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EDGE ARTICLE

Synthetic diversification of natural products: semi-synthesis and evaluation of triazole jadomycins†

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Growth of *Streptomyces venezuelae* ISP5230 with *O*-propargyl-L-serine led to the efficient production of an alkyne-containing jadomycin. The installed alkyne functionality provided a uniquely reactive handle within the natural product and was subsequently reacted with a series of azides to afford an eight-member library of jadomycin triazoles. The compounds were evaluated for their DNA cleavage, antibacterial and cytotoxic properties.

Introduction

It is hard to underestimate the importance of natural products given their long-standing ability to give rise to clinically approved medicines for treatment of debilitating diseases including bacterial infection and cancer.[1] The generation of natural product analogues remains an important goal to enhance activity. Numerous approaches exist to diversify natural products including glycodiversification,[2,3] mutasynthesis,[4–6] combinatorial,[7] or chemogenetic biosynthesis.[8]

Non-enzymatic steps in natural products biosynthesis occur infrequently, but include some chlorination processes,[9] oxidative coupling in indolocarbazole natural products,[10] macro-dimerization of sisomicin,[11] betalain aldimine formation,[12] cyclization of cathenamine derivatives,[13] late steps in urdamycin C and D biosynthesis,[14] and in aldimine formation in jadomycin biosynthesis.[15–18] Herein, we present a method to install a unique chemical handle in a natural product, based on a non-enzymatic biosynthetic step and to facilitate selective synthetic functionalization of the resulting natural product. Using the non-enzymatic biosynthetic step in jadomycin biosynthesis, a terminal alkyne functionality was introduced into the jadomycin scaffold by using an alkyne-functionalized amino acid as a sole nitrogen source for *S. venezuelae* ISP5230 VS1099 cultures. Jadomycin production was induced by ethanol shock,[19] and the resulting jadomycin derivative was used in a number of copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) reactions[20,21] with a variety of azides to produce a small library of triazole-containing jadomycins (Scheme 1).

Results and discussion

Synthesis

O-Propargyl-L-serine (**1**), an amino acid derivative containing a terminal alkyne functionality, was synthesized from *N*-Boc-L-serine in a 70% yield over two synthetic steps. This was achieved *via* selective alkylation at the hydroxyl position with propargyl bromide and removal of the Boc-protecting group using hydrochloric acid. *O*-Propargyl-L-serine was routinely synthesized on a 7 g scale without any need for chromatographic purification (ESI†).

Eight azides were synthesized for use in CuAAC reactions: one alkyl, one aryl and six glycosyl derivatives (**3–10**). All azides were synthesized from their bromide precursors *via* reaction with sodium azide. The glycosyl azides generated were identified as either α- or β-anomers according to their distinct anomeric C–H direct bond coupling constants ($^1J_{C1,H1}$; 170 Hz for α-glycosyl azide, 158 Hz for β-glycosyl azides).[22]

Published reaction conditions for the formation of 1*H*-[1,2,3]-triazoles[23] that gave exclusively 1,4-regioisomers were tested using *O*-propargyl-L-serine and azides **3**, **5** and **8**, prior to evaluation of **2**. When alkyne **1** was reacted with azides **3**, **5** or **8** under the copper(I) catalysis conditions, the anticipated 1,3-dipolar cycloaddition products were observed in excellent yields (>90%, ESI). Next, we proceeded to produce **2** through fermentation of *S. venzuelae* ISP5230 VS1099 with *O*-propargyl-L-serine as the sole nitrogen source. We used culture conditions optimized previously[24] to produce a variety of jadomycin

