gain access to jadomycins derived from non-proteinogenic amino acids.[6,8,22] Incorporation of *S*-phenylglycine yields **3**, which exhibited a markedly different DNA-cleaving profile relative to other jadomycins investigated (Figure 4). While **3** did not show DNA cleavage under the conditions employed for **1** and **2**, photoactivation of this particular jadomycin elicited DNA damage in the form of single-strand breaks. Irradiation of **3** with 313 nm light for 15 min produced detectable traces of nicked DNA at concentrations much less

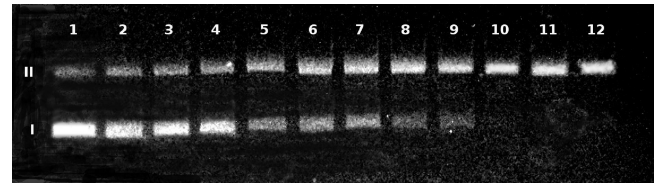

Figure 4. DNA photocleavage by **3**. Reaction mixtures (20 μL total volume) contained 400 ng of form I DNA in 10 mM MOPS buffer, pH 7.4, and were irradiated at 25 °C for 15 min with 313 nm light: lane 1, 0 μM **3**, $-h\nu$; lane 2, 0 μM **3**; lane 3, 1.0 μM **3**; lane 4, 2.5 μM **3**; lane 5, 5.0 μM **3**; lane 6, 7.5 μM **3**; lane 7, 10 μM **3**; lane 8, 12.5 μM **3**; lane 9, 15 μM **3**; lane 10, 20 μM **3**; lane 11, 40 μM **3**; lane 12, 80 μM **3**.

than 1 μM. The rich photochemistry associated with quinone-containing systems suggests that the anthraquinone-like core of **3** may play a pivotal role in DNA-strand scission. For example, irradiation of quaternary alkyl ammonium-linked anthraquinones at 350 nm is known to populate the triplet state of the anthraquinone, which reacts with DNA either by hydrogen atom abstraction from a DNA deoxyribose or by oxidation of a DNA base.[23] These DNA lesions, in turn, prompt various intramolecular thermal reactions that ultimately sever the DNA backbone. The lack of double-strand cleavage for **3** indicates that the quinone may not be reformed after this sequence or the jadomycin may exhibit only a transient binding interaction with DNA. Regardless, it is interesting to note that when the phenyl substituent is replaced with a hydrogen atom as in **4**, no discernible photoreactivity with DNA occurs. In fact, **4** did not cleave DNA with copper ions, heat, or photons, underscoring the importance of the functionality incorporated at C1 for invoking DNA damage.

Together, these select examples illustrate that tuning of the DNA damaging profiles within a given jadomycin series has been achieved by controlling the identity of the substituent at C1 through precursor-directed biosynthesis. DNA damage has been tuned from complete inhibition, as observed for **4**, to double-strand breaks as shown for **2**. Moreover, selective activation, which is of paramount importance in drug design, can also be accomplished through precursor-directed biosynthesis. The presence of exogenous copper ions in the case of **1** or of photons as in **3** offers drastically different avenues for effectively "turning on" the DNA damaging properties of a particular jadomycin. Such command in the area of rational drug design is unprecedented and demonstrates that nature has much to offer not only in achieving structural diversity but also in fine-tuning biological activity. Mechanistic studies are underway to discern the mode of DNA cleavage and to understand why such minor structural changes effected through precursor-directed biosynthesis have such a profound influence on the observed DNA-damaging properties of the jadomycins.

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Supporting Information Available: Experimental details for gel-mobility shift assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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