Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Journal Name

ARTICLE

Investigations into the binding of jadomycin DS to human topoisomerase IIβ by WaterLOGSY NMR spectroscopy

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Camilo F. Martinez-Farina,[a] Nicole McCormick,[b] Andrew W. Robertson,[a] Helen Clement,[b] Alison Jee,[b] Anna Ampaw,[a] Nei-Li Chan,[c] Ray T. Syvitski,[ad] David L. Jakeman*[ab]

The jadomycins are a family of secondary metabolites produced by *S. venezuelae* ISP5230. Specific jadomycins have been shown to possess a variety of anticancer, antifungal, and antibacterial properties, with different molecular mechanisms of action. Herein we demonstrate qualitative and quantitative direct binding between the validated anticancer target human topoisomerase IIβ and jadomycin DS using WaterLOGSY NMR spectroscopy. Additionally, we report for the first time, that jadomycin DS also binds a variety of other proteins, likely in a non-specific manner. Such interactions may rationalize the potential polypharmacology of jadomycin DS.

.

Introduction

Identifying, isolating, and characterizing novel natural products is of significance as many clinically relevant medicines are derived from natural products. [1, 2] Natural products come from a variety of sources, with bacteria being a major contributor, and the genus *Streptomyces* being responsible for an estimated 32% of all known bioactive metabolites. [3, 4] The soil bacteria *Streptomyces venezuelae* ISP5230 (ATCC 10712) is an example, producing chloramphenicol[5] jadomycins, [6, 7] venezuelin, [8] and baduredin. [9] *S. venezuelae* ISP5230 produces the jadomycins under stress conditions including heat, phage, or ethanol shock. [10, 11] It was these conditions that led to the serendipitous discovery of jadomycin A[6] (1), followed closely by jadomycin B (2) [10] (Figure 1). Since their initial discovery the jadomycin scaffold has been shown to be amenable to alteration through precursor-directed biosynthesis using proteinogenic and non-proteinogenic amino acids. [12-14] This is accomplished by exploiting a non-enzymatic step within the biosynthetic pathway where an imine is postulated to form through reaction of a biosynthetic aldehyde and an amino acid, followed by cyclization and decarboxylation of the intermediate to yield an oxazolone ring, followed by glycosylation to furnish the jadomycin of interest. [13, 15-18] This has been successful in furnishing a wide variety of jadomycin analogues with varying structures. [11, 19] Total synthesis has provided supporting evidence for the non-enzymatic imine formation and cyclization. [15, 20]

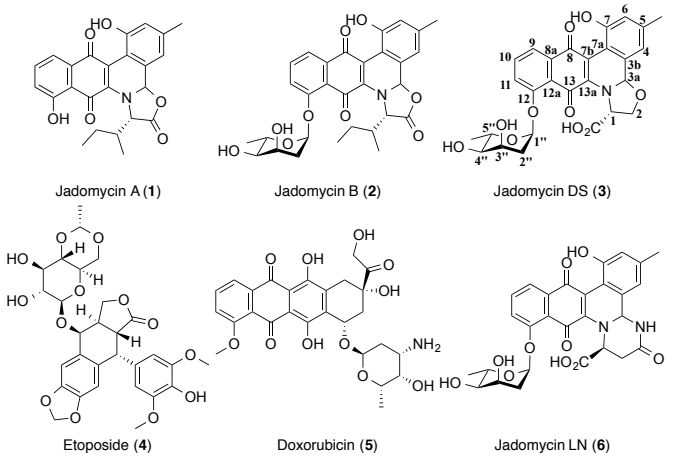

Figure 1 Structures of jadomycin A, jadomycin B, jadomycin DS, etoposide and doxorubicin.