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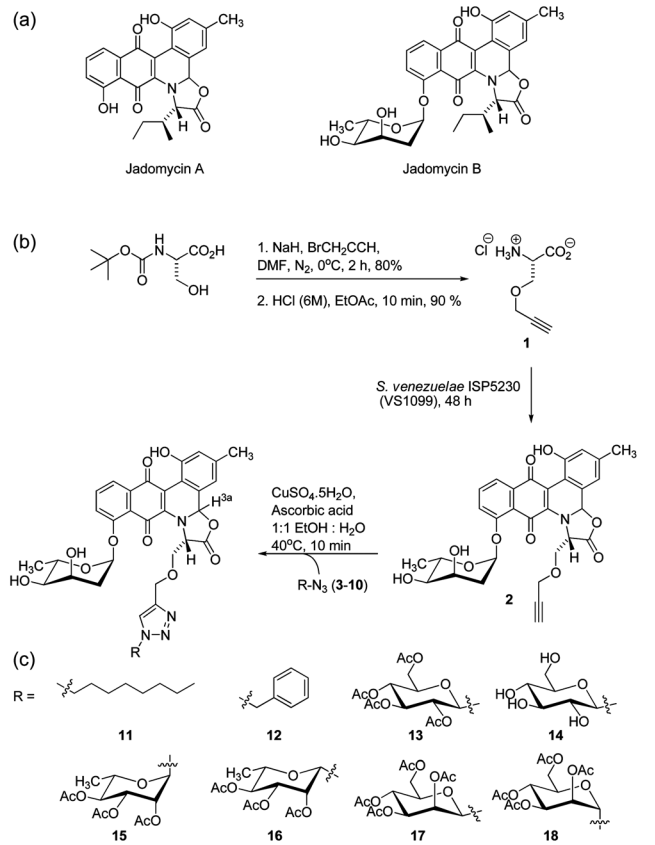
† Electronic supplementary information (ESI) available: Full experimental details, analytical data, DNA-binding UV-vis curves, cytotoxicity data for each triazole and spectroscopic characterization data (HPLC and NMR). See DOI: 10.1039/c2sc00663d



Scheme 1 (a) Parent angucycline antibiotics jadomycin A and jadomycin B; (b) synthesis of non-natural amino acid (**2**) and biosynthetic incorporation for post-fermentation synthetic diversification of the jadomycins and (c) azides used to prepare jadomycin triazoles **11–18**.

analogues.[16,25–28] Indirect evidence for the production of a jadomycin analogue included the development of a burgundy colour in the culture medium. After 48 h, the cells were removed by filtration and the culture media passed through a reversed-phase C18 column. The column was washed with water, and the crude natural product extract was eluted from the column with methanol. The crude product was analyzed for the presence of a jadomycin by thin layer chromatography (TLC) and mass spectrometry (MS). The presence of a prominent burgundy band suggested a jadomycin derivative was present in the extract. The mass of the predicted jadomycin derivative, Jadomycin OPS (**2**; 562 *m*/*z*), was observed by MS analysis, and MS/MS of the observed parent ion revealed the expected fragmentation to the aglycone consistent with the literature (Fig. 1).[25] Purification of crude Jadomycin OPS (**2**) was accomplished by preparative TLC on normal-phase silica gel plates. The structure and purity of Jadomycin OPS (**2**) was confirmed by NMR, LC-MS, and HR-MS analyses.

We next turned our attention to synthetically derivatizing the crude natural product because of challenges associated with the purification of **2** and to demonstrate the orthogonality of the CuAAC reactions to all the remaining materials. Eight CuAAC reactions were performed, using azides **3–10**. The reactions were monitored by TLC analysis and all were complete within 10 min as observed by the change in R_f of the burgundy band (ESI†).

