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Expanding the Chemical Space of Withaferin A by Incorporating Silicon To Improve Its Clinical Potential on Human Ovarian Carcinoma Cells

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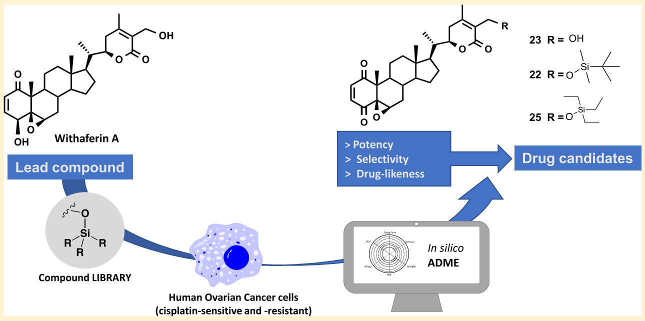
Supporting Information



ABSTRACT: Ovarian cancer represents the seventh most commonly diagnosed cancer worldwide. Herein, we report on the development of a withaferin A (WA)-silyl ether library with 30 analogues reported for the first time. Cytotoxicity assays on human epithelial ovarian carcinoma cisplatin-sensitive and -resistant cell lines identified eight analogues displaying nanomolar potency (IC_{50} ranging from 1 to 32 nM), higher than that of the lead compound and reference drug. This cytotoxic potency is also coupled with a good selectivity index on a nontumoral cell line. Cell cycle analysis of two potent analogues revealed cell death by apoptosis without indication of cell cycle arrest in G0/G1 phase. The structure−activity relationship and in silico absorption, distribution, metabolism, and excretion studies demonstrated that the incorporation of silicon and a carbonyl group at C-4 in the WA framework enhances potency, selectivity, and drug likeness. These findings reveal analogues **22**, **23**, and **25** as potential candidates for clinical translation in patients with relapsed ovarian cancer.

INTRODUCTION

Ovarian cancer (OC) represents the seventh most commonly diagnosed cancer worldwide.[1] The current treatment entails cytoreductive surgery followed by platinum- or taxane-based chemotherapy.[2] Initially, OC responds positively in 70−80% of the cases. However, nearly 70% of patients suffer a relapse within 6 months of the last chemotherapeutic cycle, which is attributed to patients eventually developing resistance to carboplatin and paclitaxel adjuvant chemotherapy.[3] Therefore, chemotherapy resistance, whether primary (i.e., intrinsic) or secondary (i.e., acquired), represents a major hurdle in OC treatment.[4] Additionally, platinum-based chemotherapy is associated with multiple severe side effects (e.g., nausea, myelosuppression, neurotoxicity, nephrotoxicity, hepatotoxicity, and ototoxicity).[5] Thus, there is an urgent need for new second-line therapies to improve the prognosis of patients with relapsed OC. As an alternative treatment strategy to reduce the side effects and resistance caused by cis-platinum-based chemotherapy, a number of combinations with other compounds have been explored. In this sense, natural products (NPs) are ideal candidates for OC chemoprevention or adjuvants of conventional chemotherapy. Recently, Pistollato and co-workers[6] have reviewed NPs targeting OC, describing the molecular mechanisms underlying their effects. These NPs, which include curcumin, epigallocatechin 3-gallate, resveratrol, sulforaphane, and Withaferin A, are characterized by long-term safety and negligible and/or inexistent side effects and have been proposed as possible adjuvants to traditional chemotherapy.

Withaferin A (WA), a natural steroidal lactone, is a promising drug candidate multitargeting various cancer hallmarks.[7] WA downregulates the Notch, Akt, and bcl-2 pathways and causes growth inhibition and apoptosis induction in ovarian carcinoma cell lines, CaOV3 and SKOV3.[8] Studies conducted on various epithelial cancer cell lines (cisplatin-sensitive A2780, cisplatin-resistant variant A2780/CP70, and p53 mutant CaOV3) revealed a synergetic effect of WA in combination with doxorubicin[9] and cisplatin[10] on cell death through the generation of reactive oxygen species-mediated autophagy, leading to DNA damage and induction of apoptosis. The authors suggest that this synergetic therapy could minimize/eliminate the side effects and induction of drug resistance associated with

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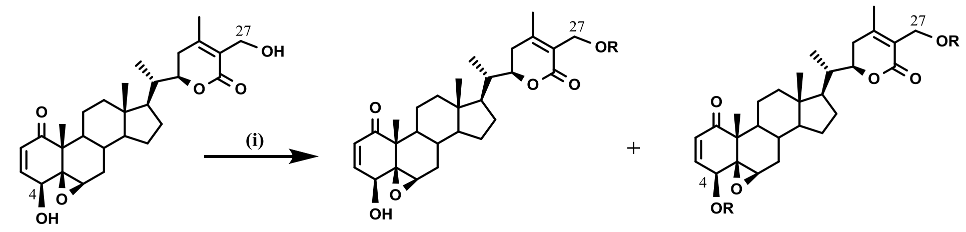
Scheme 1. Synthesis of Withaferin A-Silyl Ether Analogues 2–16[a]



WA (1)

2 R= TES, **3** R= TPRS, **4** R= TIPS, **5** R= TBS, **6** R= THS, **7** R= DMOS, **8** R= DMVS, **9** R= MDPS, **10** R= TBDPS

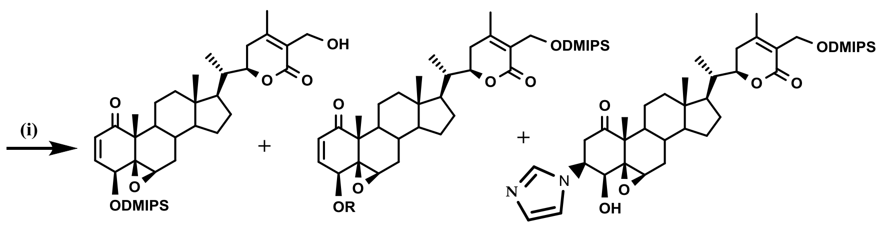
11 R= TES, **12** R= TPRS, **13** R= DMOS, **14** R= DMVS, **15** R= DMPS, **16** R= MDPS

M, methyl; E, ethyl; IP, *iso*-propyl; PR, propyl; B, butyl; TB, *tert*-butyl; H, hexyl; O, octyl; P, phenyl; V, vinyl; S, silicon

[a]Reagents and conditions: (i) $R_1R_2R_3SiCl$, imidazole, 4-(*N*,*N*-dimethylamino)pyridine (DMAP), Et_3N, CH_2Cl_2, room temperature (rt).

Scheme 2. Synthesis of Withaferin A-Silyl Ether Analogues 17–20[a]

WA (**1**)



17

18 R= H
19 R= DMIPS

20

[a]Reagents and conditions: (i) DMIPSiCl, imidazole, DMAP, Et_3N, CH_2Cl_2, rt.

high drug doses. In addition, WA in combination with liposomal preparation of doxorubicin targets aldehyde dehydrogenase I positive cancer stem cells in OC.[11] Moreover, WA alone and in combination with cisplatin targets putative cancer stem cells,[12,13] suggesting that this may present a more efficacious therapy for OC.

A large number of studies have demonstrated the ability of WA to suppress the in vivo growth of various human cancer xenograft models, including prostate, breast, lung, and colon.[7] Also, experimentally induced carcinogenesis in rodent models strongly suggests the chemopreventive potential of WA.[14] Furthermore, the health benefits of Ashwaganda (*Withania somnifera*), mainly attributed to its most abundant and therapeutically effective component WA, are supported by clinical trials for inflammation, immune modulation, and reducing anxiety and arthritis pain. However, to our knowledge, no clinical trials have been carried out with Ashwaganda on cancer or cancer biomarkers as end points.[15] Moreover, in spite of evidence from current preclinical studies suggesting WA to be a promising anticancer drug, its application in clinical oncology is nonexistent.

Furthermore, the medicinal applications of organosilicon molecules are particularly interesting because differences in their chemical properties can contribute to enhancing potency and improving a pharmacological profile.[16] These differences offer the potential for unique and/or specific interactions between an organosilicon molecule and a biological macromolecule. In fact, there are many examples demonstrating that the incorporation of silyl groups provides a general strategy to increase size and lipophilicity for drug design. With the exception of simethicone, a mixture of poly(dimethylsiloxane) and hydrated silica gel used as an antifoaming agent,[17] there are no marketed drugs containing silicon, although currently at least nine silicon-containing compounds have entered human clinical trials.[18] Although still a growing area, the incorporation of silicon in drug scaffolds may well offer great potential for enlarging the chemical space of medicinal chemistry. Moreover, computational methods have become a promising tool for identifying active lead compounds and are being used with the pipeline of drug discovery in most pharmaceutical companies.[19] Thus, theoretical prediction of pharmacokinetic properties i.e., absorption, distribution, metabolism, excretion, and toxicity (ADMET), play a key role in drug discovery, since an unfavorable ADMET has been identified as the major cause of failure of candidate molecules in drug development.[20]

Previously, we have reported that incorporation of silyl ether substituents in the WA framework enhances its cytotoxic effect on HeLa (carcinoma of the cervix), A-549 (lung carcinoma), and MCF-7 (breast adenocarcinoma) human cancer cell lines, whereas a ketone group at C-4 increases selectivity.[21,22] Moreover, the induction of apoptosis by 27-*O*-(*tert*-butyldimethylsilyl)-4-dehydroxy-4-oxo-withaferin A without necrosis under extreme experimental conditions has drawn our attention to these organosilicon analogues.[21]

Therefore, encouraged by previous works highlighting that WA targets various ovarian cancer cell lines[6,8] and the expectation of bio-organosilicon in drug design, efforts to enlarge the chemical space of WA to improve its clinical potential as an anticancer agent are continuing. The current study reports the design, synthesis, and evaluation of a WA-silyl

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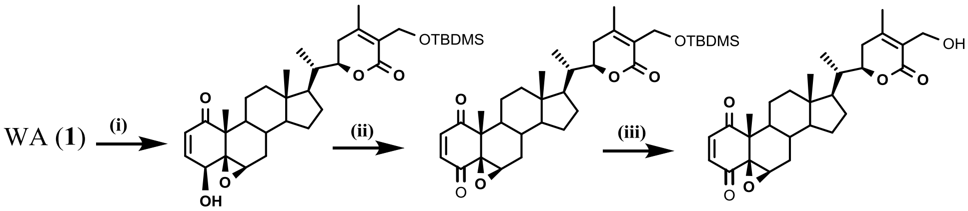
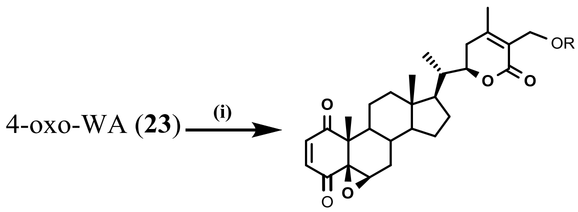
Scheme 3. Synthesis of Withaferin A-Analogues 21−23[a]



[a]Reagents and conditions: (i) TBDMSiCl, imidazole, DMAP, CH_2Cl_2, rt; (ii) CrO_3, py, CH_2Cl_2, rt, 5 min; (iii) Dowex (50WX8-200), acetone, rt, 24 h.

Scheme 4. Synthesis of 4-Oxo-withaferin A-Silyl Ether Analogues 24−34[a]



24 R= TMS
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27 R= TIPS
28 R= TBS
29 R= THS
30 R= DMIPS
31 R= DMOS
32 R= DMVS
33 R= DMPS
34 R= MDPS

[a]Reagents and conditions: (i) $R_1R_2R_3SiCl$, imidazole, DMAP, Et_3N, CH_2Cl_2, rt.

Table 1. Cytotoxic Activity (IC_{50}, nM)[a] of WA Analogues[b] on Human Ovarian Carcinoma Cell Lines,[c] and on Noncarcinoma Cancer Cells (ARPE19)

compd	A2780	A2780/CP70	ARPE19	SI,[d] A2780	SI,[d] A2780/CP70
1	32.7 ± 0.2	32.0 ± 2.0	37.0 ± 14.0	1.1	1.2
2	>100	12.8 ± 2.0	30.0 ± 10.0		2.3
3	32.0 ± 2.0	3.6 ± 1.4	30.0 ± 0.6	0.9	8.3
4	30.0 ± 2.0	62.0 ± 20.0	62.0 ± 30.0	2.1	1.0
5	27.0 ± 4.0	22.0 ± 0.3	260.0 ± 210.0	9.6	11.8
7	10.0 ± 0.7	33.0 ± 10.0	32.0 ± 5.0	3.2	1.0
8	27.0 ± 10.0	31.0 ± 2.0	86.0 ± 16.0	3.2	2.8
9	22.0 ± 5.0	28.3 ± 0.3	70.0 ± 10.0	3.2	2.5
10	46.0 ± 9	29.0 ± 6.0	2295.0 ± 25.0	49.9	79.1
13	1.5 ± 0.5	24.9 ± 10.0	318.0 ± 61.0	212.0	12.8
14	33.0 ± 0.5	27.5 ± 5.0	92.0 ± 30.0	2.8	3.4
15	2.9 ± 1.0	29 ± 0.005	309.0 ± 40.0	106.6	10.7
17	34.0 ± 1.0	23.0 ± 9.0	310.0 ± 200.0	9.1	13.5
18	34.0 ± 0.6	35.0 ± 0.4	6500.0 ± 150.0	191.2	185.7
20	20.0 ± 7.0	19.0 ± 10.0	52.0 ± 10.0	2.6	2.7
21	47.5 ± 19.0	>100	12.4 ± 1.0	0.3	0.1
22	17.0 ± 10.0	>100	1660.0 ± 90.0	97.7	11.3
23	7.3 ± 6.0	< 1	32.0 ± 2.0	4.3	>32.0
24	41.0 ± 5.0	68.8 ± 8.0	820.0 ± 300.0	20.0	11.9
25	35.0 ± 10.0	20.0 ± 0.2	1870.0 ± 70.0	53.5	93.5
26	69.0 ± 20.0	21.0 ± 6.0	1740.0 ± 340.0	25.2	82.9
27	35.0 ± 6.0	35.0 ± 1.0	37.5 ± 4.0	1.1	1.1
28	53.0 ± 40.0	>100	617.0 ± 500.0	11.6	
30	43.0 ± 0.2	34.0 ± 0.2	2140.0 ± 100.0	49.8	62.9
33	59.0 ± 10.0	>100	2305.0 ± 270.0	39.1	
34	35.0 ± 10.0	>100	2050.0 ± 350.0	58.6	

[a]IC_{50} values (nM) of WA-silyl analogues were determined as described in the Biological Evaluation section. Carboplatin was used as a reference drug (IC_{50} 2.6, 44.9, and 4.6 μm on A2780, A2780/CP70, and ARPE19 cell lines, respectively). Results are expressed as the mean ± standard deviation of three independent experiments performed in duplicate. [b]WA analogues exhibiting IC_{50} values ≤100 nM. [c]Human ovarian carcinoma cisplatin-sensible (A2780) and cisplatin-resistant (A2780/CP70) cell lines. [d]SI, selectivity index.

ether library with enhanced ovarian cancer cell cytotoxicity compared with the lead compound and reference drug. Furthermore, two analogues were investigated for their ability to induce apoptosis, confirming previous studies on this type of scaffold.[22] In addition, extensive structure−activity relationship (SAR) and in silico ADME studies were employed to understand the pharmacokinetic properties of this series of WA analogues.