Various jadomycin bioactivities have been measured including cytotoxicity profiles in the National Cancer Institute 60-cell line screening,[21] copper-mediated DNA cleavage,[22] photodynamic inactivation of bacteria, [19, 23] and aurora B kinase inhibition, [24] indicating that the jadomycins may function as polypharmacologic agents. [25-27] The copper-mediated DNA cleavage activity, the presence of a lactone ring, and a quinone functionality, each a structural feature consistent with either etoposide (**4**) or doxorubicin (**5**) both known anti-cancer agents that act *via* inhibition of human topoisomerase IIβ, led us to hypothesize that the jadomycins

[a] Department of Chemistry, Dalhousie University, Halifax, Nova Scotia Canada.
[b] College of Pharmacy, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada, Email: david.jakeman@dal.ca
[c] Institute of Biochemistry and Molecular Biology, College Medicine, National Taiwan University, Taipei City 100, Taiwan
[d] Institute of Marine Biosciences, National Research Council of Canada, Halifax, Nova Scotia, Canada

Electronic Supplementary Information (ESI) available: Jadomycin DS production and isolation methodology, recombinant human topoisomerase IIβ production, WaterLOGSY methodology, and binding curves. See DOI: 10.1039/x0xx00000x

may bind human topoisomerase IIβ. Topoisomerase IIβ is an enzyme that is responsible for relieving DNA tension during DNA replication, and inhibition of the enzyme leads to cell death. The structural basis for topoisomerase IIβ inhibition by etoposide has recently been reported. [28] For this reason, we chose to examine binding to human topoisomerase IIβ of a jadomycin, to provide insight into how this natural product interacts with a specific cytotoxic protein target.

Results and Discussion

Jadomycin DS was chosen to test as a potential binder to human topoisomerase IIβ because of its high water solubility in contrast to other jadomcyins. Binding studies were conducted using Water-Ligand Observed *via* Gradient SpectroscopY (WaterLOGSY) Nuclear Magnetic Resonance (NMR) spectroscopy. [28] This experiment was chosen because of the good signal-to-noise and the clarity between binders and non-binders: opposite phasing is observed between binders and non-binders in a single NMR spectrum. Furthermore, it does not require highly purified materials since each assigned proton is interrogated independently. Indeed, the presence of an impurity may function as a non-binding control. We have recently used WaterLOGSY NMR spectroscopy to measure enzyme-substrate and enzyme-inhibitor interactions. [29, 30]

Incubation of jadomycin DS and recombinant human topoisomerase IIβ[28] allowed for the qualitative assessment that human topoisomerase IIβ binds jadomycin DS, as a result of the change of phase of the jadomycin DS resonances in the WaterLOGSY NMR spectrum (Figure 2). This interaction was then quantified using a series of WaterLOGSY spectra. Dissociation constants (K_D) can be obtained for enzyme-ligand complexes under fast exchange processes using WaterLOGSY NMR. [29, 31] This quantitation is accomplished by monitoring the change in signal intensity as a function of ligand concentration in a two-part experiment. The first experiment is conducted by monitoring the change in signal intensity as a function of increasing ligand concentration in the presence of constant enzyme, producing a binding-curve. [29] However, signals are dampened by the presence of ligand that has not yet bound to the enzyme. [32] To correct for this intensity, a second control experiment is conducted by monitoring change in signal intensity as a function of increasing ligand concentration in the absence of enzyme, producing a non-binding negative linear-standard. The standard is subtracted from the binding-curve in order to correct for any possible signal dampening, to produce a dose-response curve. Regression analysis is used to fit Equation 1 to the data, where I is the observed signal intensity, I_{max} is the maximal signal intensity, L is the ligand concentration, and K_D is the dissociation constant.[32]

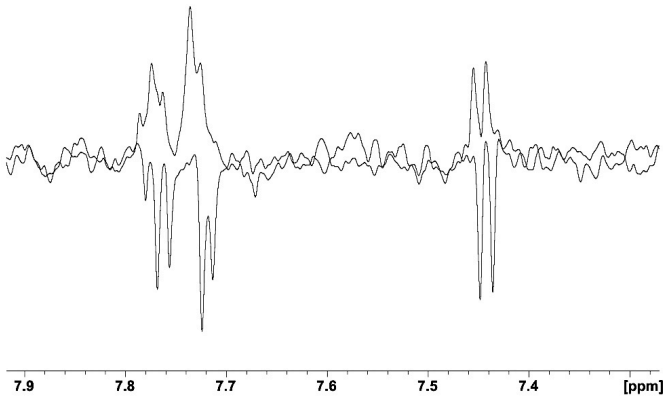

Figure 2 Two WaterLOGSY NMR spectra illustrating jadomycin DS (1 mM) binding in the presence of human topoisomerase IIβ (0.02 mM) (positive signal) and non-binding in its absence (negative signal). Signals highlighted correspond to protons at positions 10, 9, and 11.