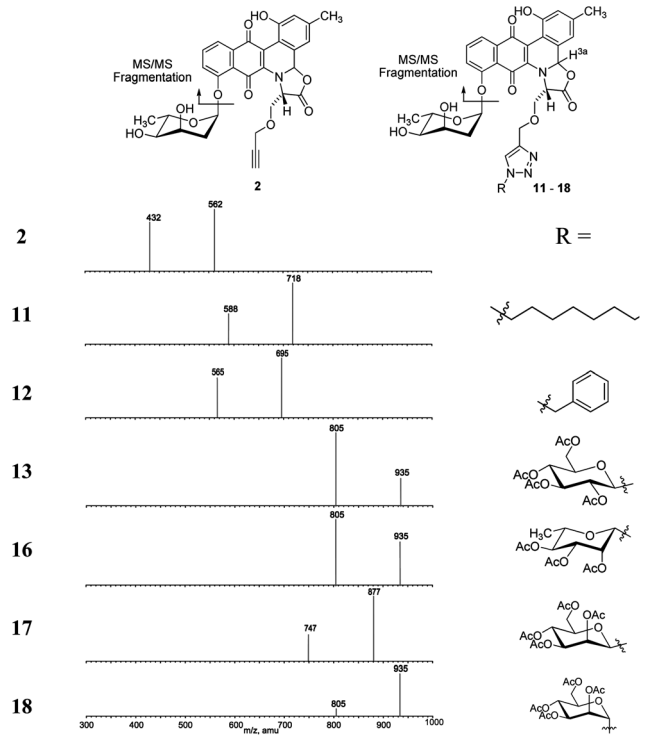


Fig. 1 MS/MS analysis of jadomycin triazole analogues demonstrating fragmentation of the glycosidic linkage.

Products were purified *via* preparative TLC and characterized (Table 1 and ESI†). All products exhibited the predicted fragmentation pattern when analyzed by MS/MS (Fig. 1), demonstrating the cleavage of the glycosidic linkage. The jadomycins exist as two diastereomers at C3a,[15,29] and the preference for the 3a*S* diastereomer ranged between 3 : 1 and 1 : 1 depending on the triazole congener. In general, the ^{1}H chemical shifts for each diastereomer differed by less than 0.1 ppm. One exception is the two signals for the proton in the triazole ring, which were separated by 1 ppm. The deshielding observed for the 3a*S* diastereomer is potentially due to hydrogen bonding or stacking effects to the aromatic moiety. Evidently, this is not possible for the 3a*R* diastereomer.

Table 1 Diastereomeric ratios for jadomycin triazole analogues and selected characterization data

Product	3a*S* : 3a*R*	Yield/mg	High res. MS
2	64 : 20	12 mg L^{-1}	454.0897
11	55 : 35	3.5	739.2886
12	60 : 36	3.7	695.2328
13	65 : 22	4.8	957.2651
14		4.0[a]	
15	52 : 30	7.1	877.2770
16	63 : 20	5.8	899.2540
17	60 : 31	9.0	957.2641
18	41 : 35	5.0	957.2632

[a] Compound **14** was prepared but could not be purified to homogeneity.

Biological evaluation

Jadomycin derivatives (**2**, **11–13** and **15–18**), as well as the three non-jadomycin triazoles (ESI†), were tested for DNA-cleavage, antibiotic and cytotoxicity activity.

DNA cleavage evaluation

We have recently reported the ability of the jadomycins to promote copper-mediated DNA-cleavage.[28,30] Thus, the triazole derivatives were evaluated for their ability to promote the cleavage of supercoiled pUC19 plasmid DNA in the presence of stoichiometric quantities of Cu(II). At 100 μM, the jadomycins produced no detectable relaxation of supercoiled (form I) plasmid DNA. However, in the presence of equimolar $Cu(OAc)_2$, all jadomycins yielded single-strand breaks (form II) in a concentration dependant manner (Fig. 2). The calculated EC50 values for single-stranded scission are listed and the order, from lowest to highest (most to least cleavage), was **12** > **11** ≈ **13** > **18** > **16** > **17**. Jadomycin **12**, in the presence of equimolar $Cu(OAc)_2$, caused complete single-strand scission at approximately 60 μM. None of the compounds evaluated demonstrated double-stranded DNA cleavage, in contrast to jadomycin L.[28] UV-visible titrations were carried out with each of the analogues, titrating in either $Cu(OAc)_2$ or calf-thymus DNA. In all instances, no significant interaction was observed (ESI†).

