

Synthesis and Cytotoxicity of Semisynthetic Withalongolide A Analogues

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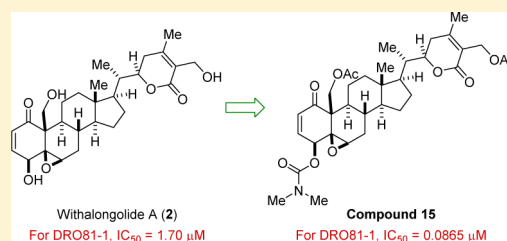
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S Supporting Information

ABSTRACT: The natural product withaferin A exhibits potent antitumor activity and other diverse pharmacological activities. The recently discovered withalongolide A, a C-19 hydroxylated congener of withaferin A, was recently reported to possess cytotoxic activity against head and neck squamous cell carcinomas. Semisynthetic acetylated analogues of withalongolide A were shown to be considerably more cytotoxic than the parent compound. To further explore the structure–activity relationships, 20 new semisynthetic analogues of withalongolide A were synthesized and evaluated for cytotoxic activity against four different cancer cell lines. A number of derivatives were found to be more potent than the parent compound and withaferin A.

KEYWORDS: Withalongolide A, withaferin A, triacetate, jaborosalactone, macrocycle, DRO81-1



Withanolides are a group of naturally occurring C28 steroidal lactones assembled on an ergostane skeleton. Most of the withanolides are highly oxygenated members of the Solanaceae family.^{1,2} Withaferin A (1; Figure 1) has a wide

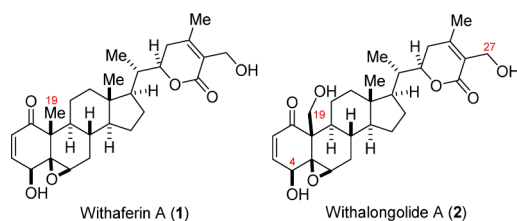


Figure 1. Chemical structures of withaferin A and its C-19 oxygenated analogue, withalongolide A.

range of biological activities, such as antitumor, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, protective, and antiangiogenic effects.^{1–6} The antitumor activity of withaferin A results from targeting multiple signaling pathways such as HSP90 and NF-κB that may circumvent the development of resistance among cancer cells.^{7–11}

Recently, Zhang et al. reported the isolation, structures, and cytotoxic activities of withanolides featuring the presence of a rare C-19 hydroxy group.^{12,13} Withalongolide A (2; Figure 1) was found to be less potent than withaferin A against the carcinoma and melanoma cell lines tested. However, the

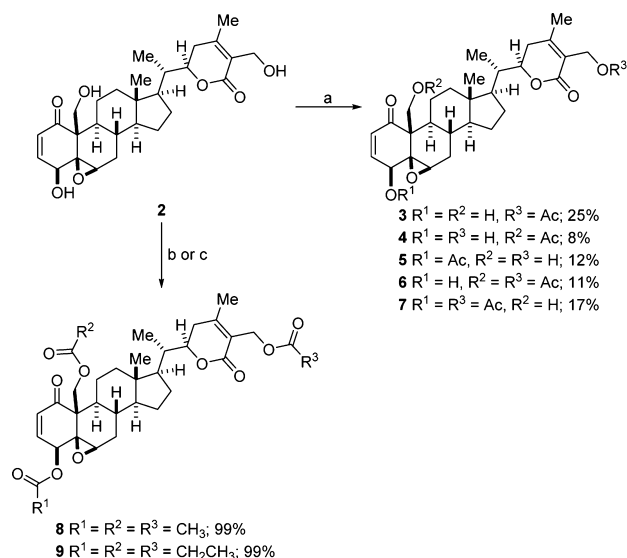
semisynthetic derivatives withalongolide A 4,27-diacetate 7 and withalongolide A 4,19,27-triacetate 8 (Scheme 1) showed improved potency and selectivity against the melanoma cell line B16F10 over both the parent compounds 2 and 1.¹² This led us to initiate a study to examine preliminary structure–activity relationships (SARs) for compound 2. In this letter, we report the synthesis and biological evaluation of 20 new semisynthetic analogues of this fascinating class of cytotoxic agents.