$$I = \frac{-I_{max}}{1+\left(\frac{L}{K_D}\right)} + I_{max} \quad \text{Equation 1}$$

To determine the K_D value for jadomycin DS binding to human topoisomerase IIβ, a series of experiments were conducted, varying the jadomycin DS concentration (1.0 – 3.33 mM, the limit of jadomycin DS water solubility, see ESI) in the presence (0.02 mM) or absence of human topoisomerase IIβ. These experiments were carried out in phosphate buffered saline (PBS, 80% v/v, pH 7.6), deuterated PBS (10% v/v), and DMSO-d_6 (10% v/v). DMSO-d_6 was important to prevent aggregation of the jadomycin and enhance its solubility. Six different proton resonances, comprising of protons on the L-digitoxose (H1'', 5''-CH_3), the D-ring (H9, H10, H11) and the oxazolone ring (H3a), provided consistent data for fitting. Changes to the intensity of resonances close in chemical shift to the HOD resonance (H3'', H5'') were difficult to quantify due to the size of the HOD signal. Other signals appeared dispersive, and could not be quantified. Equation 1 was fitted to these data for each of the six individual resonances to determine I_{max} and K_D values (Figure 3 & Table 1). These individual K_D values were averaged to obtain a mean K_D value for jadomycin DS (Table 1). The mean K_D calculated for the jadomycin DS–human topoisomerase IIβ complex was 9.4 mM. This suggests that human topoisomerase IIβ binds jadomycin DS, although not very tightly, demonstrating that the WaterLOGSY method is appropriate to analyse K_D values of this magnitude. By contrast, measuring the strength of the etoposide–human topoisomerase IIβ interaction by WaterLOGSY was not feasible due to observation of tight binding (data not shown). It is known from a nitrocellulose filter assay that the etoposide-human topoisomerase IIβ interaction is 5 μM, albeit this was determined using a different protein source.[33]WaterLOGSY NMR spectroscopy was also attempted using jadomycin LN (at concentrations of 1 – 5 mM) in the presence of 0.02 mM human topoisomerase IIβ. Binding was not observed between jadomycin LN and human topoisomerase IIβ (data not shown). This demonstrates that not all jadomycins are able to bind human topoisomerase IIβ.

The millimolar binding constant calculated for jadomycin DS binding to human topoisomerase IIβ, and the fact that jadomycin LN was not found to bind human topoisomerase IIβ meant that jadomycin DS may exhibit promiscuous binding that may lend itself to the polypharmacologic nature of the jadomycins. Thus, we turned our attention to probing the qualitative interaction between jadomycin DS and a series of unrelated enzymes to hand in our laboratory, and also bovine serum albumin, a known promiscuous binder. To this end, WaterLOGSY NMR spectroscopy was conducted using jadomycin DS (0.5 mM and 2 mM) along with either β-phosphoglucomutase,[34] a family 3 glucosidase, DesR,[35] a thymidylyltransferase, Cps2L,[36] or bovine serum albumin, each at 0.02 mM. Jadomycin DS appeared to bind all the proteins investigated (Figure 4). Interactions of jadomycin DS with plasma proteins, including bovine serum albumin, will potentially effect disposition in *in vivo* environments.[37] The evidence for this is the change of phase of the jadomycin DS resonances in the WaterLOGSY NMR spectrum. Thereby confirming the promiscuous protein binding nature of jadomycin DS.