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■ RESULTS AND DISCUSSION

Chemistry. When silicon is incorporated into an organic compound, the chemical and physical differences contributed by the silyl group can provide compounds with unique properties that are relevant for medicinal chemistry.[16]

Withaferin A is a C28 ergosterane-type steroid with a δ-lactone ring between C-22 and C-26 in the side chain. To refine structural features and enhance the anticancer profile of WA, a suitable starting material from *Withania aristata*,[21] a library of WA-silyl ether analogues (**2**−**22** and **24**−**34**) were designed and synthesized (Schemes 1−4 and detailed in the Experimental Section).

The synthesis of this WA library was carried out using silyl chloride analogues with different electronic and steric properties, such as hydrophobicity, size, and aromaticity. The first step in this task was to investigate the modification of the hydroxyl groups at C-4 and C-27 by converting them into silyl ethers. Thus, 27-silyl ether (**2**−**10**) and 4,27-disilyl ether (**11**−**16**) analogues were synthesized following the strategy outlined in Scheme 1.

It is worth noting that treatment of WA (**1**) with dimethylisopropylsilyl chloride (DMIPSiCl) afforded, in addition to the expected silyl ethers **18** and **19**, analogues **17** and **20**, which were formed by selective silylation of the secondary alcohol at C-4 and Michael addition of imidazole to the enone system in the WA framework, respectively (Scheme 2).

Previously reported structure−activity relationship (SAR) studies on withanolides[21] indicated that compounds bearing a ketone at C-4 have a selective pharmacological profile. Encouraged by these results, the synthesis of analogue **23** was carried out following the strategy outlined in Scheme 3. First, selective protection of the primary alcohol in WA (**1**) with *tert*-butyldimethylsilyl chloride (TBDMSiCl) yielded the corresponding silyl ether derivative **21**, whose oxidation by treatment with Collins reagent afforded the ketone analogue **22**. Further cleavage of the protecting group in **22** with carboxylic acid resin led to the 4-dehydro-WA analogue **23**.

Derivatives **24**−**34** bearing both a 4-ketone and a 27-silyl ether group were prepared from compound **23** by silylation at C-27 with different silyl chloride reagents, as shown in Scheme 4.

Among the former synthetic analogues, 30 out of 33 are reported for the first time. The structures of the new compounds were elucidated by high-resolution mass spectrometry and NMR analysis (Figures S2−S31, Supporting information), whereas those of the previous reported analogues, compounds **21**−**23** were elucidated by comparison of their spectral data with those reported in the literature.[21]

Biological Evaluation. *Antiproliferative Activity.* The in vitro antiproliferative activity of lead compound **1** and derivative **23** (4-oxo-WA) and their silyl analogues **2**−**22** and **24**−**34**, respectively, were evaluated on two human epithelial ovarian tumor cell lines, cisplatin-sensitive (A2780) and cisplatin-resistant (A2780/CP70) cells, and on the noncarcinoma cell line ARPE19 (human retinal pigment epithelial), wherein the latter is used to test for selectivity.

Cytotoxic evaluation (Table 1 and Table S32 in Supporting Information) against the cisplatin-sensitive cell line revealed that the cytotoxicity of 11 analogues (compounds **3**−**5**, **7**−**9**, **13**, **15**, **20**, **22**, and **23**) was higher than that of the widely known anticancer WA (**1**, IC_{50} 32.7 nM) and the reference drug (carboplatin, IC_{50} 2.6 μm), exhibiting IC_{50} values ranging from 7.3 to 32 nM. Moreover, it is noteworthy that silyl ether analogues bearing a dimethyloctyl (**7** and **13**, IC_{50} 10 and 1.5 nM, respectively) or a dimethylphenyl (**15**, IC_{50} 2.9 nM) moiety as well as oxidation at C-4 (**23**, IC_{50} 7.3 nM) are favorable trends for optimal cytotoxicity against A2780 cells, improving activity by 3.2- to 21.8-fold compared with lead compound **1**. Curiously, potency of the imidazole derivative **20** (IC_{50} 20 nM) was slightly higher than that of the lead compound **1**, which is opposite to previously reported SAR of withanolides in which the enone system is essential for the anticancer activity.[23] On the other hand, functional group interconversion of alcohols in WA by a trihexylsilyl (analogues **6** and **29**) or a tripropylsilyl (TPRS) (derivative **12**) moiety was particularly detrimental, furnishing completely inactive analogues.

Drug resistance is a major obstacle for first line chemotherapy in ovarian cancer treatment.[4] Taking into consideration the promising results obtained for WA-silyl analogues assayed on A2780 cisplatin-sensitive cell line, this series of compounds was tested for efficacy on a human ovarian carcinoma cisplatin-resistant (A2780/CP70) cell line. The results (Table 1) indicated that 15 analogues showed from similar (**7**−**10**, **14**, **15**, **18**, **27**, and **30**) to slightly improved (**5**, **13**, **17**, **20**, **25**, and **26**) profile than the lead compound, WA (IC_{50} 32 nM). Moreover, potencies of target compounds **2**, **3**, and **23** on A2780/CP70 cells, exhibiting IC_{50} values ranging from 1 to 12.8 nM, were significantly improved from 2- to 30-fold compared with compound **1**. Regarding analogues with a drastic loss of activity, again analogues **6** and **29** and the disilyl ether derivatives **12** and **16** were from 794- to 364-fold less active than WA.

After confirming that some of the newly synthesized analogues showed potent activity against both human epithelial ovarian tumor cell lines, cytotoxicity against a noncarcinoma cell line (ARPE19, human retinal pigment epithelial cells) was evaluated for all derivatives to test for selectivity (Table 1). We assume that a selectivity index (SI) value higher than two indicates a good selectivity for inducing cytotoxicity in tumor cell lines as compared to those in noncancerous cells, according to Suffness.[24] Among the evaluated compounds, 25 of them showed selectivity to some extent (SI > 2) in the noncarcinoma (ARPE19) cell line with respect to the A2780 cell line. Selectivity was observed not only for the most potent analogues, **7**, **8**, **13**, **15**, **20**, **22**, and **23** (SI ranging from 2.6 to 212.0), but also for those compounds equipotent to WA, analogues **18**, **25**, and **34** (SI 191.2, 53.5, and 58.6, respectively). Similarly, SI was higher than two for 21 analogues regarding the A2780/CP70 cell line. The most active analogues on this cell line, compounds **2**, **3**, **17**, **20**, **23**, **25**, and **26**, showed SI values from 2.3 to 93.5. In addition, analogues **10**, **18**, and **30** with a similar profile to WA were not cytotoxic on the noncarcinoma cell line (SI 79.1, 185.7, and 62.9, respectively).

The overall results of the biological assays identified analogues **13**, **15**, **22**, and **23** on the cisplatin-sensitive cell line, and even more noteworthy, analogues **3**, **17**, **23**, **25**, and **26** on the cisplatin-resistant cell line as having significantly improved activity profiles compared with lead compound **1**. These profiles were coupled with remarkable selectivity on the nontumoral cell line and therefore are suitable for further studies.

Cell Cycle Assay. Our previous studies have indicated an apoptotic effect associated with withanolide-type steroids and reported the first examples of WA-silyl ether analogues, **21** and **22**,[21] in HeLa cells. Moreover, WA has been reported to trigger the apoptotic cascade by extrinsic or intrinsic pathways, e.g., in promyelocytic leukemia HL-6022[25] and U937 cells,[26] prostate

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cancer cells,[27] head and neck squamous carcinoma cells,[28] and Caki cells.[29] In the present study, the cell cycle event mediated by the two potent analogues, **21** and **22**, was investigated on the human ovarian cancer cell line A2780. It should be noted that **22** showed a very good selectivity on noncancerous cells (SI 97.68) (Table 1 and Table S32 in Supporting Information). Cell cycle analysis was carried out using NucleoCounter NC-3000 system by rapid quantification of DNA content, which was measured using fluorescent 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI)-stained cells. This assay will determine cell sorting at different phases of the cell cycle. Results showed that both analogues, **21** and **22**, induce a dose-dependent increase in DNA fragmentation, as evidenced by the increase in the number of cells with low-intensity DAPI signal in sub-G0/G1 as compared with control cells (Figures 1 and S34). This is suggestive of cell death by apoptosis without indication of a cell cycle arrest in G0/G1 over 48 h contact time with the compounds. It is well known that apoptosis is often associated with growth arrest. However, cell death can be induced without the initiation of cell cycle arrest. Indeed, cell cycle arrest does not always lead to cell death.[30]

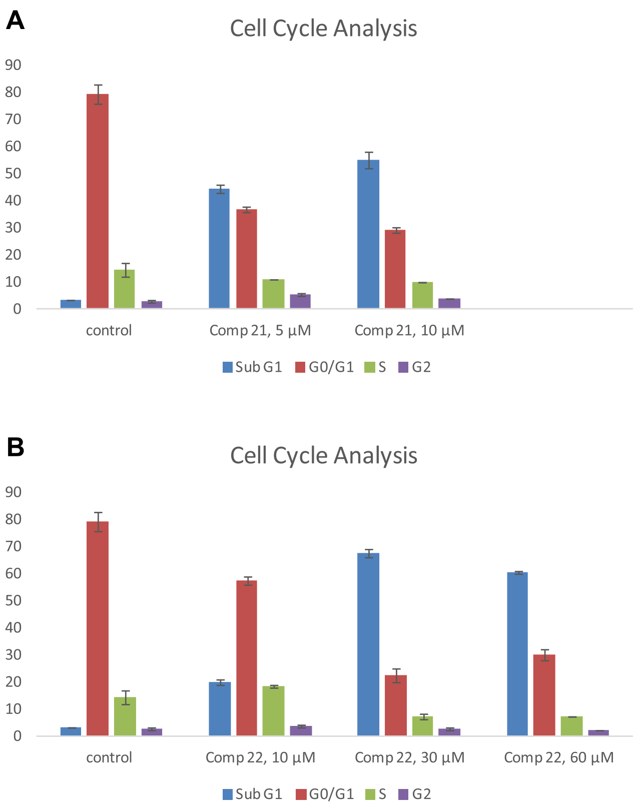

Figure 1. Percentage of cells in sub-G1, G0/G1, S, and G2 phases after performing a two-step cell cycle assay on human ovarian cancer cells (A2780 cell line) with compounds **21** (A) and **22** (B) at different concentrations. Data are based on 48 h exposure to compounds or vehicle control. Each column represents mean ± standard error of the mean (SEM) of $n = 2$.

Structure–Activity Relationship Analysis. The previous reported SAR studies agree that an α,β-unsaturated ketone on ring A, a 5β,6β-epoxide in ring B, and an α,β-unsaturated δ-lactone on side chain in the WA framework[23] are antitumor structural feature requirements, and more recently, acylation is reported to enhance cytotoxicity.[22]

In this work, chemical modulation by incorporation of silicon on the WA framework was investigated. Thus, taking into consideration the IC_{50} values against both cancer cell lines assayed, the effect of silyl ether substituents was analyzed for each synthesized analogue (**2–34**), according to the nature of the group attachment to silicon. The trends of the SAR study from this series of WA-silyl analogues on the A2789 cell line were as follows. Compounds carrying heterogeneous alkyl substituents on the silyl ether (**7–10**) displayed higher cytotoxicity than those with a homogeneous silyl ether (**2–6**). Indeed, replacement of dimethyloctyl or dimethylphenyl groups in the potent analogues **13** and **15** by a trihexyl, triisopropyl, or methyldiphenyl moiety led to the loss of activity (**6**, **12**, and **29**). Previous work revealed that oxidation of the secondary alcohol at C-4 of the WA framework plays an important role in cytotoxicity.[21] Oxidation changes H-bonding ability and lipophylicity and confers a pseudoplanar spatial arrangement of the A-ring. These features seem to modulate the 4-oxo-analogue cytotoxicity profile. Surprisingly, the 4-oxo-WA derivative **23** (7.3 nM) showed a 4.4-fold increase in activity as compared with **1** (32.7 nM). Furthermore, to explore the effect of replacement the primary hydroxyl group at C-27 by a silyl ether, derivatives **24–34** were prepared from **23** (Scheme 4). These analogues showed a broad profile of inhibitory activities with IC_{50} values ranging from 35 nM to 25.17 μm, although all of them were significantly less potent than the congener **23** (IC_{50} 7.3 nM) and lead compound **1** (IC_{50} 32.7 nM). In general, silyl analogues with a hydroxyl group at C-4 (**3–9** and **18**) are more potent than those with a ketone group (**26–29**, **31**, and **32**) at this position.

Regarding the cisplatin-resistant cell line (A2780/CP70), SAR studies of this series of analogues revealed that compounds carrying a heterogeneous alkyl substituent on the silyl ether (**7–10** and **13–15**) displayed similar profiles (IC_{50} 24.9–33.0 nM) to **1**, indicating that activity was not greatly influenced by their corresponding silyl ether moiety on the withanolide skeleton. Analogues with a homogeneous alkyl substituent on the silyl ether (**2–6**, **11**, and **12**) showed a great range of cytotoxic activity. In fact, replacement of the triethyl or tripropyl substituent in potent compounds **2** and **3** by a trihexyl, di-tripropyl, or dimethyldiphenyl moiety (compounds **6**, **12**, and **16**) led to a significant loss of activity. Moreover, analogue **23** was 30-fold more potent than the parent against the cisplatin-resistant cell line, whereas silyl analogues **24–34** (Scheme 4) were significantly less potent than their congener **23**, as occurred on the A2780 cell line. Therefore, a heterogeneous alkyl substituent on the silyl ether is favorable versus a homogenous one for the A2789 cell line, with the dimethyloctylsilyl (DMOS) and dimethylphenylsilyl (DMPS) being the best functional groups. On the other hand, the homogenous alkyl substituents, triethylsilyl (TES) and TPRS are the best functional groups on the A2789/CP70 cell line. Compounds with a hydroxyl group at C-4 are favorites versus those oxidized at C-4 on both cell lines, except for compound **23**.

