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Synthesis and Biological Evaluation of (−)-Laulimalide Analogues

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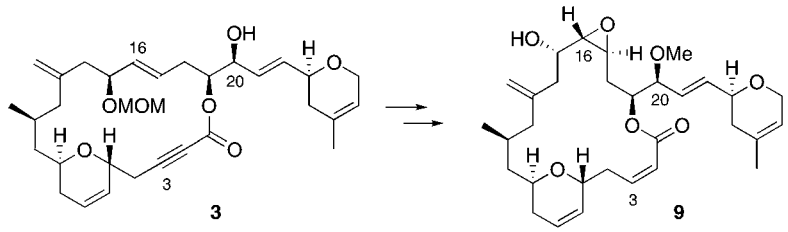
ABSTRACT



(IC_{50}: 0.24 μM MDA-MB-435)

The syntheses of five laulimalide analogues are described, incorporating modifications at the C_{16}–C_{17}-epoxide, the C_{20}-alcohol, as well as the C_1–C_3-enoate of the parent natural product. The resultant analogues are active in drug-sensitive HeLa and MDA-MB-435 cell lines. Significantly, like laulimalide, these analogues are poor substrates for the drug transport protein P-glycoprotein (Pgp) and are thus effective against Taxol-resistant cell lines.

Laulimalide (**1**)[1] is a structurally novel 20-membered macrolide that promotes abnormal tubulin polymerization and apoptosis *in vitro* with a mode of action similar to Taxol.[2] However, unlike Taxol, laulimalide binds tubulin at a different site[3] and is less susceptible to multidrug resistance.[2,3] Its unique biological profile has attracted considerable synthetic interest, resulting in ten total syntheses from seven synthetic groups.[4] Despite this intense synthetic effort, there have been few reports aimed at the over-arching goal of designing and synthesizing more efficacious analogues.[5]

A major goal in advancing the laulimalide lead is to eliminate its intrinsic instability arising from its facile conversion under acidic conditions to the more stable but biologically less potent isomer, isolaulimalide **2** (Figure 1). In 2002, we reported a concise and flexible synthesis of (−)-laulimalide, which provided a facile route to analogues that were selected to maintain laulimalide's activity against drug-resistant cell lines but to exhibit better stability. As shown in Figure 1, it was felt that the degradation pathway of **1** to

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(2) Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1977**, *37*, 159.

(3) Pryor, D. E.; O'Brate, A.; Bilcer, G.; Diaz, J. F.; Wang, Yu.; Wang, Yo.; Kabaki, M.; Jung, M. K.; Andreu, J. M.; Ghosh, A. K.; Gianna-kakou, P.; Hamel, E. *Biochemistry* **2002**, *41*, 9109.

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(5) For the preparation and evaluation of laulimalide analogues, see refs 3 and 4f.

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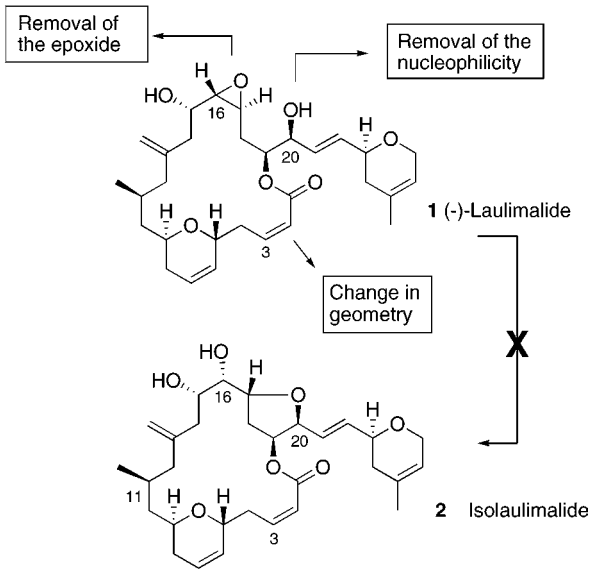