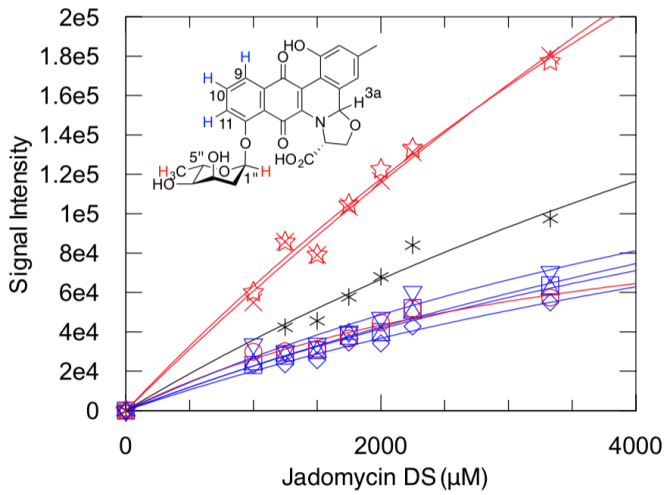

Figure 3 WaterLOGSY NMR peak intensity as a function of jadomycin DS concentration, in the presence of 0.02 μM human topoisomerase IIβ, for protons H3a (✳), H9 (▽), H10 (◇), H11 left signal (□), H11 right signal (Δ), H1'' (O), 5''-CH_3 left resonance (★), and 5''-CH_3 right resonance (×).

Table 1 Dissociation constant obtained for each proton signal analysed by WaterLOGSY NMR.

Proton	Chemical Shift δ (ppm)	K_D (mM)
3a	5.59	11.89
9	7.56	7.97
10	7.61	7.89
11	7.28	12.74
1''	5.71	3.82
5''-CH_3	1.24	12.35
Average		9.44

Conclusions

Qualitative application of WaterLOGSY NMR spectroscopy determined that jadomycin DS binds human topoisomerase IIβ. Although quantification of this relationship resulted in individual K_D values for different proton signals, it was not apparent that any one portion of the molecule was responsible for the binding observed. Instead, a global K_D for the jadomycin DS–human topoisomerase IIβ complex was determined to be 9.44 mM. By contrast jadomycin LN did not appear to bind human topoisomerase IIβ indicating that the different ring features of the jadomycins may govern interactions with protein targets. The extremely efficient acquisition of qualitative binding interactions between the jadomycins and human topoisomerase IIβ provides opportunities to efficiently evaluate other jadomycin congeners, or other potential ligands, of human topoisomerase IIβ, for the development of structure-activity relationships.

Qualitative WaterLOGSY NMR spectroscopy was also conducted using four different proteins. Since all of them show the ability to bind jadomycin DS, this indicates that jadomycin DS is a non-specific protein binding natural product. This may lend itself towards the polypharmacologic nature of this specific natural product.