These results reveal that silylation of the WA framework leads to a wide range of cytotoxic activity, since minor modifications on the silyl ether substituent had noteworthy repercussions on compound activity. Furthermore, silylation of the WA framework leads to selectivity on both tumor cell lines. The potential benefit of silicon in medicinal chemistry is due to its physical–chemical properties. The atomic size and covalent radius of silicon alters the bond lengths and bond angles having an important influence on the conformation and reactivity.

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Table 2. In Silico ADME Profile Predictions of Selected WA Analogues[a] and Their Range/Recommended Values[b]

property/descriptor	1	2	8	17	20	22	23	25	range/recommended values
#stars	0	1	1	1	1	1	0	0	0−5
QP log BB	−1.311	−1.072	−1.113	−1.253	−1.258	−087	−1.267	−1.156	−3.0 to 1.2
QPPCaco	258.201	1060.23	759.53	550.181	600.427	996.046	243.685	711.663	<25 poor, >500 great
QPPMDCK	114.484	526.993	367.48	259.339	285.031	492.595	107.544	342.512	<25 poor, >500 great
QP log Khsa	0.329	1.189	1.017	1.136	1.1	0.892	−0.13	0.827	−1.5 to 1.5
QP log Po/w	3.046	5.959	5.291	5.752	5.573	5.464	2.519	5.391	−2.0 to 6.5
QP log Kp	−3.842	−2.271	−2.635	−3.075	−2.816	−2.615	−3.977	−2.74	−8.0 to −1.0
QP log *S*	−4.999	−7.586	−7.336	−7.379	−7.844	−6.713	−3.974	−6.489	−6.5 to 0.5
#metab	4	4	4	4	5	3	3	3	1−8
%HOA	87.948	90.068	83.565	83.76	83.39	86.682	84.413	83.643	>80% high <25% poor
PSA	96.36	85.36	85.36	85.36	103.18	82.2	93.2	82.2	7.0−200.0
SASA	718.234	906.765	874.31	88.418	949.884	877.558	711.717	882.911	300.0−1000.0
mol MW	470.605	584.867	554.8	570.84	638.918	582.851	468.589	582.851	130.0−725.0
#rotor	5	9	7	7	7	6	4	8	0−15
donorHB	1	1	1	0	1	0	0	0	0.0−6.0
accptHB	9.4	9.55	9.55	8.55	11.55	9.85	9.7	9.85	2.0−20.0
volume	1396.689	1803.47	1709.1	1757.66	1903.72	1778.773	1383.64	1785.65	500.0−2000.0

[a]WA analogues exhibiting IC_{50} values lower than those for WA on A2780 and/or A2780 cell lines (IC_{50} < 32.7 nM) and #stars values between 0 and 1. [b]#star (number of property values that fall outside the 95% range of similar values for known drugs), QP log BB (predicted brain/blood partition coefficient), QPPCaco2 (predicted human epithelial colorectal adenocarcinoma cell line permeability in nm/s), QPPMDCK (predicted Madin−Darby canine kidney permeability in nm/s), QP log Khsa (predicted binding to human serum albumin), QP log Po/w (predicted octanol/water partition coefficient), QP log Kp (skin permeability), QP log *S* (predicted aqueous solubility), #metab (number of likely metabolic reactions), % HOA (predicted human oral absorption on 0−100%), PSA (van der Waals surface area of polar nitrogen and oxygen atoms and carbonyl atoms), SASA (total solvent accessible surface area), MW (molecular weight), #rotor (number of nontrivial, nonhindered rotable bonds).

Moreover, the silicon enhanced cell distribution due to the increase in lipophilicity whereas the electropositive nature of silicon enhances H-bonding ability.[16,18,31] Furthermore, additional lipophilic, steric, and stereoelectronic synergetic effects contributed by the substituted silyl ether moiety seem to guide cytotoxicity, selectivity, and ADME profiles of analogues. Taking into consideration these features, WA-silyl analogues could present advantages as drug candidates.

In Silico ADME Predictions. Understanding pharmacokinetic properties (ADME, absorption, distribution, metabolism, and excretion molecular properties) is an important step in drug discovery to select new lead/drug candidates, since potent in vitro activity along with enhanced ADME profiles increases the probability of clinical success.[32] Moreover, despite a great deal of research conducted on the potential anticancer properties of WA-related withanolides, there are only two reports of ADME studies.[33,34]

The QikProp module of Schrödinger software[35] was used for analyzing physicochemical and pharmacokinetic descriptors (ADME properties) of selected compounds (IC_{50} values $\leq 1\ \mu$m on A2780 and A2780/CP70 cell lines) with the aim of increasing the success rate of compounds reaching further stages of development. A detailed account of these parameters is given in Table S35 of the Supporting Information. These parameters provide insights into key aspects, such as drug likeness, solubility, permeability, bioavailability predictions, oral absorption, metabolism, etc. One of the primary descriptors that was taken into account was #stars. The #stars descriptor informs about the number of properties of each compound that fail to remain within the recommended ranges; therefore, a lower number of #stars denotes a better druglike molecule.[35]

Thus, taking into consideration the IC_{50} values of the assayed series of analogues with higher cytotoxic effect than WA (IC_{50} < 32.7 nM on A2780 cell line) and #stars values (0 or 1), compounds **2**, **8**, **17**, **20**, **22**, **23**, and **25** were selected to analyze their predicted pharmacokinetic properties (Table 2). Analogue **23** and the silyl ether analogue **25** as well as WA lie within the recommended range of known drugs for all analyzed parameters (#stars = 0), whereas silyl ether analogue **2**, **8**, **17**, **20**, and **22** fail in the QP log *S* displaying low aqueous solubility, although values for these analogues were near the upper limit of the recommended range. Nevertheless, these silyl analogues showed a predicted intestinal absorption rate (QPPCaco) and apparent cell permeability (QPPMDK) greater than those of the lead compound WA, thus predicting good oral bioavailability. The lipophilicity is also an important physicochemical property requirement for a potential drug. It is expressed as QP log Po/w and plays a crucial role in absorption, bioavailability, hydrophobic drug−receptor interactions, metabolism, and toxicity.[36] All selected compounds' log Po/w values lay within the permissible range. The predicted values for properties, such as octanol/water partition (QP log Po/w), gut−blood barrier permeability (QPPCaco, QP log BB, and QPPMDCK), human serum albumin binding (QP log Khsa), and percent of human oral absorption (>83%), were within ideal ranges. Therefore, compounds under study were predicted to have good drug likeness, since they have mostly favorable pharmacokinetic properties, especially regarding membrane permeability and oral absorptivity.

■ CONCLUSIONS

The current study reports on our efforts to find new drug candidates for OC resistant to current treatments. Therefore, the synthesis and evaluation of a WA-silyl ether library, together with structure−activity relationship and in silico ADME studies, were employed to find drug candidates for the treatment of ovarian cancer. We have successfully identified a new generation of potent and selective WA analogues with a significantly improved cytotoxic profile. In fact, 10 WA analogues exhibited higher potency than the lead compound and reference drug on the cisplatin-sensitive cell line, and more notably, 15 analogues

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enhanced the cytotoxic profile on the cisplatin-resistant cell line. Cell cycle analysis of two potent analogues revealed cell death by apoptosis without cell cycle arrest in G0/G1. Furthermore, the predicted pharmacokinetic properties highlight three analogues with great potential to become drug candidates: the 4-oxo-WA (**23**) exhibiting single-digit nanomolar potency on both cancer cell lines and two potent silyl ether analogues, **22** and **25**, on A2780 and A2780/CP70 cells, respectively. This potency is accompanied by an excellent selectivity index and favorable drug likeness. Thus, analogues reported herein are promising candidates in anticancer drug development for OC that deserve further investigation.

In summary, the current study provides an insight into the anticancer potential of WA analogues on OC, whether alone or in combination with clinical drugs, and supports the increasingly important role that silicon will play in drug design.

EXPERIMENTAL SECTION

General Methods for Chemistry. Optical rotations were measured on a PerkinElmer 241 automatic polarimeter, in $CHCl_3$ at 25 °C; the $[\alpha]_D$ values are given in units of $10^{-1°}$ cm^2/g. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a Bruker Avance 400 spectrometer; chemical shifts are given in ppm and coupling constants in hertz. Solutions were typically prepared in $CDCl_3$ with chemical shifts referenced to deuterated solvent as an internal standard. Electron ionization mass spectrometry (EIMS) and high-resolution EIMS (HREIMS) were measured on a Micromass AutoSpec spectrometer, and electrospray ionization mass spectrometry (ESIMS) and high-resolution ESIMS (HRESIMS) (positive mode) were measured on a LCT Premier XE Micromass AutoSpec spectrometer. Silica gel 60 used for column chromatography (particle size 15−40 and 63−200 μm), Polygram Sil G/UV_{254} used for analytical and preparative thin-layer chromatography (TLC), and HPTLC-Platten Nano-Sil 20 UV_{254} were purchased from Macherey-Nagel. Reactions were monitored by TLC, the spots were visualized by UV light and heating silica gel plates sprayed with $H_2O-H_2SO_4-AcOH$ (1:4:20). Varian high-performance liquid chromatography (HPLC) equipment consisted of a ProStar 210 solvent delivery module, ProStar 335 photodiode array detector, using an analytical Pursuit C18 column (2.0×100 mm^2, 3 μm) with a flow rate of 0.3 mL/min, and mixtures of acetonitrile−H_2O as eluent. The degree of purity of the compounds was over 95%, as indicated by the appearance of a single peak using HPLC. Unless otherwise noted, solvents and reagents were obtained from commercial suppliers and used without further purification. Anhydrous tetrahydrofuran and Cl_2CH_2 were distilled from sodium/benzophenone and calcium hydride ketyl under nitrogen, respectively. All solvents used were of analytical grade from PanReac, and the reagents were purchased from Sigma-Aldrich. Withaferin A (WA, **1**), used as starting material, was isolated from the leaves of *W. aristata*, as previously described.[21]

General Procedure for the Preparation of Silyl Ether Derivatives. Compounds **2**−**21** and **24**−**34**: To a solution of **1** or **23** in dry CH_2Cl_2 (5−10 mL) was added imidazole (7−9 mg, 0.1−0.13 mmol), 4-(*N*,*N*-dimethylamino)pyridine (6−10 mg, 0.05−0.08 mmol), two drops of triethylamine, and the corresponding silyl chloride. The reaction mixture was stirred at room temperature and under an argon atmosphere until all starting material was consumed. The progress of the reaction was monitored by TLC using CH_2Cl_2/acetone (9:1). After the mixture was concentrated to dryness under reduced pressure, the residue was purified by column chromatography on silica gel and eluted with CH_2Cl_2/acetone mixtures of increasing polarity (from 10:0 to 7:3), affording the desired compounds **2**−**21** and **24**−**34**.

*Preparation of 27-O-(Triethylsilyl)withaferin A (**2**).* A solution of **1** (20 mg, 0.04 mmol) and triethylsilyl chloride (15 μL, 0.09 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **2** (8.5 mg, 37%). $[\alpha]^{20}_D$ +61.7 (*c* 0.65, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.95 (1H, m, H-14), 0.99 (3H, d, *J* = 6.7 Hz, Me-21), 1.03 (1H, m, H-9), 1.08 (1H, m, H-17), 1.12 (1H, m, H-12), 1.15 (1H, m, H-15), 1.28 (1H, dd, *J* = 11.2, 14.2 Hz, H-7), 1.38 (1H, m, H-16), 1.42 (3H, s, Me-19), 1.47 (1H, m, H-11), 1.52 (1H, dd, *J* = 3.9, 11.2 Hz, H-8), 1.62 (1H, m, H-15), 1.68 (1H, m, H-16), 1.83 (1H, ddd, *J* = 3.4, 6.9, 13.9 Hz, H-11), 1.96 (1H, dd, *J* = 3.3, 17.5 Hz, H-23α), 1.97 (1H, m, H-12), 2.08 (3H, s, Me-28), 2.01 (1H, m, H-20), 2.16 (1H, m, H-7β), 2.47 (1H, dd, *J* = 13.5, 17.5 Hz, H-23β), 3.24 (1H, br s, H-6), 3.77 (1H, d, *J* = 5.8, H-4), 4.39 (1H, dt, *J* = 3.5, 13.4 Hz, H-22), 4.39, 4.52 (2H, d_{AB}, *J* = 11.6 Hz, H-27), 6.21 (1H, d, *J* = 10.0 Hz, H-2), 6.94 (1H, dd, *J* = 5.8, 10.0 Hz, H-3), OTES [0.64 (6H, t, *J* = 8.0 Hz), 0.96 (9H, t, *J* = 8.0 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.5 (CH_3, C-19), 20.6 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.7 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 125.9 (C, C-25), 132.3 (CH, C-2), 141.9 (CH, C-3), 154.6 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), OTES [4.3 ($3 \times CH_3$), 6.8 ($3 \times CH_2$)]; EIMS *m/z* 584 $[M]^+$ (2), 555 (100), 537 (1), 417 (1), 299 (3), 255 (21) 211 (8), 123 (11), 103 (20), 95 (12), 75 (15); HREIMS *m/z* 584.3509 $[M]^+$ (calcd for $C_{34}H_{52}O_6Si$, 584.3533).