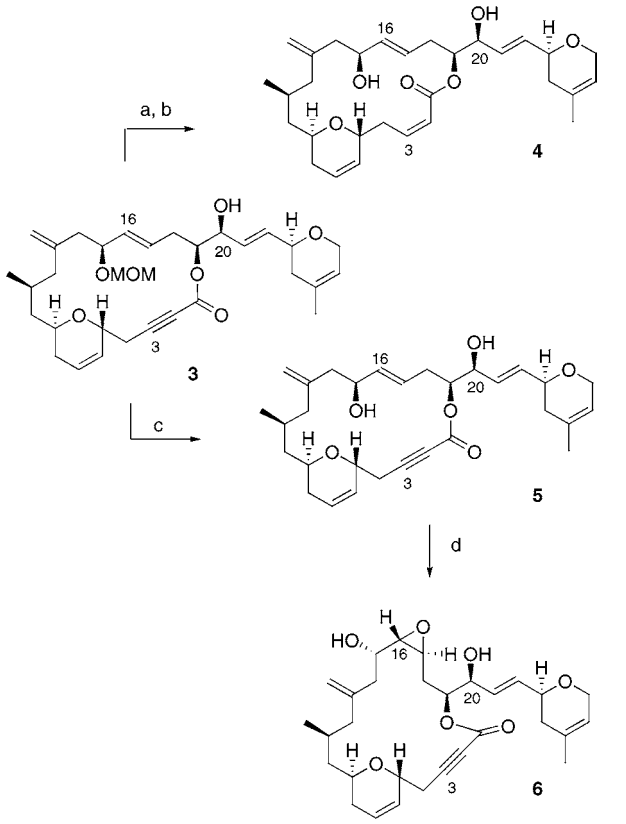
Figure 1. Design of laulimalide analogues.

2 could be blocked in three ways: by removing either the C_{16}-C_{17}-*trans*-epoxide, thereby eliminating the C_{17}-electrophilic center, alkylating the C_{20}-hydroxyl, thereby attenuating its nucleophilicity, or modification of the C_2-C_3-enoate to alter the orbital alignment of the C_{20}-OH and the C_{17}-epoxide. Herein, we report the syntheses and biological activities of structurally novel analogues designed to explore these structural changes.

Macrolactone **3**, prepared as previously reported,[6] was used as a common intermediate for the synthesis of the analogues **4**, **5**, and **6** (Scheme 1). *des*-Epoxy laulimalide **4**,[7] which served as the direct precursor of **1** in our total synthesis effort, was readily prepared via a Lindlar reduction[8] of the alkynoate to afford the corresponding *Z*-enoate (91%) and subsequent removal of the C_{15}-MOM group with use of Me_2BBr (76%).[9] Considering the synthetic difficulties associated with the chemoselective synthesis of the base-sensitive, enolizable *Z*-macrolactone moiety, we next targeted a *des*-epoxy alkynoate analogue **5**, in which the *Z*-C_2-C_3-alkene is replaced by a more robust and synthetically tractable C_2-C_3-alkyne. Under the above MOM deprotection conditions, alkyne diol **5** was readily generated from **3** in good yield (78%). Finally, treatment of **5** under the original Sharpless asymmetric epoxidation conditions led to the regio- and diastereoselective formation of analogue **6** in moderate yield (36%).

Analogues **8** and **9** (Scheme 2) were selected to mitigate the inherent nucleophilicity of C_{20}-hydroxyl. The methyl ether was chosen to minimize potential steric effects and

Scheme 1[a]



[a] Reagents and conditions: (a) 25 mol % of Lindlar catalyst, 1 equiv of quinoline, 1 atm of H_2, 1:1 EtOAc:1-hexene (91%); (b) Me_2BBr, Et_3N, CH_2Cl_2, −78 °C (76%); (c) Me_2BBr, Et_3N, CH_2Cl_2, −78 °C (78%); (d) Ti(O*i*Pr)$_4$, (+)-DIPT, *t*-BuOOH, 4 Å MS, CH_2Cl_2, −20 °C (36%).

maximize stability. Toward this end, Lindlar reduction of **3** afforded the *Z*-alkenoate, which after extensive reaction screening was converted with Meerwein's salt in the presence of a non-nucleophilic base[10] to the desired methyl ether **7** in *94% yield*. Deprotection of the C_{15}-MOM group yielded *des*-epoxy analogue **8**, which upon epoxidation yielded the C_{20}-methoxy laulimalide analogue **9** as a single diastereomer in 83% yield.

The anti-proliferative and cytotoxic activities of analogues **4**, **5**, **6**, **8**, and **9** were evaluated in two drug-sensitive cell lines, HeLa and MDB-MB-435, using the SRB assay. The dose response curves indicated that in both cell lines all of the analogues were effective inhibitors of cell proliferation and were cytotoxic. Differences were observed in the potencies of the compounds as reflected in the IC_{50} values for the inhibition of proliferation summarized in Table 1. *des*-Epoxy laulimalide (**4**) is the most potent analogue with IC_{50} values averaging 0.11 μM, which is roughly 19-fold less potent than the activity reported for laulimalide. The C_{20}-methoxy analogue **8** exhibited a significant decrease in potency (0.11 μM to 5.1 μM). However, potency can be

(6) For the synthesis of **3**, see ref 4*l*.

(7) Compound **4** was also prepared and evaluated by Ghosh and Mulzer, see refs 3 and 4f.