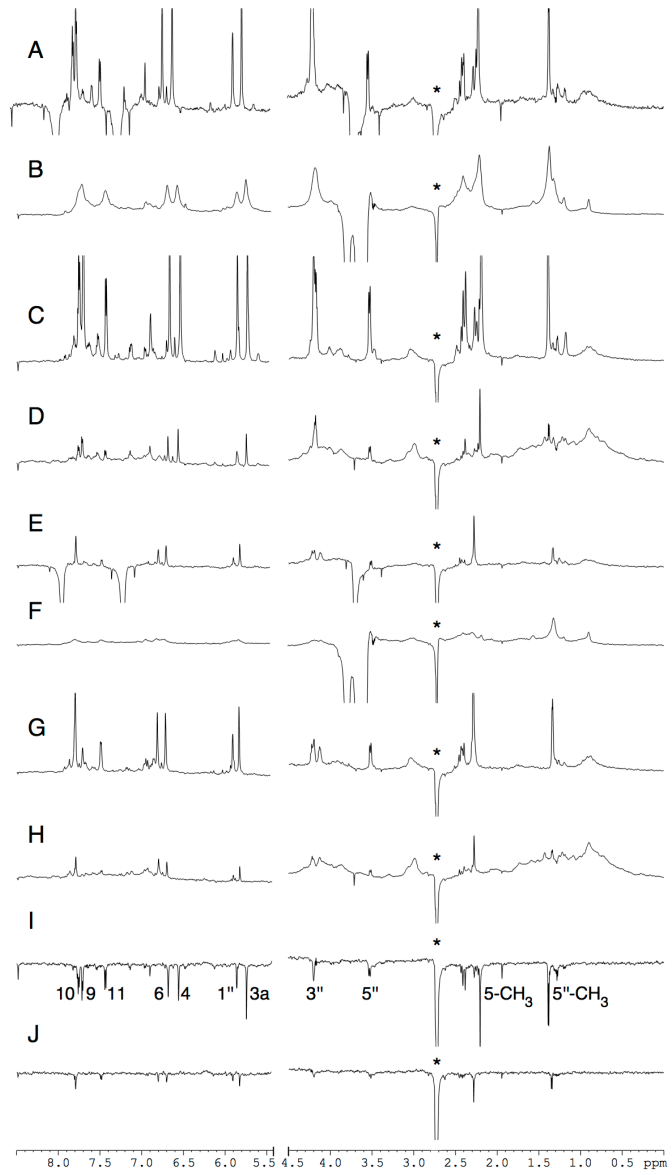

Figure 4 WaterLOGSY NMR spectra of (A) 2 mM jadomycins DS with β-phosphoglucomutase, (B) 2 mM jadomycin DS with bovine serum albumin, (C) 2 mM jadomycin DS with cps2L, (D) 2 mM jadomycin DS with DesR, (E) 0.5 mM jadomycin DS with β-phosphoglucomutase, (F) 0.5 mM jadomycin DS with bovine serum albumin, (G) 0.5 mM jadomycin DS with Cps2L, (H) 0.5 mM jadomycin DS with DesR, (I) 2 mM jadomycin DS in the absence of protein, and (J) 0.5 mM jadomycin DS in the absence of protein. All protein was present at 0.02 mM. The DMSO signal has been highlighted in all cases (*). The non-binding negatively phased signals in spectra A to H correspond to the buffer in which the protein was suspended. The intensity of spectra I and J were increased two-fold, in comparison to the other spectra, for clarity. All other spectra were processed identically.

Acknowledgements

We thank the NSERC, CIHR and the NSHRF for funding.