Previous authors have asserted that a 2-ene-1-one, a 5β,6β-epoxide, and a 17β-oriented δ-lactone are essential for biological activity.^{2,14,15} Accordingly, we initially sought to minimize modifications at these positions. Moreover, since the di- and triacetate of compound 2 displayed significant selectivity against melanoma cell line B16F10 with a potency in nanomolar range, we first focused on examining the effects of different derivatives of the free hydroxyl group of 2.

Accordingly, we prepared a series of aliphatic esters of 2 (Scheme 1). After slight modifications of the standard acetylation procedures, it was possible to isolate mono- (3–5) and diacetylated analogues 6 and 7¹² from the same reaction along with some recovered 2 (acetic anhydride was added in two portions; see the Supporting Information for details). Withalongolide A 4,19,27-triacetate 8¹² and withalongolide A

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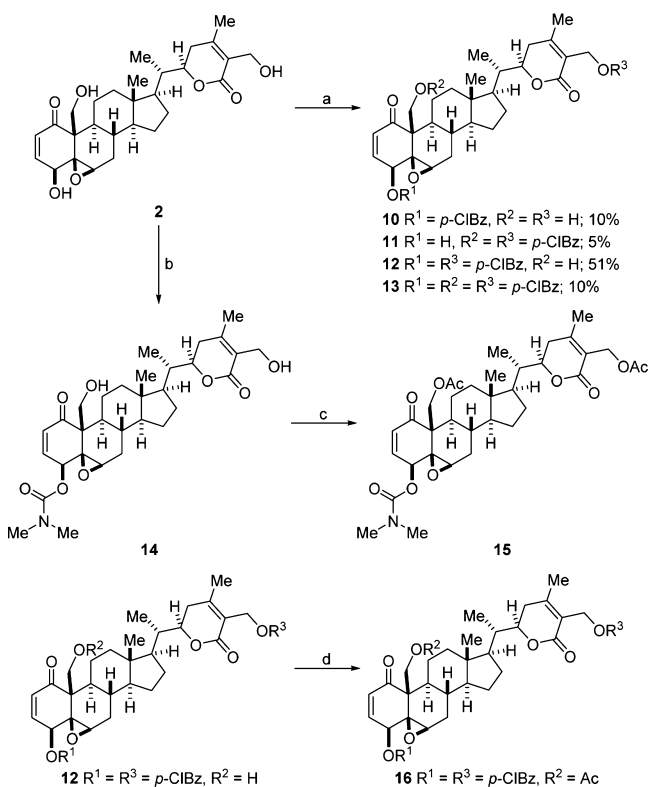
Scheme 1. Synthesis of Acetylated and Propionylated Analogues of Withalongolide A (2)^a

^aReagents and conditions: (a) For 3–7, $(CH_3CO)_2O$, pyridine, rt, 73% combined yield; (b) for 8, $(CH_3CO)_2O$, pyridine, DMAP, rt, 99%; (c) for 9, $(CH_3CH_2CO)_2O$, pyridine, DMAP, rt, 99%.

4,19,27-propionate 9 were obtained by treating 2 with appropriate anhydrides in the presence of pyridine and a catalytic amount of 4-(dimethylamino)pyridine (DMAP).

The second series of analogues entailed the synthesis of acetates and aromatic esters in assorted combinations along with carbamates (Scheme 2). The reaction of 2 with *p*-chlorobenzoyl chloride under typical acylation conditions afforded a mixture from which mono- (10), di- (11 and 12), and tri-*p*-chlorobenzoate ester 13 were isolated.¹⁶ The free C-19 hydroxyl group of 12 was further acetylated with acetic anhydride to afford compound 16. Carbamoylation only occurred at the C-4 hydroxyl, even after treating 2 with excess dimethylcarbamoyl chloride, to afford monosubstituted dimethylcarbamate analogue 14. Subsequent acetylation of the remaining two hydroxyl groups with acetic anhydride delivered compound 15 in good yield. Efforts to make carbonate analogues from phenylchloroformate led to a multitude of products, which seemed to be unstable.

