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Synthesis, conformational preferences, and biological activity of conformational analogues of the microtubule-stabilizing agents, (–)-zampanolide and (–)-dactylolide†

 Jeffrey L. Henry,^a Matthew R. Wilson,^b Michael P. Mulligan,^a Taylor R. Quinn,^a
 Dan L. Sackett^c and Richard E. Taylor ^{*,a}

Zampanolide and dactylolide are microtubule-stabilizing polyketides possessing potent cytotoxicity towards a variety of cancer cell lines. Using our understanding of the conformational preferences of the macrolide core in both natural products, we hypothesized that analogues lacking the C17-methyl group would maintain the necessary conformation for bioactivity while reducing the number of synthetic manipulations necessary for their synthesis. Analogues **3**, **4** and **5** were prepared *via* total synthesis, and their conformational preferences were determined through computational and high-field NMR studies. While no observable activities were present in dactylolide analogues **3** and **4**, zampanolide analogue **5** exhibited sub-micromolar cytotoxicity. Herein, we describe these efforts towards understanding the structure- and conformation-activity relationships of dactylolide and zampanolide.

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Introduction

Marine polyketide natural products offer an immense amount of structural diversity which often correlates to powerful and diverse biological activities.¹ Capitalizing on the potential of marine-derived natural products remains a challenge due to the scarcity of the isolated compounds. Therefore, chemical and biological synthetic techniques are often valuable complementary avenues for material supply. Zampanolide (**1**) and dactylolide (**2**) are marine polyketides that have been isolated from a variety of marine sponges from the Pacific Ocean (Fig. 1).² Both polyketides are microtubule-stabilizing agents with IC₅₀'s in the nanomolar and micromolar range, respectively.³ Structurally, they contain an identical 20-membered macrolactone core, however, zampanolide contains an unusually stable acyl hemiaminal side chain. Compounds **1** and **2** covalently bind to His229 in α,β-tubulin dimers through conjugate addition to C9 of the enone functionality, as characterized by X-ray crystallography.⁴ Although there have been several total syntheses reported on dactylolide and zampanolide,⁵ efficient and stereoselective gen-

eration of the acyl hemiaminal has limited access to material for extensive biological studies. Though the biosynthesis of **1** and **2** have not been elucidated, recent isolation of the marine polyketides from the same marine sponge, *Cacospongia mycofijiensis*, by Northcote and co-workers^{2c} provides some support for their expected biosynthetic relationship.

Fig. 1 (–)-Zampanolide and (–)-dactylolide are marine polyketide natural products that covalently stabilize microtubules to promote tubulin polymerization and apoptosis. **3**, **4**, and **5** are the proposed analogues lacking the C13-methylene and C17-methyl groups and their corresponding GC₅₀ values.

^a The Warren Family Research Center for Drug Discovery and Development and the Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670, USA. E-mail: rtaylor@nd.edu

^b Vertex Pharmaceuticals, 50 Northern Ave, Boston, MA 02210, USA

^c Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

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As polyketides are biosynthetically constructed by polyketide synthase (PKS) machinery, alkyl substituents are typically installed at even-numbered positions through the use of methylmalonyl-CoA extender units or by *S*-adenosyl-*L*-methionine (SAM)-dependent methyl transferase enzymes.⁶ Although less common, β -branching occurs when alkyl substituents are incorporated at odd-numbered carbons. These alkyl substituents are incorporated into polyketide scaffolds by genes encoding HMG-CoA synthase (3-hydroxy-3-methylglutaryl-CoA) cassette proteins.⁷ Zampanolide and dactylolide's macrolactone core possesses three different β -branched functionalities: methyl groups at C5 and C17 and a C13-exocyclic methylene. Whether individual structural motifs, within a natural product framework, provides evolutionary value or are vestigial artifacts of larger gene transfers are uncertain. However, identification of a complex target's extraneous functional groups provides the synthetic chemist an opportunity to prepare a simpler target while maintaining desired biological activity.⁸