*Preparation of 27-O-(Tripropylsilyl)withaferin A (**3**).* A solution of **1** (20 mg, 0.04 mmol) and tripropylsilyl chloride (10 μL, 0.05 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **3** (19.6 mg, 78%) as an amorphous solid. $[\alpha]^{20}_D$ +66.1 (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.95 (1H, H-14), 0.99 (3H, d, *J* = 6.7 Hz, Me-21), 1.03 (1H, m, H-9), 1.10 (1H, m, H-17), 1.13 (1H, m, H-12), 1.16 (1H, m, H-15), 1.26 (1H, dd, *J* = 11.6, 14.9 Hz, H-7), 1.39 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.48, (1H, m, H-11), 1.53 (1H, m, H-8), 1.65 (1H, m, H-15), 1.70 (1H, m, H-16), 1.84 (1H, m, H-11), 1.93 (1H, m, H-12), 1.94 (2H, dd, *J* = 3.4, 17.5 Hz, H-23α), 2.01 (1H, H-20), 2.06 (3H, s, Me-28), 2.15 (1H, ddd, *J* = 2.5, 3.9, 14.9 Hz, H-7β), 2.46 (1H, dd, *J* = 13.4, 17.7 Hz, H-23β), 2.62 (1H, t, *J* = 2.5 Hz, OH-4), 3.24 (1H, br s, H-6), 3.76 (1H, dd, *J* = 2.5, 5.9 Hz, H-4), 4.39 (1H, dt, *J* = 3.5, 13.2 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, *J* = 11.7 Hz, H-27), 6.21 (1H, d, *J* = 10.0 Hz, H-2), 6.94 (1H, dd, *J* = 5.9, 10.0 Hz, H-3), OTPRS [0.63 (6H, br t, *J* = 8.5 Hz), 0.96 (9H, t, *J* = 7.3 Hz), 1.37 (6H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.4 (CH_3, C-19), 20.5 (CH_3, C-28), 22.1 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.6 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 125.9 (C, C-25), 132.3 (CH, C-2), 141.9 (CH, C-3), 154.5 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), OTPRS [16.3 ($3 \times CH_2$), 16.8 ($3 \times CH_2$), 18.4 ($3 \times CH_3$)]; ESIMS *m/z* 649 $[M + Na]^+$ (100); HRESIMS *m/z* 649.3906 $[M + Na]^+$ (calcd for $C_{37}H_{58}O_6NaSi$, 649.3900).

*Preparation of 27-O-(Triisopropylsilyl)withaferin A (**4**).* A solution of **1** (20 mg, 0.04 mmol) and triisopropylsilyl chloride (10 μL, 0.05 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **4** (7.9 mg, 21%) as an amorphous solid. $[\alpha]^{20}_D$ +64.1 (*c* 0.49, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.94 (1H, m, H-14), 1.00 (3H, d, *J* = 6.7 Hz, Me-21), 1.11 (1H, m, H-9), 1.13 (1H, m, H-17), 1.16 (1H, m, H-12), 1.19 (1H, m, H-15), 1.27 (1H, ddd, *J* = 3.5, 11.0, 14.3 Hz, H-7), 1.40 (1H, m, H-16), 1.42 (3H, s, Me-19), 1.47 (1H, m, H-11), 1.53 (1H, m, H-8), 1.60 (1H, m, H-15), 1.68 (1H, m, H-16), 1.84 (1H, ddd, *J* = 3.5, 6.9, 13.9 Hz, H-11), 1.97 (1H, d, *J* = 17.5 Hz, H-23α), 1.98 (1H, m, H-12), 2.01 (1H, m, H-20), 2.09 (3H, s, Me-28), 2.16 (1H, dt, *J* = 2.6, 14.9, H-7β), 2.47 (1H, dd, *J* = 13.2, 17.5 Hz, H-23β), 3.25 (1H, br s, OH-4), 3.36 (1H, br s, H-6), 3.77 (1H, d, *J* = 5.8 Hz, H-4), 4.38 (1H, dt, *J* = 3.1, 13.2 Hz, H-22), 4.49, 4.60 (2H, d_{AB}, *J* = 11.7 Hz, H-27), 6.21 (1H, d, *J* = 10.0 Hz, H-2), 6.94 (1H, dd, *J* = 5.8, 10.0 Hz, H-3), OTIPS [0.90−1.20 (3H, m), 1.07 (18H, d, *J* = 6.5 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.5 (CH_3, C-19), 20.6 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.2 (CH, C-9), 47.7 (C, C-10), 52.1 (CH, C-17),

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56.1 (CH, C-14), 57.4 (CH_2, C-27), 62.7 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.2 (CH, C-22), 126.1 (C, C-25), 132.3 (CH, C-2), 141.8 (CH, C-3), 154.6 (C, C-24), 165.9 (C, C-26), 202.3 (C, C-1), OTIPS [12.0 (6 × CH_3), 18.0 (3 × CH)]; ESIMS m/z 649 $[M + Na]^+$ (100); HRESIMS m/z 649.3893 $[M + Na]^+$ (calcd for $C_{37}H_{58}O_6NaSi$, 649.3900).

*Preparation of 27-O-(Tributylsilyl)withaferin A (**5**).* A solution of **1** (22 mg, 0.05 mmol) and tributylsilyl chloride (15 μL, 0.06 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **5** (6.1 mg, 18%) as an amorphous solid. $[\alpha]_D^{20}$ +64.3 (c 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.95 (1H, m, H-14), 1.00 (3H, d, J = 6.8 Hz, Me-21), 1.04 (1H, m, H-9), 1.09 (1H, m, H-17), 1.14 (1H, m, H-12), 1.17 (1H, m, H-15), 1.28 (1H, m, H-7), 1.36 (1H, m, H-16), 1.42 (3H, s, Me-19), 1.47 (1H, m, H-11), 1.53 (1H, m, H-8), 1.63 (1H, m, H-15), 1.68 (1H, m, H-16), 1.84 (1H, ddd, J = 3.4, 7.1, 14.1 Hz, H-11), 1.95 (1H, m, H-12), 1.96 (1H, dd, J = 2.4, 17.5 Hz, H-7), 2.01 (1H, m, H-20), 2.07 (3H, s, Me-28), 2.16 (1H, ddd, J = 2.6, 3.9, 14.7 Hz, H-7β), 2.47 (1H, dd, J = 13.6, 17.7 Hz, H-23β), 3.24 (1H, br s, H-6), 3.77 (1H, d, J = 5.8, H-4), 4.39 (1H, dt, J = 3.5, 13.3 Hz, H-22), 4.38, 4.51 (2H, d_{AB}, J = 11.7 Hz, H-27), 6.21 (1H, d, J = 10.0 Hz, H-2), 6.94 (1H, dd, J = 5.8, 10.0 Hz, H-3), OTBS [0.63 (6H, br t, J = 7.3 Hz), 0.89 (9H, t, J = 6.9 Hz), 1.32 (12H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.3 (CH_3, C-21), 17.4 (CH_3, C-19), 20.5 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.2 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.7 (CH_2, C-27), 62.7 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 126.0 (C, C-25), 132.3 (CH, C-2), 141.8 (CH, C-3), 154.4 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), OTBS [13.2 (3 × CH_3), 13.7 (3 × CH_2), 25.4 (3 × CH_2), 26.6 (3 × CH_2)]; ESIMS m/z 691 $[M + Na]^+$ (100); HRESIMS m/z 691.4382 $[M + Na]^+$ (calcd for $C_{40}H_{64}O_6NaSi$, 691.4370).

*Preparation of 27-O-(Trihexylsilyl)withaferin A (**6**).* A solution of **1** (20.0 mg, 0.04 mmol) and trihexylsilyl chloride (15 μL, 0.4 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **6** (17.7 mg, 59%) as an amorphous solid. $[\alpha]_D^{20}$ +55.1 (c 1.50, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.94 (1H, m, H-14), 0.99 (3H, d, J = 6.7 Hz, Me-21), 1.01 (1H, m, H-9), 1.07 (1H, m, H-17), 1.10 (1H, m, H-12), 1.11 (1H, m, H-7), 1.15 (1H, m, H-15), 1.26 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.46 (1H, m, H-11), 1.52 (1H, m, H-8), 1.63 (1H, m, H-15), 1.69 (1H, m, H-16), 1.83 (1H, dd, J = 2.9, 14.2 Hz, H-11), 1.95 (1H, d, J = 17.1 Hz, H-23α), 1.97 (1H, m, H-12), 2.00 (1H, m, H-20), 2.06 (3H, s, Me-28), 2.15 (1H, br d, J = 14.6 Hz, H-7β), 2.46 (1H, dd, J = 13.3, 17.1 Hz, H-23β), 2.61 (1H, d, J = 2.5 Hz, OH-4), 3.24 (1H, br s, H-6), 3.76 (1H, d, J = 5.9 Hz, H-4), 4.38 (1H, dt, J = 3.2, 13.2 Hz, H-22), 4.36, 4.50 (2H, d_{AB}, J = 11.7 Hz, H-27), 6.21 (1H, d, J = 10.0 Hz, H-2), 6.94 (1H, dd, J = 5.9, 10.0 Hz, H-3), OTHS [0.62 (6H, br t, J = 7.7 Hz), 0.88 (9H, t, J = 6.7 Hz), 1.29 (24H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.4 (CH_3, C-19), 20.5 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.7 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 126.0 (C, C-25), 132.3 (CH, C-2), 141.9 (CH, C-3), 154.4 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), OTHS [13.5 (3 × CH_2), 14.2 (3 × CH_2), 22.6 (3 × CH_2), 23.1 (3 × CH_2), 31.6 (3 × CH_2), 33.4 (3 × CH_2)]; ESIMS m/z 775 $[M + Na]^+$ (100); HRESIMS m/z 775.5315 $[M + Na]^+$ (calcd for $C_{46}H_{76}O_6NaSi$, 775.5309).

*Preparation of 27-O-(Dimethyloctylsilyl)withaferin A (**7**).* A solution of **1** (20.0 mg, 0.04 mmol) and dimethyloctylsilyl chloride (10 μL, 0.04 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **7** (5.2 mg, 20%) as an amorphous solid. $[\alpha]_D^{20}$ +85.0 (c 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.96 (1H, m, H-14), 0.99 (3H, d, J = 6.6 Hz, Me-21), 1.03 (1H, m, H-9), 1.08 (1H, m, H-17), 1.14 (1H, m, H-12), 1.17 (1H, m, H-15), 1.28 (1H, m, H-7), 1.39 (1H, m, H-16), 1.42 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.51 (1H, m, H-8), 1.62 (1H, m, H-15), 1.67 (1H, m, H-16), 1.84 (1H, ddd, J = 3.3, 6.9, 14.3 Hz, H-11), 1.96 (1H, m, H-12), 2.01 (1H, m, H-20), 2.06 (3H, s, Me-28), 2.16 (1H, m, H-7β), 2.47 (1H, dd, J = 13.3, 17.2 Hz, H-23β), 2.51 (1H, d, J = 2.4 Hz, OH-4), 3.24 (1H, br s, H-6), 3.77 (1H, dd, J = 2.4, 5.8 Hz, H-4), 4.41 (1H, dt, J = 3.3, 13.2 Hz, H-22), 4.36, 4.49 (2H, d_{AB}, J = 11.6 Hz, H-27), 6.21 (1H, d, J = 10.0 Hz, H-2), 6.94 (1H, dd, J = 5.8, 10.0 Hz, H-3), ODMOS [0.13 (6H, s), 0.63 (2H, m), 0.88 (3H, t, J =7.8 Hz), 1.23–1.35 (12H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.3 (CH_3, C-21), 17.4 (CH_3, C-19), 20.4 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.7 (CH, C-8), 30.0 (CH_2, C-23), 31.1 (CH_2, C-7), 38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.5 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.4 (CH_2, C-27), 62.6 (CH, C-6), 63.8 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 125.8 (C, C-25), 132.3 (CH, C-2), 141.8 (CH, C-3), 154.5 (C, C-24), 165.7 (C, C-26), 202.3 (C, C-1), ODMOS [−2.10 (2 × CH_3), 14.1 (CH_3), 16.3 (CH_2), 22.7 (CH_2), 23.1 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 31.9 (CH_2), 33.5 (CH_2)]; ESIMS m/z 663 $[M + Na]^+$ (100); HRESIMS m/z 663.4055 $[M + Na]^+$ (calcd for $C_{38}H_{60}O_6NaSi$, 663.4057).

*Preparation of 27-O-(Dimethylvinylsilyl)withaferin A (**8**).* A solution of **1** (20 mg, 0.04 mmol) and dimethylvinylsilyl cholride (10 μL, 0.07 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **8** (12.0 mg, 47%). $[\alpha]_D^{20}$ +76.3 (c 0.94, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.94 (1H, m, H-14), 0.99 (3H, d, J = 6.7 Hz, Me-21), 1.03 (1H, m, H-9), 1.08 (1H, m, H-17), 1.13 (1H, m, H-12), 1.16 (1H, m, H-15), 1.28 (1H, dd, J = 3.7, 11.3 Hz, H-7), 1.37 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.52 (1H, m, H-8), 1.63 (1H, m, H-15), 1.67 (1H, m, H-16), 1.83 (1H, ddd, J = 3.6, 7.3, 14.3 Hz, H-11), 1.96 (1H, dd, J = 3.3, 17.8 Hz, H-23α), 1.97 (1H, m, H-12), 2.00 (1H, m, H-20), 2.05 (3H, s, Me-28), 2.15 (1H, ddd, J = 2.6, 4.0, 14.9 Hz, H-7β), 2.47 (1H, dd, J = 13.5, 17.8 Hz, H-23β), 2,57 (1H, d, J = 2.5 Hz, OH-4), 3.24 (1H, br s, H-6), 3.77 (1H, dd, J = 2.5, 5.9 Hz, H-4), 4.40 (1H, dt, J = 3.5, 13.3 Hz, H-22), 4.36, 4.50 (2H, d_{AB}, J = 11.6 Hz, H-27), 6.21 (1H, d, J = 9.9 Hz, H-2), 6.94 (1H, dd, J = 5.9, 9.9 Hz, H-3), ODMVS [0.22 (6H, s), 5.80 (1H, dd, J = 3.9, 20.6 Hz), 6.03 (1H, dd, J = 3.9, 15.2 Hz), 6.17 (1H, dd, J = 15.2, 20.6 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.3 (CH_3, C-21), 17.4 (CH_3, C-19), 20.5 (CH_3, C-28), 22.1 (CH_2, C-11), 24.2 (CH_2, C-15), 27.2 (CH_2, C-16), 29.7 (CH, C-8), 30.0 (CH_2, C-23), 31.1 (CH_2, C-7), 38.7 (CH, C-20), 39.3 (CH_2, C-12), 42.5 (C, C-13), 44.1 (CH, C-9), 47.6 (C, C-10), 52.0 (CH, C-17), 56.0 (CH, C-14), 56.5 (CH_2, C-27), 62.6 (CH, C-6), 63.8 (C, C-5), 69.9 (CH, C-4), 78.0 (CH, C-22), 125.7 (C, C-25), 132.3 (CH, C-2), 141.8 (CH, C-3), 154.6 (C, C-24), 165.7 (C, C-26), 202.3 (C, C-1), ODMVS [−2.2 (2 × CH_3), 133.3 (CH), 137.3 (CH_2)]; ESIMS m/z 577 $[M + Na]^+$ (100); HRESIMS m/z 577.2957 $[M + Na]^+$ (calcd for $C_{32}H_{46}O_6NaSi$, 577.2961).