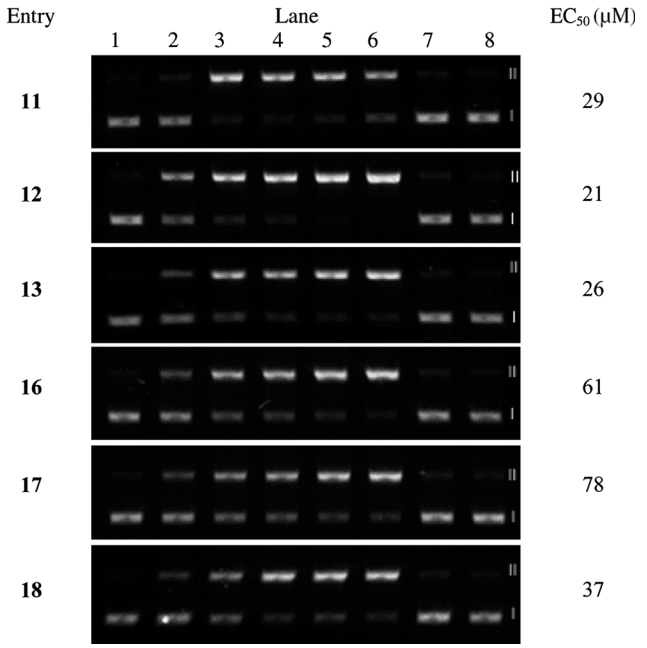


Fig. 2 Jadomycin-copper mediated DNA cleavage. Total reaction volume 20 μL, comprising pUC19 plasmid (20 μM) in Tris buffer (10 mM), pH 7.4 and incubation at 37 °C for 2 h: lane 1, DNA only; lane 2, 20 μM complex; lane 3, 40 μM complex; lane 4, 60 μM complex, lane 5, 80 μM complex; lane 6, 100 μM complex; lane 7, 100 μM jadomycin only; lane 8 100 μM $Cu(OAc)_2$ only; complex = equimolar jadomycin : $Cu(OAc)_2$; I, form I (supercoiled DNA); II, form II (nicked DNA).

Antibacterial evaluation

Jadomycins have been shown to have antibacterial activity against gram positive organisms.[31] We screened the compounds against five strains of Gram-positive bacteria (*Staphylococcus aureus* C622, *Staphylococcus aureus* MRSA C623, *Staphylococcus epidermidis* C960, *Bacillus subtilis* C971, and *Enterococcus faecalis* C625) and three strains of Gram-negative bacteria (*Salmonella enterica typhimurium* C587, *Escherichia coli* C498, and *Pseudomonas aeruginosa* H187) using Clinical and Laboratory Standard Institutes methods. The activity was measured as the minimum inhibitory concentration (MIC), the lowest concentration of compound that inhibited bacterial growth (Table 2). Only compound **2** showed any activity against a Gram-negative strain. The non-jadomycin triazoles (ESI†) failed to have any activity whatsoever, indicating the importance of the jadomycin angucycline functionality for antibacterial activity. Compounds **11–13**, the non-glycosylated derivatives, were most active. All compounds exhibited the most pronounced activity against *S. epidermidis* C960, yet only triazole derivative **12** outperformed the parent compound **2**, potentially indicating the need to further evaluate structurally related triazole derivatives for antibacterial activity.