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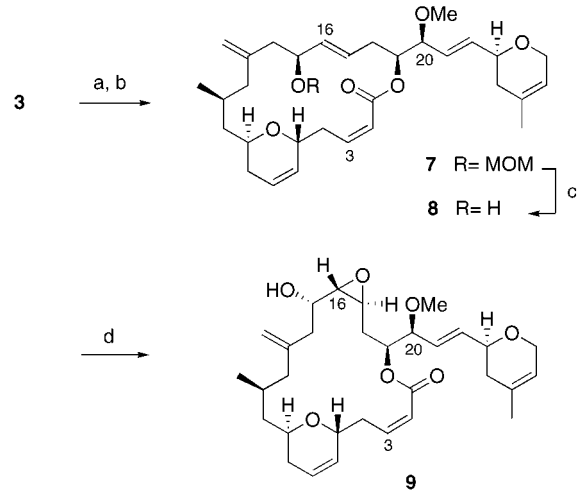
(10) Evans, D. A.; Ratz, A. M.; Huff, B. E.; Sheppard, G. S. *J. Am. Chem. Soc.* **1995**, *117*, 3448.

Scheme 2[a]



[a] Reagents and conditions: (a) 25 mol % of Lindlar catalyst, 1 equiv of quinoline, 1 atm of H_2, 1:1 EtOAc:1-hexene (91%); (b) Me_3OBF_4, CH_2Cl_2, 2,6-di-*tert*-butyl-4-methylpyridine, CH_2Cl_2 (94%); (c) Me_2BBr,CH_2Cl_2, −78 °C (82%); (d) Ti(O*i*-Pr)$_4$, (+)-DIPT, *t*-BuOOH, 4 Å MS, CH_2Cl_2, −20 °C (83%).

regained by incorporation of C_{16}-C_{17}-epoxide (analogue **9**), suggesting that modifications at the C_{20}-hydroxyl are moderately tolerated. A similar effect is also observed in alkynoate analogues with the epoxy analogue **6** being roughly 6−8-fold more potent than the *des*-epoxy analogue **5**.

Table 1. IC_{50}Values of the Natural Laulimalides and Synthesized Analogues

	IC_{50} (μM)		
compd	HeLa	MDA-MB-435	NCI/ADR[a]
1		0.0057 ± 0.0006[14]	
2		1.97 ± 0.097[14]	
4	0.10 ± 0.01	0.12 ± 0.004	0.37 ± 0.007 (3.1)
5	18.3 ± 4.0	16.5 ± 2.5	25.4 ± 3.0 (1.5)
6	2.20 ± 0.84	2.50 ± 0.29	13.7 ± 0.34 (5.5)
8	5.86 ± 0.85	4.41 ± 0.58	22.0 ± 2.5 (5.0)
9	0.18 ± 0.014	0.24 ± 0.009	0.47 ± 0.052 (2.0)

[a] Resistance factors are given in parentheses.

Cell lines with a high expression of the drug-transport protein P-glycoprotein are resistant to the effects of drugs that are substrates of this transporter. Taxol is a well-known substrate for Pgp and cell lines over-expressing this protein are resistant. The ability of the analogues to circumvent Pgp-mediated resistance was next evaluated by using the NCI/ADR cells, which have a high level of Pgp expression. This cell line was previously known as MCF7/ADR.[11] The resistance factors, shown in parentheses in Table 1, for each compound were calculated by dividing the mean IC_{50} value in the NDI/ADR cell line by the mean IC_{50} value in the drug-sensitive MDA-MB-435 cell line. In the presence of verapamil, an inhibitor of Pgp, the IC_{50} values obtained in the NCI/ADR cell line are equal to the values obtained in the MDA-MB-435 drug-sensitive cell line.[12] The calculated resistance factor for Taxol in these two cell lines is 827 (data not shown). As shown in Table 1,[13,14] the laulimalide-based analogues showed a range of resistance values of 1.5−5.5, indicating that all of the analogues, like the parent compound, are poor substrates for Pgp and are therefore candidates for treating Taxol-resistant tumor cells.

In summary, we have disclosed the syntheses and biological activities of structurally novel (−)-laulimalide analogues that were prepared to determine whether biological activity against Taxol-resistant cell lines could be maintained while minimizing instability. Biological evaluations of these analogues have provided valuable information allowing the generation of preliminary structure activity relationship data, which in turn could facilitate the realization of rationally designed, structurally simplified, clinically superior analogues as anticancer therapeutic agents.

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Supporting Information Available: Experimental details and analytical data for **4**, **5**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) Cells were treated with a range of concentrations of the analogues for 48 h and the inhibition of proliferation was determined by using the SRB assay. The resistance factors were calculated by dividing the IC_{50} of the drug-resistant cell line (NCI/ADR) by the IC_{50} of the (MDA-MB-435) drug-sensitive cell line. The values represent the means of 3 experiments ± the SD.

(14) The values are from ref 2.