*Preparation of 27-O-(Methyldiphenylsilyl)withaferin A (**9**).* A solution of **1** (20 mg, 0.04 mmol) and methyldiphenylsilyl chloride (20 μL, 0.09 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **9** (13.8 mg, 52%). $[\alpha]_D^{20}$ +48.4 (c 1.13, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.90 (1H, m, H-14), 0.96 (3H, d, J = 6.6 Hz, Me-21), 0.99 (1H, m, H-9), 1.03 (1H, m, H-17), 1.10 (1H, m, H-12), 1.16 (1H, m, H-15), 1.27 (1H, m, H-7), 1.36 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.52 (1H, m, H-8), 1.62 (1H, m, H-15), 1.65 (1H, m, H-16), 1.83 (1H, ddd, J = 3.4, 6.9, 14.2 Hz, H-11), 1.87 (1H, dd, J = 3.0, 17.6 Hz, H-23α), 1.94 (1H, m, H-12), 1.95 (1H, m, H-20), 1.96 (3H, s, Me-28), 2.15 (1H, dt, J = 3.5, 14.5 Hz, H-7β), 2.36 (1H, dd, J = 13.7, 17.6 Hz, H-23β), 2.57 (1H, br s, OH-4), 3.24 (1H, br s, H-6), 3.76 (1H, d, J = 6.2 Hz, H-4), 4.25 (1H, dt, J = 3.4, 13.2 Hz, H-22), 4.48, 4.61 (2H, d_{AB}, J = 11.7 Hz, H-27), 6.21 (1H, d, J = 10.0 Hz, H-2), 6.94 (1H, dd, J = 5.9, 10.0 Hz, H-3), OMDPS [0.70 (3H, s), 7.34–7.43 (6H, m), 7.60 (4H, d, J = 7.6 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.3 (CH_3, C-21), 17.5 (CH_3, C-19), 20.5 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.4 (CH_2, C-16), 29.8 (CH, C-8), 29.9 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 57.3 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.0 (CH, C-22), 125.5 (C, C-25), 132.3 (CH, C-2), 141.9 (CH, C-3),

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154.8 (C, C-24), 165.6 (C, C-26), 202.3 (C, C-1), OMDPS [−3.2 (CH_3), 127.8 (4 × CH), 129.8 (2 × CH), 134.4 (4 × CH), 135.9 (C), 136.0 (C)]; ESIMS *m/z* 689 [M + Na]$^+$ (100); HRESIMS *m/z* 689.3283 [M + Na]$^+$ (calcd for $C_{41}H_{50}O_6NaSi$, 689.3274).

*Preparation of 27-O-(tert-Butyldiphenylsilyl)withaferin A (**10**).* A solution of **1** (20.0 mg, 0.04 mmol) and *tert*-butyldiphenylsilyl chloride (100 μL, 0.04 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **10** (18.7 mg, 66%) as an amorphous solid. $[\alpha]_D^{20}$ +48.4 (*c* 1.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.95 (1H, m, H-14), 0.99 (3H, d, *J* = 6.8 Hz, Me-21), 1.02 (1H, m, H-9), 1.07 (1H, m, H-17), 1.12 (1H, m, H-12), 1.18 (1H, m, H-15), 1.29 (1H, dd, *J* = 11.6, 14.6 Hz, H-7), 1.42 (3H, s, Me-19), 1.47 (1H, m, H-11), 1.53 (1H, m, H-8), 1.69 (1H, m, H-16), 1.84 (1H, ddd, *J* = 3.5, 7.2, 14.3 Hz, H-11), 1.89 (1H, m, H-23α), 1.95 (1H, m, H-12), 1.92 (3H, s, Me-28), 1.97 (1H, m, H-20), 2.17 (1H, dt, *J* = 3.3, 14.9 Hz, H-7β), 2.41 (1H, dd, *J* = 13.2, 17.6 Hz, H-23β), 2.56 (1H, d, *J* = 2.4 Hz, OH-4), 3.25 (1H, s, H-6), 3.77 (1H, dd, *J* = 2.4, 5.8 Hz, H-4), 4.27 (1H, dt, *J* = 3.3, 13.2 Hz, H-22), 4.44, 4.57 (2H, d_{AB}, *J* = 11.8 Hz, H-27), 6.22 (1H, d, *J* = 10.0 Hz, H-2), 6.94 (1H, dd, *J* = 5.8, 10.0 Hz, H-3), OTBDPS [1.05 (9H, s), 7.36−7.45 (6H, m), 7.71 (4H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.5 (CH_3, C-19), 20.5 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.0 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.2 (CH, C-9), 47.7 (C, C-10), 52.1 (CH, C-17), 56.1 (CH, C-14), 57.9 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 125.8 (C, C-25), 132.3 (CH, C-2), 141.9 (CH, C-3), 154.1 (C, C-24), 165.6 (C, C-26), 202.3 (C, C-1), OTBDPS [19.3 (C), 26.9 (3 × CH_3), 127.6 (2 × CH), 127.7 (2 × CH), 129.6 (CH), 129.7 (CH), 133.5 (C), 133.6 (C), 135.6 (2 × CH), 135.7 (2 × CH)]; ESIMS *m/z* 731 [M + Na]$^+$ (100); HRESIMS *m/z* 731.3754 [M + Na]$^+$ (calcd for $C_{44}H_{56}O_6NaSi$, 731.3744).

*Preparation of 4,27-O-Di-(triethylsilyl)withaferin A (**11**).* A solution of **1** (20 mg, 0.04 mmol) and triethylsilyl chloride (15 μL, 0.09 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **11** (5.6 mg, 20%). $[\alpha]_D^{20}$ +64.0 (*c* 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.69 (3H, s, Me-18), 0.80 (1H, dt, *J* = 4.4, 12.0 Hz, H-9), 0.89 (1H, m, H-14), 0.98 (3H, d, *J* = 6.7 Hz, Me-21), 1.02 (1H, m, H-12), 1.07 (1H, m, H-17), 1.13 (1H, m, H-15), 1.23 (1H, dd, *J* = 11.6, 13.9 Hz, H-7), 1.37 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.46 (1H, m, H-11), 1.49 (1H, m, H-8), 1.62 (1H, m, H-15), 1.66 (1H, m, H-16), 1.70 (1H, m, H-11), 1.95 (2H, dd, *J* = 3.4, 17.7 Hz, H-23α), 1.96 (1H, m, H-12), 1.99 (1H, m, H-20), 2.07 (3H, s, Me-28), 2.15 (1H, m, H-7β), 2.46 (1H, dd, *J* = 13.1, 17.7 Hz, H-23β), 3.08 (1H, br s, H-6), 3.58 (1H, d, *J* = 6.2, H-4), 4.39 (1H, dt, *J* = 3.8, 13.1 Hz, H-22), 4.39, 4.52 (2H, d_{AB}, *J* = 11.6 Hz, H-27), 6.15 (1H, d, *J* = 9.8 Hz, H-2), 6.91 (1H, dd, *J* = 6.2, 9.8 Hz, H-3), OTES [0.58 (6H, dq, *J* = 3.3, 8.1 Hz), 0.64 (6H, q, *J* = 7.8 Hz), 0.94 (9H, t, *J* = 7.9 Hz), 0.96 (9H, t, *J* = 7.9 Hz)];^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.4 (CH_3, C-21), 16.3 (CH_3, C-19), 20.5 (CH_3, C-28), 21.1 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.4 (CH_2, C-7), 38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.6 (C, C-13), 44.3 (CH, C-9), 48.1 (C, C-10), 52.0 (CH, C-17), 56.2 (CH, C-14), 56.7 (CH_2, C-27), 59.8 (CH, C-6), 63.5 (C, C-5), 71.1 (CH, C-4), 78.1 (CH, C-22), 125.9 (C, C-25), 132.1 (CH, C-2), 143.7 (CH, C-3), 154.6 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), OTES [4.3 (3 × CH_3), 4.9 (3 × CH_3), 6.7 (3 × CH_2), 6.8 (3 × CH_2)]; EIMS *m/z* 698 [M]$^+$ (4), 669 (96), 641 (1), 555 (5), 385 (5), 265 (3), 239 (6), 149 (4), 103 (100), 95 (11), 75 (85); HREIMS *m/z* 698.4370 [M]$^+$ (calcd for $C_{40}H_{66}O_6Si_2$, 698.4398).

*Preparation of 4,27-O-Di-(tripropylsilyl)withaferin A (**12**).* A solution of **1** (20.0 mg, 0.04 mmol) and tripropylsilyl chloride (20 μL, 0.09 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **12** (22.4 mg, 72%) as an amorphous solid. $[\alpha]_D^{20}$ +93.1 (*c* 1.95, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.68 (3H, s, Me-18), 0.79 (1H, m, H-9), 0.89 (1H, m, H-14), 0.96 (3H, d, *J* = 6.7 Hz, Me-21), 1.00 (1H, m, H-12), 1.06 (1H, m, H-17), 1.23 (1H, dd, *J* = 11.9, 14.9 Hz, H-7), 1.33 (1H, m, H-15), 1.35 (1H, m, H-16), 1.39 (3H, s, Me-19), 1.42 (1H, m, H-11), 1.47 (1H, m, H-8), 1.62 (1H, m, H-15), 1.66 (1H, m, H-16), 1.68 (1H, m, H-11), 1.94 (1H, dd, *J* = 3.3, 17.6 Hz, H-7), 1.95 (1H, m, H-12), 1.99 (1H, m, H-20), 2.05 (3H, s, Me-28), 2.15 (1H, ddd, *J* = 2.3, 4.1, 14.9 Hz, H-7β), 2.45 (1H, dd, *J* = 13.3, 17.6 Hz, H-23β), 3.07 (1H, br s, H-6), 3.56 (1H, d, *J* = 6.1 Hz, H-4), 4.36, 4.50 (2H, d_{AB}, *J* = 11.7 Hz, H-27), 4.38 (1H, dt, *J* = 3.5, 13.3 Hz, H-22), 6.15 (1H, d, *J* = 9.8 Hz, H-2), 6.90 (1H, dd, *J* = 6.1, 9.8 Hz, H-3), OTPRS [0.55−0.65 (12H, m), 0.94 (9H, t, *J* = 7.4 Hz), 0.95 (9H, t, *J* = 7.4 Hz), 1.29−1.42 (12H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.3 (CH_3, C-21), 17.8 (CH_3, C-19), 20.5 (CH_3, C-28), 21.1 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.5 (CH_2, C-7), 38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.6 (C, C-13), 44.3 (CH, C-9), 48.1 (C, C-10), 52.0 (CH, C-17), 56.2 (CH, C-14), 56.6 (CH_2, C-27), 59.7 (CH, C-6), 63.4 (C, C-5), 71.1 (CH, C-4), 78.1 (CH, C-22), 126.0 (C, C-25), 132.1 (CH, C-2), 143.7 (CH, C-3), 154.4 (C, C-24), 165.8 (C, C-26), 202.2 (C, C-1), OTPRS [16.3 (3 × CH_2), 16.6 (3 × CH_2), 16.7 (3 × CH_2), 16.8 (3 × CH_2), 18.4 (3 × CH_3), 18.4 (3 × CH_3)]; ESIMS *m/z* 805 [M + Na]$^+$ (100); HRESIMS *m/z* 805.5240 [M + Na]$^+$ (calcd for $C_{46}H_{78}O_6NaSi_2$, 805.5235).

*Preparation of 4,27-O-Di-(dimethyloctylsilyl)withaferin A (**13**).* A solution of **1** (20.0 mg, 0.04 mmol) and dimethyloctylsilyl chloride (15 μL, 0.06 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **13** (27.2 mg, 84%) as an amorphous solid. $[\alpha]_D^{20}$ +84.0 (*c* 2.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.68 (3H, s, Me-18), 0.80 (1H, m, H-9), 0.89 (1H, m, H-14), 0.97 (3H, d, *J* = 6.6 Hz, Me-21), 1.01 (1H, m, H-12), 1.07 (1H, m, H-17), 1.23 (1H, m, H-7), 1.34 (2H, m, H-15, H-16), 1.38 (3H, s, Me-19), 1.44 (1H, m, H-11), 1.49 (1H, m, H-8), 1.61 (1H, m, H-15), 1.66 (1H, m, H-16), 1.70 (1H, m, H-11), 1.93 (1H, dd, *J* = 3.0, 17.7 Hz, H-23α), 1.94 (1H, m, H-12), 1.99 (1H, m, H-20), 2.05 (3H, s, Me-28), 2.14 (1H, br d, *J* = 15.1 Hz, H-7β), 2.46 (1H, dd, *J* = 13.2, 17.7 Hz, H-23β), 3.08 (1H, br s, H-6), 3.56 (1H, d, *J* = 6.1 Hz, H-4), 4.35, 4.48 (2H, d_{AB}, *J* = 11.6 Hz, H-27), 4.39 (1H, dt, *J* = 3.3, 13.2 Hz, H-22), 6.14 (1H, d, *J* = 9.8 Hz, H-2), 6.88 (1H, dd, *J* = 6.1, 9.8 Hz, H-3), ODMOS [0.08 (3H, s), 0.09 (3H, s), 0.11 (6H, s), 0.55 (2H, t, *J* = 7.4 Hz), 0.61 (2H, t, *J* = 7.6 Hz), 0.88 (6H, t, *J* =7.5 Hz), 1.18−1.33 (24H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.3 (CH_3, C-21), 16.7 (CH_3, C-19), 20.4 (CH_3, C-28), 21.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.0 (CH_2, C-23), 31.4 (CH_2, C-7), 38.7 (CH, C-20), 39.2 (CH_2, C-12), 42.5 (C, C-13), 44.3 (CH, C-9), 48.1 (C, C-10), 51.9 (CH, C-17), 56.1 (CH, C-14), 56.4 (CH_2, C-27), 59.9 (CH, C-6), 63.2 (C, C-5), 71.0 (CH, C-4), 78.0 (CH, C-22), 125.8 (C, C-25), 132.1 (CH, C-2), 143.6 (CH, C-3), 154.5 (C, C-24), 165.7 (C, C-26), 202.2 (C, C-1), ODMOS [−2.1 (2 × CH_3), −1.5 (CH_3), −1.4 (CH_3), 14.1 (2 × CH_3), 16.3 (2 × CH_2), 22.6 (2 × CH_2), 23.0 (CH_2), 23.2 (CH_2), 29.1 (CH_2), 29.2 (2 × CH_2), 29.3 (CH_2), 31.9 (CH_2), 32.0 (CH_2), 33.3 (CH_2), 33.4 (CH_2)]; ESIMS *m/z* 833 [M + Na]$^+$ (100), HRESIMS *m/z* 833.5538 [M + Na]$^+$ (calcd for $C_{48}H_{82}O_6NaSi_2$, 833.5548).