Limited work on the structure–activity relationship (SAR) of zampanolide/dactylolide has been exclusively conducted by Altmann.⁵ⁿ Their efforts revealed that the C13 exomethylene group in dactylolide proved inconsequential to the overall activity of the molecule. Altmann and co-workers were also successful in synthesizing dactylolide and zampanolide analogues lacking the tetrahydropyran ring (THP). Despite the elimination of two stereocenters (C11 and C15) and a major source of rigidity in the macrolide, the analogues still exhibited, albeit reduced, cytotoxic activity compared to their respective natural products. In 2018, Northcote and co-workers reported^{2c} the isolation of several new zampanolide structures from marine sponge extracts. These analogues include structural isomers of zampanolide with alkene geometric isomers about the C4–C5, C8–C9, C2'–C3', and C4'–C5' bonds. Despite the likely major differences in solution conformations adopted by the isomers, all compounds retained potent cytotoxic activity. These studies suggest that the binding site of the molecule permits some degree of conformational flexibility without loss of binding affinity.

High field NMR and accompanying computational studies conducted in our laboratory on (–)-dactylolide demonstrated that the C15–C19 region of the molecule controls the conformational preferences for the macrolide.⁹ The relative rigidity of this region is attributed to the $A^{1,3}$ -strain arising from the C17 methyl group of the trisubstituted olefin and the C15 stereogenic center. Efficiently and selectively installing the C16–C17 trisubstituted olefin has remained a major hurdle in the previous total syntheses of dactylolide and zampanolide. We hypothesized that analogues lacking the C17 methyl group would have simplified the synthesis while still retaining a comparable conformational preference and potential biological activity.¹⁰

In classical SAR studies, medicinal chemists generally focus on optimization of the binding affinity of the protein and ligand. Due to limited number of rotatable bonds, the impact that these changes have on the ligand's conformational pref-

erences and the ligand-protein interaction is often disregarded.¹¹ Targeting structures that lack the C17 methyl substituent is predicted to simplify the synthetic route. However, it is important to keep in mind that elimination of a key rigidifying element may induce major changes to the macrolide's conformational preference, entropic contributions to binding affinity and less optimal ligand-protein interactions.

Results and discussion

In designing an analogue to test our hypothesis, our group began by considering the data obtained by Altmann and co-workers. We designed our initial analogue without the C13 *exo*-methylene group, which would reduce the required synthetic operations. Altmann also revealed that replacing the aldehyde with a fully reduced alcohol exhibited similar cytotoxic activity, while still providing a molecular handle for further analogue generation. To compare the conformational preferences of **2** to the conformational preferences of **4**, we conducted a 50 000-step Monte Carlo conformational search employing the MM3* force field¹² and GB/SA solvation model for water with **4**.¹³ Conformers within a 4.7 kcal mol^{–1} global minimum populated two conformational families (conformers **4a** and **4b**) in regard to the C14–C19 region of the molecule. Fig. 2 shows **4** in a low energy conformational family that adopts the -120° torsional angle (conformer **4a**) about the 14–15–16–17 carbons, but now a 0° torsional angle (conformer **4b**), eclipsing the C14 alkyl and the C17–H, represents a low energy structure shown in conformer **4b**. The lowest energy structure of the different families were within the margin of error, which correlate well with classic allylic strain interactions between an alkyl group and a hydrogen of models, *E*-2-pentene and 3-methyl-1-butene.^{13,14} Analysis of the potential energy surface suggests that a larger degree of torsional flexibility exists for compound **4** compared to the natural product, where the C17-Me and C14-alkyl eclipsing interaction^{15a} is likely not accessible. In dactylolide, the C17-Me also controls the torsional preference of the 17–18–19–O dihedral *via* allylic strain induced by the trisubstituted olefin. As shown in conformer **4b**, the greater flexibility obtained from removal of the C17-Me affects the torsional preferences for the 16–17–18–19 dihedral allowing for different conformational preferences along the C14–C19 section of the macrolide. The pyran and C14–C19 portions of conformer **4a** overlay nicely with experimentally derived conformer **2a** of dactylolide and the active conformer of zampanolide covalently bound to tubulin (Fig. 2).⁴ Analogues lacking the C17-Me are less likely to adopt the conformation observed in the tubulin bound structure of zampanolide and the solution conformer of dactylolide. However, the low energy conformations resembling conformer **2a** are still energetically accessible. With this preliminary analysis in hand, we sought to examine our hypothesis by synthesizing simplified dactylolide