Cytotoxicity evaluation

Jadomycins have been shown to have cytotoxic properties,[16,27,32] and thus a series of the jadomycin compounds were analyzed at the National Cancer Institute (NCI) as part of the developmental therapeutics program. Compounds were screened initially in a single dose response at 10 μM against the standard 60-cell line panel. All jadomycin triazole derivatives were sufficiently active to proceed to the five-dose evaluation, however, the non-triazole jadomycin (**2**) was terminated after the single dose response,

Table 2 Selected MIC_{50} values (μg mL^{-1}) for jadomycin triazoles

Triazole	*E. coli* C498	*S. aureus* C622	*S. aureus* MRSA C623	*B. subtilis* C971	*S. epidermidis* C960
2	64	64	32	64	4
11	>128	16	32	32	4
12	>128	32	32	64	2
13	>128	32	16	32	4
16	>128	64	64	128	4
17	>128	64	32	128	4
18	>128	64	32	64	4
Van[a]	>128	8	<1	<1	1

[a] Vancomycin control.

Table 3 GI_{50}, TGI and LC_{50} values for jadomycin triazoles in the NCI 60 cancer cell line screen[a]

Triazole	Concentration range (nM–μM)	GI_{50} (μM) Median (range)	*n*	TGI (μM) Median (range)	*n*	LC_{50} (μM) Median (range)	*n*
2	3.2–32	18 (9.9–31.1)	3	NA	0	NA	0
11	2.5–25	3.8 (0.5–9.4)	58	7.6 (1.0–3.8)	56	14.9 (2.0–24.5)	39
12	2.5–25	6.1 (0.7–23.6)	51	11.9 (2.6–4.6)	28	14.9 (13–21.4)	10
13	1.6–16	2.2 (0.3–6.5)	59	4.6 (0.6–15)	56	9.6 (1.1–16.1)	45
15	1.6–16	3.1 (0.6–10)	55	6.8 (2.3–15.1)	46	11.6 (8.2–16.3)	25
16	1.6–16	3.1 (0.5–15.6)	57	6.3 (2.1–16)	51	9.8 (1.0–16.5)	26
17	1.6–16	3.5 (0.4–14.9)	54	7.2 (1.3–14.9)	40	11.8 (7.7–16.3)	17
18	1.6–16	3.7 (0.5–9.3)	54	6.6 (1.6–15.6)	42	10.9 (8.3–15)	19

[a] GI_{50}, concentration required to inhibit cell growth by 50%; TGI, concentration required to inhibit growth by 100%; LC_{50}, concentration required to cause lethality in 50% of the cells. All values are expressed as median (range), where *n* = the number of cancer cell lines out of a maximum of 59 in which GI_{50}, TGI, LC_{50} were quantifiable below the maximal jadomycin concentration of 32 μM for **2**, 25 μM for **11–12** and 16 μM for **13**, **15–18**. NA, not applicable.