Jaborosalactones are withanolides isolated from *Jaborosa* species. We synthetically prepared close derivatives of known jaborosalactones by chemically manipulating 8 as shown in Scheme 3. Thus, the palladium-catalyzed, formate-mediated reduction of triacetate 8 afforded a mixture of jaborosalactone derivatives 17¹⁷ and 18^{18,19} through C-4 deoxygenation of the acetate group.²⁰ The structure of the diacetate analogue of jaborosalactone V 17 was confirmed by single-crystal X-ray diffraction analysis (Figure 2).¹⁷ Compound 18, a C-19 acetate analogue of jaborosalactone B 27-acetate was obtained via similar C-4 deoxygenation followed by subsequent epoxide ring-opening.^{18,19} Analogue 19, a C-19 acetate derivative of jaborosalactone E 27-acetate, was obtained by epoxide ring-opening of compound 17 using hydrogen chloride solution in ether.²¹ Besides being expected from a mechanistic perspective, antirelationship of the C-5 chlorine and C-6 hydroxyl groups was assigned based on NMR data comparison with the literature values for jaborosalactone E and similar compounds.^{22,23} Acetylation of a small amount of compound 19

Scheme 2. Synthesis of Benzoylated and Carbamate Analogues of Withalongolide A (2)^a

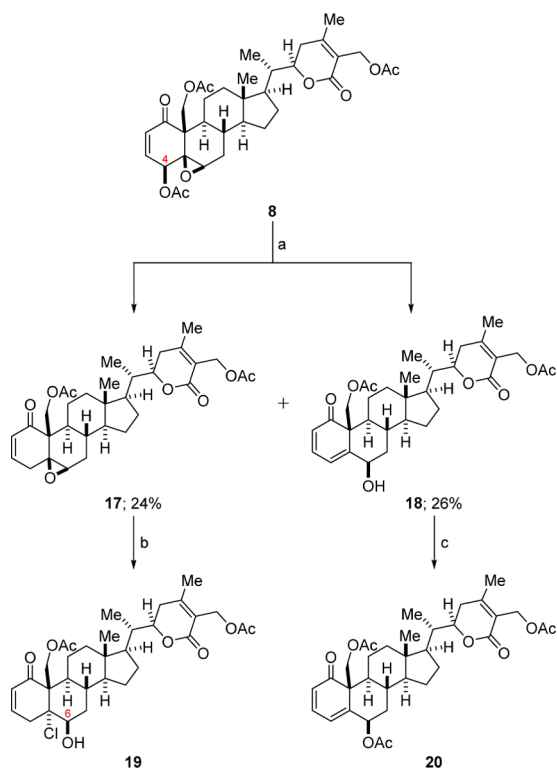
^aReagents and conditions: (a) For 10–13, *p*-chlorobenzoyl chloride, triethylamine, DMAP, CH_2Cl_2 , rt, 76% combined yield; (b) $(CH_3)_2NCOCl$, triethylamine, DMAP, CH_2Cl_2 , rt, 73%; (c) $(CH_3CO)_2O$, pyridine, DMAP, rt, 70%; (d) $(CH_3CO)_2O$, triethylamine, DMAP, CH_2Cl_2 , rt, 95%.

showed a downfield shift of H-6 from δ 3.99 to 5.12 ppm suggesting the presence of a hydroxyl group at C-6 (data not shown).^{21,24} The C-6 free hydroxyl group of compound 18 was further acetylated to provide triacetylated analogue 20.

The α,β -unsaturated ketone of withaferin A is a Michael acceptor.¹⁰ Therefore, we sought to modify the α,β -unsaturated ketone in order to modulate its reactivity. Initial attempts to perform Diels–Alder reactions on the $\Delta^{2,3}$ -olefin were unsuccessful. Subsequent efforts were directed toward the synthesis of α -substituted aryl analogues of the ring A enone of 2. Compound 2 and its triacetate analogue 8 were subjected to α -iodination conditions using iodine and DMAP to afford their corresponding α -iodoenone analogues 21 and 22 (Scheme 4).²⁵ However, attempts to modify these α -iodoenones through Suzuki cross-coupling with boronic acids^{26,27} to obtain α -aryl analogues met with no success; only decomposition of the α -iodoenones was observed.