Fig. 2 Computationally derived conformations of **2** and **4**. (a) DISCON derived solution conformation of (-)-dactylolide. (b) The lowest energy solution conformer of **4** (c) low energy conformation of **4**. (d) 2D representation of $A^{1,3}$ -strain in **4** and **2**. (e) Overlay of tubulin bound zampanolide, conformer **2a** and conformer **4a**.

and zampanolide analogues and obtaining their biological evaluations.

Fig. 3 displays the retrosynthetic fragmentation of target macrolide **3** to provide key intermediates **6** and **7** and well-precedented coupling reactions for their convergence. In previous reported synthetic efforts towards zampanolide and dactylolide, attempts to exploit cross-metathesis strategies for the installation of the *E*-trisubstituted olefin were low yielding with poor *E/Z* selectivity. However, by targeting a C17-desmethyl derivative, we envisioned that both of these issues would be alleviated.

The synthesis of southern fragment **7** (Scheme 1) began with enantiomerically pure epoxide **8**,^{5f} which can be readily prepared from commercially available (*R*)-aspartic acid using known literature procedures. Regioselective epoxide opening with but-3-en-1-yl magnesium bromide in the presence of CuI provided the terminal alkene in near quantitative yield.¹⁶ Cross-metathesis of the resulting terminal alkene product and butene diacetate **9** with Grubbs-II catalyst¹⁷ proceeded in ex-

cellent yield. Vinyl pyran **10** was then generated *via* palladium-catalyzed Tsuji–Trost cyclization¹⁸ using commercially available (*S,S*)-DACH-phenyl ligand to afford the pyran in >20:1 diastereomeric ratio and 95% yield. Notably, these steps could be performed on a multigram scale affording over 5 grams of vinyl pyran **10** with an overall yield of 71% over 6 steps from commercially available material. Pyran **10** was then

Scheme 1 Synthesis of alcohol **3** (a) but-3-en-1-yl magnesium bromide CuI, THF, -78°C , 99% (b) **9**, Grubbs II generation catalyst (5 mol%) DCM, 45°C , 98%, 8:1 *E/Z* (c) $\text{Pd}_2\text{dba}_3\text{-HCCL}_3$ (10 mol%) (*S,S*)-DACH-phenyl (30 mol%), DCM, 95%, 25:1 dr (d) **11**, Grubbs II generation catalyst (20 mol%), DCM, 45°C (e) TESCl, imidazole, DMF, 78% (90% brsm) over 2 steps (f) MeCN:H₂O:TFA 1:8:1, 93%, 8:1 *E/Z* (g) (2,4,6)-trichlorobenzoyl chloride, NEt₃, DMAP, toluene, 79% (h) HF-Pyr, THF 92% (i) DMP, DCM, 83% (j) Ba(OH)₂·8H₂O, THF/H₂O 40:1, 49%, >20:1 *E/Z* (k) DDQ, DCM/H₂O 5:1, 67%.

Fig. 3 Synthetic disconnections of function-oriented analogue design. The macrolactone is closed *via* an intramolecular HWE olefination. Lactone functionality is installed using Yamaguchi esterification. The disubstituted alkene was constructed by cross metathesis.

subjected to cross metathesis conditions using homoallylic alcohol **11** as a coupling partner.¹⁹ Unfortunately, chromatographic separation of cross metathesis product **7** and remaining homoallylic alcohol **11** proved to be exceedingly challenging. To circumvent this setback, we found that the two compounds were much easier to separate if the mixtures of alcohols were transiently protected using TESCl to afford the silyl ether²⁰ (78% yield over 2 steps, 90% brsm, 8,1 *E/Z* selectivity). The southern fragment could be completed in 93% yield after silyl deprotection using buffered trifluoroacetic acid (TFA) affording **7** as a single stereoisomer.