Notes and references

1 D. J. Newman and G. M. Cragg, *J. Nat. Prod.,* 2012, **75**, 311-335.
2 S. I. Elshahawi, K. A. Shaaban, M. K. Kharel and J. S. Thorson, *Chem. Soc. Rev.,* 2015, .
3 K. Scherlach and C. Hertweck, *Org. Biomol. Chem.,* 2009, **7**, 1753-1760.
4 J. Berdy, *J. Antibiot.,* 2005, **58**, 1-26.
5 J. He, N. Magarvey, M. Piraee and L. C. Vining, *Microbiology,* 2001, **147**, 2817-2829.
6 S. W. Ayer, A. G. McInnes, P. Thibault, L. Wang, J. L. Doull, T. Parnell and L. C. Vining, *Tetrahedron Lett.,* 1991, **32**, 6301-6304.
7 D. L. Jakeman, C. L. Graham, W. Young and L. C. Vining, *J. Ind. Microbiol. Biotechnol.,* 2006, **33**, 767-772.
8 Y. Goto, B. Li, J. Claesen, Y. Shi, M. J. Bibb and W. A. van der Donk, *Plos Biology,* 2010, **8**, e1000339.
9 J. D. Sidda, L. Song, V. Poon, M. Al-Bassam, O. Lazos, M. J. Buttner, G. L. Challis and C. Corre, *Chem. Sci.,* 2014, **5**, 86-89.
10 J. L. Doull, S. W. Ayer, A. K. Singh and P. Thibault, *J. Antibiot.,* 1993, **46**, 869-871.
11 C. N. Borissow, C. L. Graham, R. T. Syvitski, T. R. Reid, J. Blay and D. L. Jakeman, *ChemBioChem,* 2007, **8**, 1198-1203.
12 S. N. Dupuis, A. W. Robertson, T. Veinot, S. M. A. Monro, S. E. Douglas, R. T. Syvitski, K. B. Goralski, S. A. McFarland and D. L. Jakeman, *Chem. Sci.,* 2012, **3**, 1640-1644.
13 U. Rix, J. Zheng, L. L. Remsing Rix, L. Greenwell, K. Yang and J. Rohr, *J. Am. Chem. Soc.,* 2004, **126**, 4496-4497.
14 C. F. Martinez-Farina and D. L. Jakeman, *Chem. Commun.,* 2015, DOI: 10.1039/C5CC05571G.
15 M. Shan, E. U. Sharif and G. A. O'Doherty, *Angew. Chem. Int. Ed.,* 2010, **49**, 9492-9495.
16 N. Tibrewal, P. Pahari, G. Wang, M. K. Kharel, C. Morris, T. Downey, Y. Hou, T. S. Bugni and J. Rohr, *J. Am. Chem. Soc.,* 2012, **134**, 18181-18184.
17 M. K. Kharel and J. Rohr, *Curr. Opin. Chem. Biol.,* 2012, **16**, 150-161.
18 M. K. Kharel, P. Pahari, M. D. Shepherd, N. Tibrewal, S. E. Nybo, K. A. Shaaban and J. Rohr, *Nat. Prod. Rep.,* 2012, **29**, 264-325.
19 A. W. Robertson, C. Martinez-Farina, D. A. Smithen, H. Yin, S. Monro, A. Thompson, S. A. Mcfarland, R. T. Syvitski and D. L. Jakeman, *J. Am. Chem. Soc.,* 2015, **137**, 3271-3275.
20 X. Yang and B. Yu, *Chem. Eur. J.,* 2013, **19**, 8431-8434.
21 S. N. Dupuis, T. Veinot, S. M. A. Monro, S. E. Douglas, R. T. Syvitski, K. B. Goralski, S. A. McFarland and D. L. Jakeman, *J. Nat. Prod.,* 2011, **74**, 2420-2424.
22 K. Cottreau, C. Spencer, J. Wentzell, C. L. Graham, C. N. Borissow and D. L. Jakeman Mcfarland S.A., *Org. Lett.,* 2010, **12**, 1172-1175.
23 C. F. Martinez-Farina, A. W. Robertson, H. Yin, S. M. A. Monro, S. A. McFarland, R. T. Syvitski and D. L. Jakeman, *J. Nat. Prod.,* 2015, **78**, 1208-1214.
24 D. H. Fu, W. Jiang, J. T. Zheng, G. Y. Zhao, Y. Li, H. Yi, Z. R. Li, J. D. Jiang, K. Q. Yang, Y. Wang and S. Y. Si, *Mol. Cancer Ther.,* 2008, **7**, 2386-2393.
25 A. Anighoro, J. Bajorath and G. Rastelli, *J. Med. Chem.,* 2014, **57**, 7874-7887.
26 X. Jalencas and J. Mestres, *Medchemcomm,* 2013, **4**, 80-87.
27 J. Peters, *J. Med. Chem.,* 2013, **56**, 8955-8971.
28 C. Wu, T. Li, L. Farh, L. Lin, T. Lin, Y. Yu, T. Yen, C. Chiang and N. Chan, *Science,* 2011, **333**, 459-462.
29 S. M. Forget, A. Jee, D. A. Smithen, R. Jagdhane, S. Anjum, S. A. Beaton, D. R. Palmer, R. T. Syvitski and D. L. Jakeman, *Org. Biomol. Chem.,* 2015, **13**, 866-875.
30 M. W. Loranger, S. M. Forget, N. E. McCormick, R. T. Syvitski and D. L. Jakeman, *J. Org. Chem.,* 2013, **78**, 9822-9833.
31 L. Fielding, S. Rutherford and D. Fletcher, *Magn. Reson. Chem.,* 2005, **43**, 463-470.

32 C. Dalvit, G. Fogliatto, A. Stewart, M. Veronesi and B. Stockman, *J. Biomol. NMR,* 2001, **21**, 349-359.
33 P. Kingma, D. Burden and N. Osheroff, *Biochemistry,* 1999, **38**, 3457-3461.
34 Y. Jin, D. Bhattasali, E. Pellegrini, S. M. Forget, N. J. Baxter, M. J. Cliff, M. W. Bowler, D. L. Jakeman, G. M. Blackburn and J. P. Waltho, *Proc. Natl. Acad. Sci. U. S. A.,* 2014, **111**, 12384-12389.
35 A. Sadeghi-Khomami, M. D. Lumsden and D. L. Jakeman, *Chem. Biol.,* 2008, **15**, 739-749.
36 S. M. Forget, D. A. Smithen, A. Jee and D. L. Jakeman, *Biochemistry,* 2015, **54**, 1703-1707.
37 M. Otagiri, *Drug Metab. Pharmacok.,* 2005, **20**, 309-323.