*Preparation of 4,27-O-Di-(dimethylvinylsilyl)withaferin A (**14**).* A solution of **1** (20 mg, 0.04 mmol) and dimethylvinylsilyl cholride (10 μL, 0.07 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **14** (2.4 mg, 9%). $[\alpha]_D^{20}$ +79.7 (*c* 0.36, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.69 (3H, s, Me-18), 0.82 (1H, dt, *J* = 4.3, 12.0 Hz, H-9), 0.91 (1H, m, H-14), 0.98 (3H, d, *J* = 6.6 Hz, Me-21), 1.03 (1H, m, H-12), 1.06 (1H, m, H-17), 1.14 (1H, m, H-15), 1.24 (1H, ddd, *J* = 1.2, 11.7, 14.5 Hz, H-7), 1.37 (1H, m, H-16), 1.40 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.50 (1H, m, H-8), 1.62 (1H, m, H-15), 1.66 (1H, m, H-16), 1.72 (1H, ddd, *J* = 3.6, 7.3, 14.4 Hz, H-11), 1.95 (1H, dd, *J* = 3.2, 17.6 Hz, H-23α), 1.96 (1H, m, H-12), 2.00 (1H, m, H-20), 2.05 (3H, s, Me-28), 2.15 (1H, ddd, *J* = 2.4, 4.3, 15.0 Hz, H-7β), 2.47 (1H, dd, *J* = 13.2, 17.5 Hz, H-23β), 3.09 (1H, br s, H-6), 3.60 (1H, d, *J* = 6.2 Hz, H-4), 4.40 (1H, dt, *J* = 3.4, 13.2 Hz, H-22), 4.36, 4.50 (2H, d_{AB}, *J* = 11.6 Hz, H-27), 6.16 (1H, d, *J* = 9.9 Hz, H-2), 6.88 (1H, dd, *J* = 6.2, 9.9 Hz, H-3), ODMVS [0.18 (3H, s), 0.21 (3H, s), 0.23 (6H, s), 5.78 (1H, dd, *J* = 4.1, 20.1 Hz), 5.81 (1H, dd, *J* = 4.0, 20.2 Hz), 6.01 (1H, dd, *J* = 4.1, 14.9 Hz), 6.03 (1H, dd, *J* = 4.0, 14.9 Hz), 6.10 (1H, dd, *J* = 14.9, 20.1 Hz), 6.18 (1H, dd, *J* = 14.9, 20.3 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.4 (CH_3, C-21), 16.4 (CH_3, C-19), 20.5 (CH_3, C-28), 21.3 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.0 (CH_2, C-23), 31.4 (CH_2, C-7),

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38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.6 (C, C-13), 44.4 (CH, C-9), 48.1 (C, C-10), 52.0 (CH, C-17), 56.2 (CH, C-14), 56.6 (CH_2, C-27), 60.1 (CH, C-6), 63.2 (C, C-5), 71.2 (CH, C-4), 78.1 (CH, C-22), 125.7 (C, C-25), 132.2 (CH, C-2), 143.5 (CH, C-3), 154.6 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), ODMVS [−1.2 (CH_3), −1.6 (CH_3), −2.1 (CH_3), −2.2 (CH_3), 133.4 (CH), 133.6 (CH), 137.4 (CH_2), 137.4 (CH_2)]; ESIMS m/z 661 $[M + Na]^+$ (100); HRESIMS m/z 661.3365 $[M + Na]^+$ (calcd for $C_{36}H_{54}O_6NaSi_2$, 661.3357).

Preparation of 27-O-(Dimethylphenylsilyl)withaferin A (15). A solution of **1** (20.0 mg, 0.04 mmol) and dimethylphenylsilyl chloride (10 μL, 0.06 mmol) was stirred at room temperature for 4 h. The residue was purified affording compound **15** (9.7 mg, 33%) as an amorphous solid. $[\alpha]_D^{20}$ +88.5 (c 0.75, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.69 (3H, s, Me-18), 0.81 (1H, m, H-14), 0.96 (3H, d, J = 6.7 Hz, Me-21), 1.01 (1H, m, H-9), 1.05 (1H, m, H-17), 1.14 (1H, m, H-12), 1.21 (1H, m, H-7), 1.33 (2H, m, H-15, H-16), 1.43 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.50 (1H, m, H-8), 1.61 (1H, m, H-15), 1.64 (1H, m, H-16), 1.72 (1H, ddd, J = 23.4, 7.0, 14.4 Hz, H-11), 1.87 (1H, dd, J = 3.2, 17.6 Hz, H-23α), 1.88 (1H, m, H-12), 1.94 (1H, m, H-20), 1.96 (3H, s, Me-28), 2.14 (1H, ddd, J = 2.3, 4.0, 14.9 Hz, H-7β), 2.37 (1H, dd, J = 13.4, 17.6 Hz, H-23β), 3.02 (1H, br s, H-6), 3.55 (1H, d, J = 6.2 Hz, H-4), 4.28 (1H, dt, J = 3.5, 13.4 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, J = 11.6 Hz, H-27), 6.14 (1H, d, J = 9.9 Hz, H-2), 6.77 (1H, dd, J = 6.2, 9.9 Hz, H-3), ODMPS [0.37 (3H, s), 0.42 (6H, s), 0.43 (3H, s), 7.35−7.42 (6H, m), 7.55−7.62 (4H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.3 (CH_3, C-21), 16.3 (CH_3, C-19), 20.4 (CH_3, C-28), 21.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 29.9 (CH_2, C-23), 31.4 (CH_2, C-7), 38.7 (CH, C-20), 39.2 (CH_2, C-12), 42.5 (C, C-13), 44.3 (CH, C-9), 48.1 (C, C-10), 51.9 (CH, C-17), 56.1 (CH, C-14), 56.8 (CH_2, C-27), 60.0 (CH, C-6), 63.2 (C, C-5), 71.2 (CH, C-4), 77.9 (CH, C-22), 125.6 (C, C-25), 132.2 (CH, C-2), 143.3 (CH, C-3), 154.6 (C, C-24), 165.6 (C, C-26), 202.2 (C, C-1), ODMPS [−0.63 (CH_3), −1.38 (CH_3), −1.85 (CH_3), −2.00 (CH_3), 127.8 (CH), 127.8 (2 × CH), 127.9 (CH), 129.6 (CH), 129.7 (CH), 132.9 (CH), 133.5 (CH), 133.6 (2 × CH), 137.4 (C), 137.8 (C)]; ESIMS m/z 761 $[M + Na]^+$ (100); HRESIMS m/z 761.3668 $[M + Na]^+$ (calcd for $C_{44}H_{58}O_6NaSi_2$, 761.3670).

Preparation of 4,27-O-Di-(methyldiphenylsilyl)withaferin A (16). A solution of **1** (20 mg, 0.04 mmol) and methyldiphenylsilyl chloride (20 μL, 0.09 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **16** (10.5 mg, 30%). $[\alpha]_D^{20}$ +76.5 (c 0.80, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.68 (3H, s, Me-18), 0.80 (1H, m, H-8), 0.88 (1H, m, H-14), 0.94 (3H, d, J = 6.7 Hz, Me-21), 1.01 (1H, m, H-12), 1.12 (1H, m, H-17), 1.19 (1H, m, H-15), 1.33 (1H, m, H-7), 1.44 (1H, m, H-16), 1.46 (3H, s, Me-19), 1.47 (1H, m, H-11), 1.50 (1H, m, H-8), 1.60 (1H, m, H-15), 1.62 (1H, m, H-16), 1.70 (1H, ddd, J = 4.0, 7.6, 14.6 Hz, H-11), 1.85 (1H, dd, J = 3.3, 17.7 Hz, H-23α), 1.91 (1H, m, H-12), 1.94 (1H, m, H-20), 1.95 (3H, s, Me-28), 2.14 (1H, ddd, J = 2.3, 3.9, 14.9 Hz, H-7β), 2.34 (1H, dd, J = 13.2, 17.7 Hz, H-23β), 2.98 (1H, br s, H-6), 3.61 (1H, d, J = 6.2 Hz, H-4), 4.23 (1H, dt, J = 3.5, 13.3 Hz, H-22), 4.46, 4.60 (2H, d_{AB}, J = 12.3 Hz, H-27), 6.15 (1H, d, J = 9.9 Hz, H-2), 6.69 (1H, dd, J = 6.2, 9.9 Hz, H-3), OMDPS [0.67 (3H, s), 0.68 (3H, s), 7.33−7.43 (12H, m), 7.53−7.61 (8H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.3 (CH_3, C-21), 16.4 (CH_3, C-19), 20.5 (CH_3, C-28), 21.2 (CH_2, C-11), 24.2 (CH_2, C-15), 27.4 (CH_2, C-16), 29.8 (CH, C-8), 29.9 (CH_2, C-23), 31.3 (CH_2, C-7), 38.7 (CH, C-20), 39.2 (CH_2, C-12), 42.5 (C, C-13), 44.3 (CH, C-9), 48.1 (C, C-10), 51.9 (CH, C-17), 56.1 (CH, C-14), 57.2 (CH_2, C-27), 60.0 (CH, C-6), 63.2 (C, C-5), 71.5 (CH, C-4), 77.9 (CH, C-22), 125.5 (C, C-25), 132.3 (CH, C-2), 143.1 (CH, C-3), 154.7 (C, C-24), 165.6 (C, C-26), 202.1 (C, C-1), OMDPS [−3.2 (CH_3), −2.10 (CH_3), 127.7 (4 × CH), 127.8 (2 × CH), 1279 (2 × CH), 129.8 (2 × CH), 129.9 (CH), 130.0 (CH), 134.3 (2 × CH), 134.4 (4 × CH), 134.5 (2 × CH), 135.5 (C), 135.7 (C), 135.9 (C), 136.0 (C)]; ESIMS m/z 885 $[M + Na]^+$ (100); HRESIMS m/z 885.3983 $[M + Na]^+$ (calcd for $C_{54}H_{62}O_6NaSi_2$, 885.3983).

Preparation of Derivatives 17−20. A solution of **1** (40 mg, 0.08 mmol) and dimethylisopropylsilyl chloride (20 μL, 0.12 mmol) was stirred at room temperature for 1 h. The residue was purified affording compounds **17** (4.6 mg, 20%), **18** (8.0 mg, 18%), **19** (25.4 mg, 47%), and **20** (6.5 mg, 12%).

4-O-(Dimethylisopropylsilyl)withaferin A (17). $[\alpha]_D^{20}$ +43.3 (c 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.91 (1H, m, H-14), 1.00 (3H, d, J = 6.7 Hz, Me-21), 1.03 (1H, m, H-9), 1.11 (1H, m, H-12), 1.15 (1H, m, H-15), 1.24 (1H, dt, J = 2.2, 13.5 Hz, H-7), 1.38 (1H, m, H-16), 1.40 (3H, s, Me-19), 1.45 (1H, m, H-11), 1.48 (1H, m, H-8), 1.61 (1H, m, H-15), 1.64 (1H, m, H-16), 1.72 (1H, m, H-11), 1.95 (1H, m, H-12), 2.02 (1H, m, H-20), 2.04 (3H, s, Me-28), 2.15 (1H, ddd, J = 2.3, 4.0, 14.8 Hz, H-7β), 2.50 (1H, dd, J = 13.6, 17.6 Hz, H-23β), 2.87 (1H, t, J = 6.6 Hz, OH-27), 3.09 (1H, br s, H-6), 3.57 (1H, d, J = 6.1, H-4), 4.42 (1H, dt, J = 3.4, 13.2 Hz, H-22), 4.36, 4.38 (2H, dd_{AB}, J = 6.6, 11.6 Hz, H-27), 6.16 (1H, d, J = 9.9 Hz, H-2), 6.90 (1H, dd, J = 6.1, 9.9 Hz, H-3), ODMIPS [0.05 (3H, s), 0.09 (3H, s), 0.80 (1H, m), 0.92 (3H, d, J = 6.6 Hz), 0.94 (3H, d, J = 6.5 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.3 (CH_3, C-21), 16.3 (CH_3, C-19), 20.0 (CH_3, C-28), 21.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8; CH_2, C-23), 31.4 (CH_2, C-7), 38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.6 (C, C-13), 44.4 (CH, C-9), 48.1 (C, C-10), 51.9 (CH, C-17), 56.2 (CH, C-14), 57.5 (CH_2, C-27), 59.9 (CH, C-6), 63.3 (C, C-5), 71.2 (CH, C-4), 78.8 (CH, C-22), 125.7 (C, C-25), 132.1 (CH, C-2), 143.6 (CH, C-3), 152.7 (C, C-24), 167.0 (C, C-26), 202.3 (C, C-1), ODMIPS [−3.9 (CH_3), −3.6 (CH_3), 14.7 (CH), 16.7 (2 × CH_3)]; ESIMS m/z 593 $[M + Na]^+$ (100); HRESIMS m/z 593.3273 $[M + Na]^+$ (calcd for $C_{33}H_{50}O_6NaSi$, 593.3274).