With the southern fragment in hand and a large amount of carboxylic acid **6** prepared using Altmann and co-worker's procedure,⁵¹ the fragment coupling could commence. The Yamaguchi esterification proceeded smoothly, providing 79% of the corresponding ester **12**. Global deprotection employing HF·Pyr and oxidation of the resulting allylic alcohol with Dess–Martin periodinane (DMP) afforded the intramolecular Horner–Wadsworth–Emmons precursor in 75% yield over two steps. The ketoaldehyde was then reacted with Ba(OH)₂·8H₂O under high dilution in THF/H₂O (40:1) to afford the macrocyclic enone as a single diastereomer in a modest 49% yield. Finally, subsequent PMB deprotection using DDQ produced the alcohol analogue **3** in 67% yield.

With a scalable route to the alcohol established, we aimed to generate the corresponding 13-desmethylene-17-desmethyl zampanolide (**5**) and dactylolide (**4**) analogues (Scheme 2). Alcohol **3** could easily be oxidized using DMP to synthesize 13-desmethylene-17-desmethyl dactylolide (**4**). Aldehyde **4** could then be converted to an epimeric mix (1.5:1) of hemiaminal **5** using a nucleophilic source of amide originally developed by Hoyer and co-workers.^{5d}

Computational analysis suggested that **4** has a higher degree of flexibility compared to that of dactylolide **2**. With a substantial amount of **4** in hand, we sought to analyze its solution conformational preferences and compare it to our understanding of the solution conformation of **2**. Towards this aim, we used rotating-frame Overhauser effect (ROESY)²¹ experiments to understand whether **4** adopted a conformation similar to dactylolide **2**. The ROESY experiments with **4** was measured in DMSO on a 600 MHz spectrometer with a mixing time of 400 ms. A strong NOE was observed between C15–H and C17–H, providing evidence for a populated conformer **4a** with a 120° dihedral across the 14–15–16–17 dihedral (Fig. 4). Though we observed the strong through-space interaction, the ³*J*_{H15–H16} coupling value measured 4.8 Hz for

4 compared to the 8.0 Hz obtained from NMR experiments with **2**. The ROESY spectra suggests that the macrolide does adopt a conformation at ambient temperatures that enables the C15–H to be close in space to the C17–H on the NMR timescale. The smaller coupling constant indicates that the ring spends less time in that conformation than the natural product due to rapid rotation about the 14–15–16–17 dihedral enabled by the alleviated allylic strain. The resulting time-averaged coupling constant and observed NOE may be accredited to conformational averaging on the NMR timescale.

The spectral data indicates that the alleviation of allylic strain also has an impact on the C18–O region of the macrolide. The observed coupling constants for the C19–H in **4** (³*J*_{H18A–H19} = 9.5 Hz, ³*J*_{H18B–H19} = 3.7 Hz) indicates that this portion of the molecule adopts a similar conformation to solution conformer **2a** (³*J*_{H18A–H19} = 11.2 Hz, ³*J*_{H18B–H19} = 2.7 Hz). Interestingly, the ROESY data of **4** shows strong cross peaks between C16–H and both C18–H_A and C18–H_B, while **2** was only reported to show a cross peak with C18–H_A. The coupling constants observed for C17–H and C18–H_{AB} (³*J*_{H17–H18A} = 7.6 Hz, ³*J*_{H17–H18B} = 6.0 Hz) indicated that the alleviated allylic strain induces flexibility about the 16–17–18–19 dihedral and the 17–18–19–O dihedral. While conformation **4a** represents a viable low-energy conformational family, the mitigation of A^{1,3} and A^{1,2} strain enables the C18–O region of the ring to adopt other low energy torsion angles about these dihedrals. This is supported by the lack of an observed NOE between C19–H and C17–H, cross peaks between the C16–H and both hydrogens on C18, as well as the time-averaged ³*J*_{H18AB–H19} coupling constants. In conclusion, the spectral data supports the hypothesis drawn from our computational data. The relief of allylic strain in the C14–C19 portion of the macrolide does allow for solution conformations similar to that of **2a**, but it also allows for a greater amount of torsional flexibility about the region of the molecule.