The final analogue was prepared via ring-closing macrocyclization^{28,29} to afford a rare steroidal macrocycle (Scheme 5).^{30,31} Thus, bis-acylation of withalongolide monoacetate 3 with 4-pentenoic anhydride afforded 23. Ring-closing metathesis with Grubb's II catalyst exclusively afforded the 14-membered macrocycle 24 with *E*-configuration.³²

All synthesized analogues of 2, along with withaferin A as a positive control, were tested for their cytotoxic activity against four cancer cell lines: head and neck squamous cell carcinoma (HNSCC, JMAR), breast cancer cells (MDA-MB-231),

Scheme 3. Synthesis of Jaborosalactone Derivatives from Triacetate **8**^a

^aReagents and conditions: (a) Pd(OAc)₂, PPh₃, HCO₂NH₄, dioxane, reflux, 50% combined yield; (b) HCl solution in ether, CH₂Cl₂, rt, 70%; (c) (CH₃CO)₂O, pyridine, DMAP, rt, 87%.

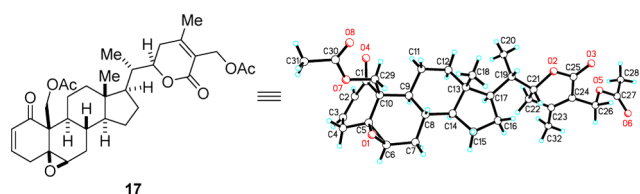
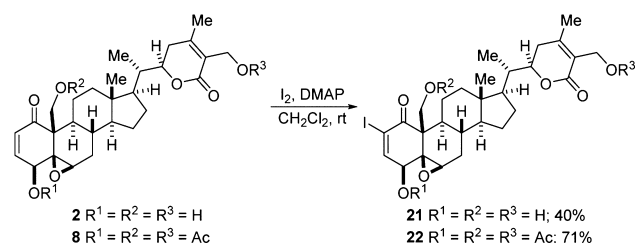
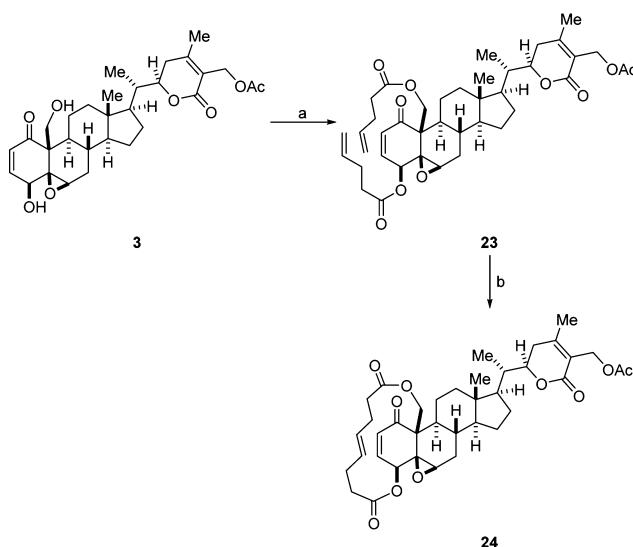


Figure 2. Single-crystal X-ray structure of analogue 17.

Scheme 4. Synthesis of α -Iodoenone Derivatives

melanoma (SKMEL-28), and colon cancer cells (DRO81-1), in addition to normal fetal lung fibroblast (MRC-5) cells (Table 1). Compound **2** was less potent than withaferin A, as previously reported.¹² Acetylation of compound **2** resulted in more potent analogues with diacetates (**6** and **7**) and triacetate **8** exhibiting enhanced cytotoxic activity than the monoacetylated analogues (**3**, **4**, and **5**). Notably, compound **7** was found to be selectively cytotoxic toward DRO81-1 with an IC₅₀ value of 0.0580 μ M.¹² The tripropionylated analogue **9** was also active with IC₅₀ values in the range of 0.130–1.00 μ M. The

Scheme 5. Synthesis of Steroidal Macrocycle **24** from Withalongolide Monoacetate **3**^a

^aReagents and conditions: (a) (H₂C=CHCH₂CH₂CO)₂O, pyridine, DMAP, rt, 92%; (b) Grubb's II catalyst, CH₂Cl₂, 37 °C, 87%.