27-O-(Dimethylisopropylsilyl)withaferin A (18). $[\alpha]_D^{20}$ +77.1 (c 0.56, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.70 (3H, s, Me-18), 0.92 (1H, m, H-14), 0.99 (3H, d, J = 6.7 Hz, Me-21), 1.03 (1H, m, H-9), 1.07 (1H, m, H-17), 1.11 (1H, m, H-12), 1.16 (1H, m, H-15), 1.28 (1H, dd, J = 11.1 14.4 Hz, H-7), 1.37 (1H, m, H-16), 1.41 (3H, s, Me-19), 1.46 (1H, m, H-11), 1.52 (1H, m, H-8), 1.63 (1H, m, H-15), 1.66 (1H, m, H-16), 1.83 (1H, ddd, J = 3.4, 7.5, 14.3 Hz, H-7), 1.96 (1H, m, H-12), 1.97 (1H, dd, J = 3.3, 17.7 Hz, H-23α), 2.01 (1H, m, H-20), 2.06 (3H, s, Me-28), 2.15 (1H, m, H-7β), 2.47 (1H, dd, J = 13.4, 17.7 Hz, H-23β), 2.55 (1H, br s, OH-4), 3.24 (1H, br s, H-6), 3.76 (1H, d, J = 5.8 Hz, H-4), 4.40 (1H, dt, J = 3.5, 13.4 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.21 (1H, d, J = 10.0 Hz, H-2), 6.93 (1H, dd, J = 5.8, 10.0 Hz, H-3), ODMIPS [0.09 (6H, s), 0.89 (1H, m), 0.95 (6H, d, J = 6.4 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 17.5 (CH_3, C-19), 20.5 (CH_3, C-28), 22.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.2 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 44.1 (CH, C-9), 47.7 (C, C-10), 52.0 (CH, C-17), 56.1 (CH, C-14), 56.6 (CH_2, C-27), 62.6 (CH, C-6), 63.9 (C, C-5), 69.9 (CH, C-4), 78.1 (CH, C-22), 125.9 (C, C-25), 132.3 (CH, C-2), 141.8 (CH, C-3), 154.5 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), ODMIPS [−4.4 (CH_3), −4.5 (CH_3), 14.4 (CH), 16.9 (2 × CH_3)]; ESIMS m/z 593 $[M + Na]^+$ (100); HRESIMS m/z 593.3265 $[M + Na]^+$ (calcd for $C_{33}H_{50}O_6NaSi$, 593.3274).

4,27-O-Di-(dimethylisopropylsilyl)withaferin A (19). $[\alpha]_D^{20}$ +59.6 (c 0.50, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.69 (3H, s, Me-18), 0.83 (1H, H-9), 0.91 (1H, H-14), 0.98 (3H, d, J = 6.6 Hz, Me-21), 1.06 (1H, H-12), 1.08 (1H, H-17), 1.15 (1H, H-15), 1.24 (1H, H-7), 1.39 (3H, s, Me-19), 1.40 (1H, H-16), 1.48 (1H, H-11), 1.51 (1H, H-8), 1.64 (1H, H-15), 1.69 (1H, H-16), 1.73 (1H, H-11), 1.96 (1H, H-12), 1.95 (1H, dd, J = 3.2, 17.7Hz, H-23α), 2.00 (1H, H-20), 2.06 (3H, s, Me-28), 2.15 (1H, ddd, J = 2.1, 4.0, 14.8 Hz, H-7β), 2.47 (1H, dd, J =3.5, 13.2 Hz, H-23β), 4.39 (1H, dt, J = 3.5, 13.2 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.15 (1H, d, J = 9.8 Hz, H-2), 6.90 (1H, dd, J = 6.1, 9.8 Hz, H-3), ODMIPS [0.05 (3H, s), 0.08 (3H, s), 0.09 (6H, s), 0.80 (1H, m), 0.90 (1H, m), 0.92 (3H, d, J = 7.0 Hz), 0.94 (3H, d, J = 6.6 Hz), 0.96 (6H, d, J = 6.6 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.5 (CH_3, C-18), 13.4 (CH_3, C-21), 16.3 (CH_3, C-19), 20.5 (CH_3, C-28), 21.2 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.8 (CH, C-8), 30.1 (CH_2, C-23), 31.5 (CH_2, C-7), 38.8 (CH, C-20), 39.3 (CH_2, C-12), 42.6 (C, C-13), 44.4 (CH, C-9), 48.1 (C, C-10), 52.0 (CH, C-17), 56.2 (CH, C-14), 56.6 (CH_2, C-27), 59.9 (CH, C-6), 63.3 (C, C-5), 71.2 (CH, C-4), 78.1 (CH, C-22), 125.9 (C, C-25), 132.1 (CH, C-2), 143.6 (CH, C-3), 154.5 (C, C-24), 165.8 (C, C-26), 202.3 (C, C-1), ODMIPS [−4.5 (CH_3), −4.4 (CH_3), −3.9 (CH_3), −3.6

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(CH_3), 14.4 (CH), 14.7 (CH), 16.7 (2 × CH_3), 16.9 (2 × CH_3)]; ESIMS *m*/*z* 693 [M + Na]$^+$ (100); HRESIMS *m*/*z* 693.3975 [M + Na]$^+$ (calcd for $C_{38}H_{62}O_6NaSi_2$, 693.3983).

*3β-(Imidazol-1-yl)-27-O-(dimethylisopropylsilyl)withaferin A (**20**).* $[\alpha]_D^{20}$ +3.8 (*c* 0.39, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.66 (3H, s, Me-18), 0.97 (3H, d, *J* = 6.6 Hz, Me-21), 1.01 (1H, m, H-14), 1.12 (1H, m, H-17), 1.16 (1H, m, H-12), 1.21 (1H, m, H-15), 1.25 (1H, m, H-9), 1.36 (1H, m, H-16), 1.37 (3H, s, Me-19), 1.41 (1H, m, H-11), 1.44 (1H, m, H-8), 1.63 (1H, m, H-15), 1.66 (1H, m, H-16), 1.70 (1H, m, H-11), 1.93 (1H, m, H-12), 1.96 (1H, dd, *J* = 3.3, 14.0 Hz, H-8), 1.97 (1H, m, H-20), 2.06 (3H, s, Me-28), 2.15 (1H, m, H-7β), 2.46 (1H, dd, *J* = 14.0, 17.5 Hz, H-23β), 2.82 (1H, br s, H-6), 2.98 (1H, dd, *J* = 6.6, 15.7 Hz, H-2α), 3.21 (1H, dd, *J* = 8.8, 15.7 Hz, H-2β), 3.50 (1H, d, *J* = 4.0 Hz, H-4), 4.37 (1H, dd, *J* = 3.3, 13.3 Hz, H-22), 4.38, 4.49 (2H, d_{AB}, *J* = 11.7 Hz, H-27), 4.70 (1H, ddd, *J* = 4.0, 6.6, 10.5 Hz, H-3), imidazole [6.93 (1H, s), 7.07 (1H, s), 7.58 (1H, s)], ODMIPS [0.07 (3H, s), 0.08 (3H, s), 0.88 (6H, d, *J* = 6.6 Hz), 0.90 (1H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.6 (CH_3, C-18), 13.4 (CH_3, C-21), 15.7 (CH_3, C-19), 20.5 (CH_3, C-28), 21.4 (CH_2, C-11), 24.3 (CH_2, C-15), 27.2 (CH_2, C-16), 29.2 (CH, C-8), 30.1 (CH_2, C-23), 31.1 (CH_2, C-7), 38.7 (CH, C-20), 39.4 (CH_2, C-2), 42.7 (CH, C-9), 43.2 (C, C-13), 50.4 (CH, C-17), 51.9 (C, C-10), 56.1 (CH, C-3), 56.2 (CH, C-14), 57.1 (CH_2, C-27), 58.5 (CH, C-6), 63.2 (C, C-5), 76.6 (CH, C-4), 78.1 (CH, C-22), 126.0 (C, C-25), 154.6 (C, C-24), 165.9 (C, C-26), 208.1 (C, C-1), imidazole [117.4 (CH), 129.8 (CH), 136.2 (CH)], ODMIPS [−5.3 (CH_3), −5.2 (CH_3), 18.4 (CH), 25.9 (2 × CH_3)]; ESIMS *m*/*z* 653 [M + 1]$^+$ (100); HRESIMS *m*/*z* 653.3994 [M + 1]$^+$ (calcd for $C_{37}H_{57}N_2O_6Si$, 653.3986).

*Preparation of 27-O-(tert-Butyldimethylsilyl)withaferin A (**21**).* To a solution of **1** (108.0 mg, 0.25 mmol) in dry dichloromethane (10 mL) were added imidazole (18.0 mg, 0.24 mmol), 4-(*N*,*N*-dimethylamino)-pyridine (28.0 mg, 0.24 mmol) and *tert*-butylchlorodimethylsilyl chloride (56.0 mg, 0.36 mmol). The reaction was stirred at room temperature for 3 h. The residue was then purified by column chromatography (dichloromethane/acetone, 9:1) to give **21**[21] (131.1 mg, 94%).

*Preparation of 27-O-(tert-Butyldimethylsilyl)-4-dehydroxy-4-oxowithaferin A (**22**).* To a solution of **21** (105.0 mg, 0.18 mmol) in dry CH_2Cl_2 (20 mL) was added drop wise a solution of chromium trioxide/pyridine complex in CH_2Cl_2, previously prepared by addition of CrO_3 (700 mg, 7.1 mmol) to dry pyridine (1 mL) and dry CH_2Cl_2 (20 mL). The reaction mixture was stirred under an argon atmosphere for 5 min. The progress of the reaction was monitored by TLC using CH_2Cl_2/acetone (95:5). The reaction was subsequently quenched with 2-propanol (0.5 mL), and the resulting black suspensions were filtered through Florisil. The filtrate was concentrated under reduced pressure, and the residue was purified by preparative TLC using CH_2Cl_2/acetone (9/1) to give the corresponding derivative **22**[21] (102.0 mg, 97%).

*Preparation of 4-Dehydroxy-4-oxowithaferin A (**23**).* To a solution of **22** (96 mg, 0.16 mmol) in dry acetone (12 mL) was added a suspension of Dowex 50WX8-200 (600 mg) in dry acetone (16 mL). The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was monitored by TLC using CH_2Cl_2/acetone (95:5). The suspension were filtered through a pad of celite, and the filtrate was concentrated under reduced pressure. The residue was purified by preparative TLC using CH_2Cl_2/acetone (95/5) to give the corresponding derivative **23**[21] (74.0 mg, 99%).

*Preparation of 27-O-(Trimethylsilyl)-4-dehydroxy-4-oxowithaferin A (**24**).* A solution of **23** (10.0 mg, 0.02 mmol) and trimethylsilyl chloride (10 μL, 0.08 mmol) was stirred at room temperature for 10 min. The residue was purified affording compound **24** (8.8 mg, 41%) as an amorphous solid. $[\alpha]_D^{20}$ +59.7 (*c* 0.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.01 (3H, d, *J* = 6.7 Hz, Me-21), 1.03 (1H, m, H-14), 1.14 (1H, m, H-17), 1.19 (1H, m, H-15), 1.26 (1H, m, H-12), 1.34 (1H, m, H-16), 1.37 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.44 (1H, m, H-11), 1.46 (1H, m, H-9), 1.58 (1H, m, H-8), 1.62 (1H, m, H-15), 1.67 (1H, m, H-16), 1.97 (1H, m, H-23α), 1.99 (1H, m, H-20), 2.01 (1H, m, H-12), 2.05 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, *J* = 3.2, 15.1 Hz, H-7β), 2.49 (1H, dd, *J* = 13.2, 17.4 Hz, H-23β), 3.43 (1H, d, *J* = 1.9 Hz, H-6), 4.42 (1H, dt, *J* = 3.4, 13.2 Hz, H-22), 4.36, 4.49 (2H, d_{AB}, *J* = 11.3 Hz, H-27), 6.86 (1H, d, *J* = 10.5 Hz, H-2), 6.88 (1H, d, *J* = 10.5 Hz, H-3), OTMS [0.16 (9H, s)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.7 (CH_3, C-18), 13.3 (CH_3, C-21), 19.1 (CH_3, C-19), 20.0 (CH_3, C-28), 23.4 (CH_2, C-11), 24.2 (CH_2, C-15), 27.1 (CH_2, C-16), 29.6 (CH, C-8), 29.8 (CH_2, C-23), 30.5 (CH_2, C-7), 38.7 (CH, C-20), 39.4 (CH_2, C-12), 42.6 (C, C-13), 43.6 (CH, C-9), 49.8 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 57.4 (CH_2, C-27), 63.5 (CH, C-6), 63.9 (C, C-5), 78.7 (CH, C-22), 125.7 (C, C-25), 139.1 (CH, C-2), 141.6 (CH, C-3), 152.7 (C, C-24), 166.9 (C, C-26), 193.8 (C, C-4), 202.1 (C, C-1), OTMS [1.90 (3 × CH_3)]; EIMS *m*/*z* 540 [M]$^+$ (53), 525 (100), 509 (12), 468 (9), 342 (5), 292 (15), 213 (24), 200 (67), 124 (17), 118 (21), 110 (27), 95 (26), 75 (33), 68 (73); HREIMS *m*/*z* 540.2909 [M]$^+$ (calcd for $C_{31}H_{44}O_6Si$, 540.2907).

*Preparation of 27-O-(Triethylsilyl)-4-dehydroxy-4-oxowithaferin A (**25**).* A solution of **23** (10.0 mg, 0.02 mmol) and triethylsilyl chloride (10 μL, 0.06 mmol) was stirred at room temperature for 2 h. The residue was purified affording compound **25** (4.8 mg, 41%) as an amorphous solid. $[\alpha]_D^{20}$ +82.4 (*c* 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.00 (3H, d, *J* = 6.8 Hz, Me-21), 1.03 (1H, m, H-14), 1.13 (1H, m, H-17), 1.19 (1H, m, H-15), 1.27 (1H, m, H-12), 1.33 (1H, m, H-16), 1.37 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.44 (1H, m, H-11), 1.47 (1H, m, H-9), 1.61 (1H, m, H-8), 1.69 (1H, m, H-15), 1.97 (1H, dd, *J* = 3.3, 17.4 Hz, H-23α), 1.98 (1H, m, H-20), 2.00 (1H, m, H-12), 2.03 (1H, m, H-11), 2.08 (3H, s, Me-28), 2.17 (1H, dt, *J* = 3.2, 15.0 Hz, H-7β), 2.37 (1H, dd, *J* = 13.1, 17.4 Hz, H-23β), 3.43 (1H, d, *J* = 2.3 Hz, H-6), 4.40 (1H, dt, *J* = 3.4, 13.1 Hz, H-22), 4.39, 4.52 (2H, d_{AB}, *J* = 11.7 Hz, H-27), 6.85 (1H, d, *J* = 10.4 Hz, H-2), 6.88 (1H, d, *J* = 10.4 Hz, H-3), OTES [0.64 (6H, q, *J* = 7.9 Hz), 0.96 (9H, t, *J* = 7.8 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.8 (CH_3, C-18), 13.4 (CH_3, C-21), 19.2 (CH_3, C-19), 20.5 (CH_3, C-28), 23.5 (CH_2, C-11), 24.3 (CH_2, C-15), 27.2 (CH_2, C-16), 29.6 (CH, C-8), 30.1 (CH_2, C-23), 30.6 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.7 (CH_2, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 126.0 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.5 (C, C-24), 165.8 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), OTES [4.3 (3 × CH_3), 6.8 (3 × CH_2)]; ESIMS *m*/*z* 647 [M + Na]$^+$ (100); HRESIMS *m*/*z* 647.3751 [M + Na]$^+$ (calcd for $C_{37}H_{56}O_6NaSi$, 647.3744).