Our last task was to examine how the observed torsional flexibility of the simplified analogues affects the overall biological activity of the synthesized analogues. The anti-proliferative effects (GI₅₀) of **3**, **4** and **5** were examined in prostate (PC-3), lung (A549) and ovarian (1A9) human carcinoma cell lines compared to taxol and their parent natural products (Table 1). Dactylolide analogues **3** and **4** were found to have no significant cytotoxic activity (>30 000 nM) towards any of the cancer cell lines. These results came as a surprise

Scheme 2 Late-stage synthesis of the corresponding dactylolide and zampanolide analogues. (l) DMP, DCM, 68% (m) DIBAL-H, THF, 49%, 1.5:1.0 dr.

Fig. 4 Observed NOEs and coupling constants for dactyloide's conformational preferences along the C14–C19 region of the macrolide evidence a conformationally rigid structure. Observed NOEs and time-averaged coupling constants of the C14–19 region of the 13-desmethylene-17-desmethyl-dactyloide analogue supports the increased flexibility of the region predicted by our computational studies.

Table 1 Antiproliferative activity of the simplified analogues compared to taxol, (–)-zampanolide and (–)-dactyloide in human cancer cell lines. (GI₅₀ values [nM])^a

Compound ^a GI ₅₀ (nM)	PC3 (prostate)	A549 (lung)	1A9 (ovarian)
Taxol	4.6 ± 2	6 ± 1.5	5.2 ± 2.0
(–)-Zampanolide	3.2 ± 0.4	5.2 ± 2.0	2.1 ± 1.0
(–)-Dactyloide	460 ± 200	950 ± 140	620 ± 170
1	>30 000	>30 000	>30 000
2	>30 000	>30 000	>30 000
3 ^b	56 ± 20	180 ± 80	120 ± 35

^a Cells were exposed to the compounds for 72 hours. ^b 1.5 : 1.0 mixture of epimers.

to us, as Altmann's des-THP-dactyloide analogues retained moderate micromolar cytotoxicity in A549 and PC-3 cell lines despite an elimination of a major sterically persuasive moiety.⁵ⁿ On the other hand, zampanolide analogue 5 displayed moderate activity towards all three cancer cell lines, losing 18–55-fold activity compared to (–)-zampanolide. This result was to be expected since Altmann's des-THP-zampanolide analogue retained moderate nanomolar cytotoxicity. It is interesting to note how the impact of the hemiaminal side chain influences the potency of the compounds. While increased flexibility in the macrolide lost any observable activity in 3 and 4, the introduction of the acyl hemiaminal side chain recovers significant cytotoxicity with an epimeric mixture of 5 having sub-micromolar GI₅₀ values. The X-ray crystal structure solved by Altmann and co-workers reveals additional interactions between the binding of zampanolide's side chain and the tubulin binding site. Thus, it is likely that the entropic penalties associated with the increase of flexibility in the macrolide are compensated by the additional enthalpic interactions.

Conclusions

We have completed an improved synthetic route to three new simplified analogues of (–)-zampanolide and (–)-dactyloide. Using the material obtained from the synthesis, we were able to analyze the conformational preference of 4 and compare it

to the conformational preferences of the common macrolide core. Our computational and NMR analysis confirms that the removal of the 17-methyl enables a greater amount of torsional flexibility in the southern region of the macrolide. Lastly, we found that the increase of torsional flexibility negatively affects the cytotoxic activity of 3 and 4 compared to 2. On the other hand, 5 retains a large portion of that activity, providing further confirmation of the importance of the acyl hemiaminal.

Conflicts of interest

There are no conflicts to declare.

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