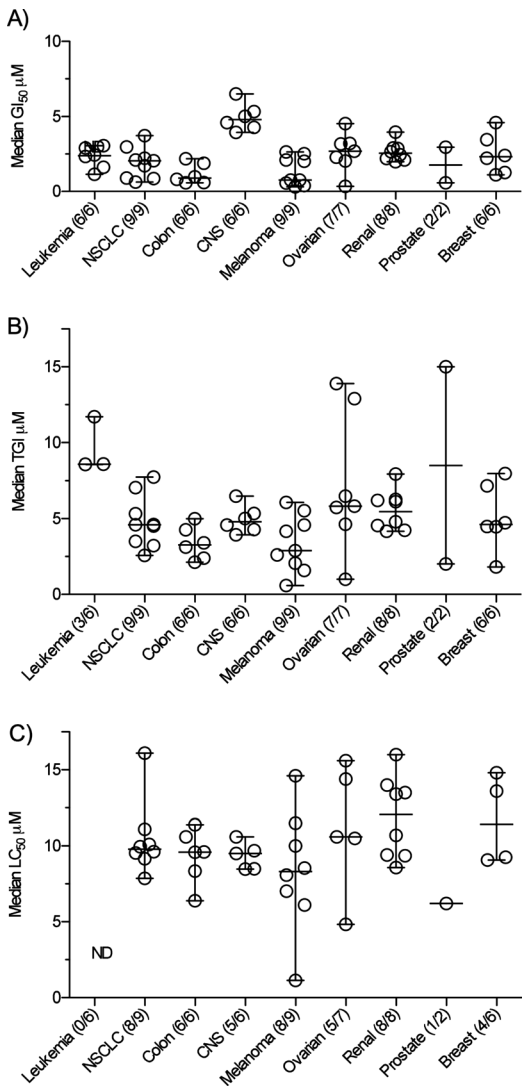


Fig. 3 GI_{50}, TGI and LC_{50} values for jadomycin triazole **13** grouped by cancer cell type. Each open circle represents the GI_{50}, TGI and LC_{50} for an individual cancer cell line. The horizontal lines represent the median and range GI_{50}, TGI and LC_{50} values for each group of cancer cells. The numbers in brackets below the *x*-axis indicate the number of cell lines out of total cell lines tested per group in which TGI, LC_{50} were quantifiable below the maximal inhibitor concentration of 16 μM. ND, not determined.

demonstrating the importance of the substituted triazole moiety for activity.

At the NCI, each compound was screened against 6 leukemia, 9 non-small cell lung carcinoma, 6 colon, 6 central nervous system, 9 melanoma, 7 ovarian, 8 renal, 2 prostate and 6 breast cancer cell lines at five doses spanning a five log 10 concentration range. At each dose the percent growth and mean optical density was determined according to the protocol described on the NCI website.[33] The optical densities were plotted *vs.* jadomycin concentration to obtain GI_{50} (the concentration at which growth inhibition is half its maximal value), TGI (the concentration of test drug that causes total growth inhibition), and LC_{50} (the concentration at which half the cells are killed). The compiled median GI_{50}, TGI and LC_{50} values for all 59-cell lines are summarized for each compound (Table 3). With the exception of compound **2**, all the compounds inhibited the growth of the majority of the 59 cancer cells that were tested. The median GI_{50} and TGI values were in the low micromolar range indicating similar growth-inhibition potency for the seven effective jadomycins. Within the concentration ranges tested, these same jadomycins demonstrated lethality in between 17–45 of the 59 cancer cell lines as measured by the LC_{50} values. Compounds **13**, **15–18** demonstrated slightly higher potency with median LC_{50} values between 9–12 μM compared to compounds **11** and **12** which were between 15–19 μM. Compound **13** is of particular interest for further testing because it had higher potency and lethality against the largest proportion 45/59 of cancer cell lines. The GI_{50}, TGI and LC_{50} of triazole **13** grouped according to cancer cell type are shown in Fig. 3, highlighting its general efficacy with respect to growth inhibition and lethality across the different classes of cancer cells. The exception was the leukemia cell lines, which were resistant to the lethal effects of **13**; these cells were also resistant to the other jadomycin triazole derivatives evaluated.

Conclusions

Jadomycin OPS (**2**), a new jadomycin derivative with terminal alkyne functionality was generated *via* precursor-directed biosynthesis utilizing a non-enzymatic step in jadomycin biosynthesis to incorporate a synthetic handle. The jadomycin was reacted with eight azides in CuAAC reactions, yielding eight triazole-containing jadomycins and marking a first-ever selective synthetic functionalization of a jadomycin derivative. This

demonstrates that the jadomycin scaffold is amenable to chemical modification post-biosynthesis and gives insight into a new, more efficient methodology to produce jadomcyin libraries. It is interesting to note that within the congeners evaluated, the most active compound was dependant on the specific assay. In the *in vitro* DNA cleavage the benzyl triazole (**12**) was most active whereas in the *in vivo* cytotoxicity analysis glucosyl triazole (**13**) was more potent. This demonstrates that the activity of the jadomycin is tuneable based on the azide incorporated, and that multiple mechanisms of action may be associated with the jadomycins.[34]

Acknowledgements

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