Table 1. Cytotoxicity Activity (IC₅₀ Values in μ M) of Withalongolide A Analogues against Five Cell Lines^a

| compd | JMAR | MDA-MB-231 | SKMEL-28 | DRO81-1 | MRC-5 |
|-------|-------|------------|----------|---------------|-------|
| 1 | 1.00 | 0.540 | 1.00 | 0.780 | 2.70 |
| 2 | 3.10 | 1.50 | 1.60 | 1.70 | 5.30 |
| 3 | 1.22 | 0.415 | 0.705 | 1.05 | 2.30 |
| 4 | 3.06 | 1.15 | 1.23 | 0.780 | 1.80 |
| 5 | 2.48 | 0.890 | 1.22 | 0.570 | 1.20 |
| 6 | 0.515 | 0.205 | 0.230 | 0.155 | 0.490 |
| 7 | 0.600 | 0.635 | 0.170 | 0.0580 | 0.295 |
| 8 | 0.655 | 0.655 | 0.110 | 0.110 | 0.310 |
| 9 | 1.00 | 0.365 | 0.285 | 0.130 | 0.615 |
| 10 | 1.72 | 1.40 | 2.25 | 1.85 | 2.55 |
| 11 | 4.64 | 2.62 | 1.11 | 1.90 | 2.35 |
| 12 | >10 | >10 | >10 | >10 | >10 |
| 13 | >10 | >10 | >10 | >10 | >10 |
| 14 | 2.09 | 0.610 | 0.515 | 0.585 | 1.75 |
| 15 | 0.970 | 0.175 | 0.205 | 0.0865 | 0.510 |
| 16 | >10 | >10 | >10 | >10 | >10 |
| 17 | 1.22 | 0.790 | 0.710 | 1.10 | 2.05 |
| 18 | >10 | >10 | >10 | >10 | >10 |
| 19 | 2.16 | 0.800 | 1.17 | 1.35 | 3.15 |
| 20 | >10 | >10 | >10 | >10 | >10 |
| 21 | >10 | >10 | 9.11 | 5.25 | 9.10 |
| 22 | 0.830 | 0.210 | 0.275 | 0.115 | 0.475 |
| 23 | 1.44 | 0.625 | 0.470 | 0.580 | 0.865 |
| 24 | 0.965 | 0.245 | 0.205 | 0.225 | 0.360 |

^aAnalogues **12**, **13**, **16**, **18**, and **20** were inactive with IC₅₀ \geq 10 μ M for all cell lines tested.

increased cytotoxic potency of the di- and triacetate analogues of **2** could be due to increased lipophilicity leading to enhanced cell permeability.^{16,33,34}

Although the mono-*p*-chlorobenzoylated derivative **10** and C-19,27-dibenzoylated analogue **11** were active, the C-4,27-dibenzoylated analogue **12** and tribenzoylated compound **13** were not. Compounds **14** and **15** exhibited higher cytotoxic activity than **2**, with compound **15** displaying 3–5 times

increased cytotoxicity against breast cancer and melanoma cells compared to **1**. Compound **15** also demonstrated modest selectivity toward DRO81-1 cells with an IC₅₀ value of 0.0865 μ M, similar to compound **7**.

Jaborosalactone V diacetate **17** was found to be equipotent to withaferin A **1**, suggesting that C-4 hydroxyl group is not crucial for activity.^{2,15} Compound **19**, containing a 5 α ,6 β -chlorohydrin, was slightly less potent than the 5 β ,6 β -epoxy analogue **17**, with IC₅₀ values in the range of 0.800–2.16 μ M against melanoma and carcinoma cells. The cytotoxic activity was abolished in epoxide-lacking analogues **18** and **20**.^{2,15}

Even though the 2-iodoenone analogue **22** displayed comparable cytotoxicity to its parent compound **8**, compound **21** showed very weak activity compared to **2**. Interestingly, macrocycle **24** exhibited increased potency compared to its acyclic analogue **23** across all cell lines tested with IC₅₀ values in the range of 0.205–0.965 μ M.^{28,29} Most of the active analogues were moderately selective toward cancer cells compared to normal fibroblast cells.

In our efforts to contribute toward the development of anticancer-related therapeutics, we have identified additional cytotoxic agents based on the natural product withalongolide A **2**. Many of these analogues were more potent than the parent compound, and the SAR profile was in good agreement with those previously reported for withanolides having similar structures.^{1,2,15,16} The selectivity of analogues **7** and **15** toward colon cancer cells (DRO81-1) is intriguing, and further efforts are underway to study these analogues as potential anticancer agents.

■ ASSOCIATED CONTENT

● Supporting Information

Experimental procedures, cytotoxicity assay, details of the crystal structure of compound **17**, characterization data, and ¹H and ¹³C NMR spectra of new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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