*Preparation of 27-O-(Tripropylsilyl)-4-dehydroxy-4-oxowithaferin A (**26**).* A solution of **23** (10.0 mg, 0.02 mmol) and tripropylsilyl chloride (10 μL, 0.05 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **26** (10 mg, 80%) as an amorphous solid. $[\alpha]_D^{20}$ +87.2 (*c* 0.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.00 (3H, d, *J* = 6.8 Hz, Me-21), 1.04 (1H, m, H-14), 1.13 (1H, m, H-17), 1.18 (1H, m, H-15), 1.28 (1H, m, H-12), 1.33 (1H, m, H-16), 1.37 (1H, m, H-7), 1.38 (3H, s, Me-19), 1.42 (1H, m, H-11), 1.46 (1H, m, H-9), 1.62 (1H, m, H-8), 1.66 (1H, m, H-15), 1.70 (1H, m, H-16), 1.97 (1H, dd, *J* = 3.5, 17.5 Hz), 2.00 (1H, m, H-20), 2.02 (1H, m, H-12), 2.04 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, *J* = 3.0, 14.8 Hz, H-7β), 2.48 (1H, dd, *J* = 13.4, 17.5 Hz, H-23β), 3.44 (1H, d, *J* = 2.1 Hz, H-6), 4.40 (1H, dt, *J* = 3.4, 13.4 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, *J* = 11.6 Hz, H-27), 6.86 (1H, d, *J* = 10.3 Hz, H-2), 6.89 (1H, d, *J* = 10.3 Hz, H-3), OTPRS [0.63 (6H, t, *J* = 8.2 Hz), 0.96 (9H, t, *J* = 7.2 Hz), 1.33−1.41 (6H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.8 (CH_3, C-18), 13.4 (CH_3, C-21), 19.2 (CH_3, C-19), 20.5 (CH_3, C-28), 23.5 (CH_2, C-11), 24.3 (CH_2, C-15), 27.2 (CH_2, C-16), 29.6 (CH, C-8), 30.1 (CH_2, C-23), 30.6 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.6 (CH_2, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 126.0 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.4 (C, C-24), 165.8 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), OTPRS [16.4 (3 × CH_2), 16.8 (3 × CH_2), 18.4 (3 × CH_3)]; ESIMS *m*/*z* 647 [M + Na]$^+$ (100); HRESIMS *m*/*z* 647.3751 [M + Na]$^+$ (calcd for $C_{37}H_{56}O_6NaSi$, 647.3744).

*Preparation of 27-O-(Triisopropylsilyl)-4-dehydroxy-4-oxowithaferin A (**27**).* A solution of **23** (10.0 mg, 0.02 mmol) and triisopropylsilyl chloride (10 μL, 0.05 mmol) was stirred at room temperature for 2 h. The residue was purified affording compound **27** (8.4 mg, 67%) as an amorphous solid. $[\alpha]_D^{20}$ +60.5 (*c* 0.57, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.02 (3H, d, *J* = 6.7 Hz, Me-21), 1.14 (1H,

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m, H-14), 1.18 (1H, m, H-17), 1.27 (1H, m, H-15), 1.32 (1H, m, H-12), 1.35 (1H, m, H-16), 1.37 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.42 (1H, m, H-11), 1.47 (1H, m, H-9), 1.62 (1H, m, H-8), 1.66 (1H, m, H-15), 1.70 (1H, m, H-16), 1.98 (1H, dd, J = 3.3, 17.5 Hz, H-23α), 1.99 (1H, m, H-20), 2.01 (1H, m, H-12), 2.02 (1H, m, H-11), 2.10 (3H, s, Me-28), 2.18 (1H, dt, J = 3.2, 15.08 Hz, H-7β), 2.49 (1H, dd, J = 13.5, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.3 Hz, H-6), 4.39 (1H, dt, J = 3.5, 13.2 Hz, H-22), 4.49, 4.60 (2H, d_{AB}, J = 11.6 Hz, H-27), 6.86 (1H, d, J = 10.4 Hz, H-2), 6.89 (1H, d, J = 10.4 Hz, H-3), OTIPS [1.07 (18H, d, J = 7.2 Hz), 2.00 (3H, m)]; ^{13}C NMR (CDCl$_3$, 125 MHz) δ 11.8 (CH$_3$, C-18), 13.4 (CH$_3$, C-21), 19.2 (CH$_3$, C-19), 20.6 (CH$_3$, C-28), 23.5 (CH$_2$, C-11), 24.3 (CH$_2$, C-15), 27.2 (CH$_2$, C-16), 29.6 (CH, C-8), 30.1 (CH$_2$, C-23), 30.6 (CH$_2$, C-7), 38.8 (CH, C-20), 39.5 (CH$_2$, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.2 (CH, C-17), 55.6 (CH, C-14), 57.4 (CH$_2$, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 78.2 (CH, C-22), 126.1 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.6 (C, C-24), 165.9 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), OTIPS [12.0 (6 × CH$_3$), 18.0 (3 × CH)]; ESIMS m/z 647 [M + Na]$^+$ (100); HRESIMS m/z 647.3758 [M + Na]$^+$ (calcd for C$_{37}$H$_{56}$O$_6$NaSi, 647.3744).

*Preparation of 27-O-(Tributylsilyl)-4-dehydroxy-4-oxowithaferin A (**28**).* A solution of **23** (10.0 mg, 0.02 mmol) and tributylsilyl chloride (10 μL, 0.04 mmol) was stirred at room temperature for 5 min. The residue was purified affording compound **28** (13.0 mg, 97%) as an amorphous solid. $[\alpha]_D^{20}$ +61.2 (c 1.30, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.00 (3H, d, J = 6.7 Hz, Me-21), 1.03 (1H, m, H-14), 1.14 (1H, m, H-17), 1.19 (1H, m, H-15), 1.28 (1H, m, H-12), 1.31 (1H, m, H-16), 1.33 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.43 (1H, m, H-11), 1.46 (1H, m, H-9), 1.59 (1H, m, H-8), 1.63 (1H, m, H-15), 1.68 (1H, m, H-16), 1.97 (1H, dd, J = 3.4, 17.7 Hz, H-23α), 1.99 (1H, m, H-20), 2.01 (1H, m, H-12), 2.03 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, J = 3.1, 14.8 Hz, H-7β), 2.47 (1H, dd, J = 13.4, 17.7 Hz, H-23β), 3.44 (1H, d, J = 2.2 Hz, H-6), 4.40 (1H, dt, J = 3.5, 13.3 Hz, H-22), 4.38, 4.51 (2H, d_{AB}, J = 11.7 Hz, H-27), 6.85 (1H, d, J = 10.4 Hz, H-2), 6.88 (1H, d, J = 10.4 Hz, H-3), OTBS [0.63 (6H, br t, J = 8.1 Hz), 0.89 (9H, t, J = 6.8 Hz), 1.32 (12H, m)]; ^{13}C NMR (CDCl$_3$, 125 MHz) δ 11.8 (CH$_3$, C-18), 13.4 (CH$_3$, C-21), 19.2 (CH$_3$, C-19), 20.5 (CH$_3$, C-28), 23.5 (CH$_2$, C-11), 24.3 (CH$_2$, C-15), 27.2 (CH$_2$, C-16), 29.6 (CH, C-8), 30.1 (CH$_2$, C-23), 30.6 (CH$_2$, C-7), 38.8 (CH, C-20), 39.5 (CH$_2$, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.7 (CH$_2$, C-27), 63.5 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 126.0 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.4 (C, C-24), 165.7 (C, C-26), 193.8 (C, C-4), 202.1 (C, C-1), OTBS [13.2 (3 × CH$_3$), 13.8 (3 × CH$_2$), 25.4 (3 × CH$_2$), 26.6 (3 × CH$_2$)]; ESIMS m/z 689 [M + Na]$^+$ (100); HRESIMS m/z 689.4219 [M + Na]$^+$ (calcd for C$_{40}$H$_{64}$O$_6$NaSi, 689.4213).

*Preparation of 27-O-(Trihexylsilyl)-4-dehydroxy-4-oxowithaferin A (**29**).* A solution of **23** (10.0 mg, 0.02 mmol) and trihexylsilyl chloride (10 μL, 0.03 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **29** (2.3 mg, 15%) as an amorphous solid. $[\alpha]_D^{20}$ +70.4 (c 0.26, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.01 (3H, d, J = 6.6 Hz, Me-21), 1.03 (1H, m, H-14), 1.13 (1H, m, H-17), 1.19 (1H, m, H-15), 1.28 (1H, m, H-12), 1.35 (1H, m, H-16), 1.38 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.43 (1H, m, H-11), 1.48 (1H, m, H-9), 1.65 (1H, m, H-15), 1.70 (1H, m, H-16), 1.97 (1H, dd, J = 3.3, 17.7 Hz, H-7), 2.00 (1H, m, H-20), 2.02 (1H, m, H-12), 2.03 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, J = 3.2, 14.9 Hz, H-7β), 2.48 (1H, dd, J = 13.4, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.3 Hz, H-6), 4.40 (1H, dt, J = 3.5, 13.4 Hz, H-22), 4.37, 4.51 (2H, d_{AB}, J = 11.8 Hz, H-27), 6.86 (1H, d, J = 10.4 Hz, H-2), 6.89 (1H, d, J = 10.4 Hz, H-3), OTHS [0.62 (6H, br t, J = 7.9 Hz), 0.96 (9H, t, J = 7.2 Hz), 1.24−1.34 (24H, m)]; ^{13}C NMR (CDCl$_3$, 125 MHz) δ 11.8 (CH$_3$, C-18), 13.4 (CH$_3$, C-21), 19.2 (CH$_3$, C-19), 20.6 (CH$_3$, C-28), 23.5 (CH$_2$, C-11), 24.3 (CH$_2$, C-15), 27.2 (CH$_2$, C-16), 29.6 (CH, C-8), 30.1 (CH$_2$, C-23), 30.6 (CH$_2$, C-7), 38.8 (CH, C-20), 39.4 (CH$_2$, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.2 (CH, C-17), 55.6 (CH, C-14), 56.7 (CH$_2$, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 126.0 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.4 (C, C-24), 165.8 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), OTHS [13.5 (3 × CH$_3$), 14.2 (3 × CH$_2$), 22.6 (3 × CH$_2$), 23.2 (3 × CH$_2$), 31.6 (3 × CH$_2$), 33.4 (3 × CH$_2$)]; ESIMS m/z 773 [M + Na]$^+$ (100); HRESIMS m/z 773.5162 [M + Na]$^+$ (calcd for C$_{46}$H$_{74}$O$_6$NaSi, 773.5152).

*Preparation of 27-O-(Dimethylisopropylsilyl)-4-dehydroxy-4-oxowithaferin A (**30**).* A solution of **23** (10.0 mg, 0.02 mmol) and dimethylisopropylsilyl chloride (10 μL, 0.06 mmol) was stirred at room temperature for 30 min. The residue was purified affording compound **30** (8.8 mg, 15%) as an amorphous solid. $[\alpha]_D^{20}$ +89.4 (c 0.63, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.01 (3H, d, J = 6.7 Hz, Me-21), 1.03 (1H, m, H-14), 1.13 (1H, m, H-17), 1.19 (1H, m, H-15), 1.27 (1H, m, H-12), 1.31 (1H, m, H-15), 1.36 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.43 (1H, m, H-11), 1.46 (1H, m, H-9), 1.63 (1H, m, H-8), 1.65 (1H, m, H-15), 1.69 (1H, m, H-15), 1.97 (1H, dd, J = 3.4, 17.7 Hz, H-7), 1.98 (1H, m, H-20), 2.01 (1H, m, H-12), 2.03 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, J = 3.3, 15.0 Hz, H-7β), 2.48 (1H, dd, J = 13.5, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.3 Hz, H-6), 4.40 (1H, dt, J = 3.6, 13.3 Hz, H-22), 4.38, 4.50 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.86 (1H, d, J = 10.3 Hz, H-2), 6.88 (1H, d, J = 10.3 Hz, H-3), ODMIPS [0.10 (6H, s), 0.90 (1H, m), 0.96 (6H, d, J = 6.5 Hz)]; ^{13}C NMR (CDCl$_3$, 125 MHz) δ 11.8 (CH$_3$, C-18), 13.4 (CH$_3$, C-21), 19.2 (CH$_3$, C-19), 20.5 (CH$_3$, C-28), 23.5 (CH$_2$, C-11), 24.3 (CH$_2$, C-15), 27.2 (CH$_2$, C-16), 29.6 (CH, C-8), 30.1 (CH$_2$, C-23), 30.6 (CH$_2$, C-7), 38.8 (CH, C-20), 39.4 (CH$_2$, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.6 (CH$_2$, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 125.9 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.5 (C, C-24), 165.8 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), ODMIPS [−4.4 (CH$_3$), −4.5 (CH$_3$), 14.4 (CH), 16.9 (2 × CH$_3$)]; ESIMS m/z 591 [M + Na]$^+$ (100); HRESIMS m/z 591.3107 [M + Na]$^+$ (calcd for C$_{33}$H$_{48}$O$_6$NaSi, 591.3118).

*Preparation of 27-O-(Dimethyloctylsilyl)-4-dehydroxy-4-oxowithaferin A (**31**).* A solution of **23** (10.0 mg, 0.02 mmol) and dimethyloctylsilyl chloride (10 μL, 0.04 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **31** (4.4 mg, 34%) as an amorphous solid. $[\alpha]_D^{20}$ +81.1 (c 0.44, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.00 (3H, d, J = 6.6 Hz, Me-21), 1.03 (1H, m, H-14), 1.13 (1H, m, H-17), 1.18 (1H, m, H-15), 1.28 (1H, m, H-12), 1.34 (1H, m, H-16), 1.38 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.44 (1H, m, H-11), 1.47 (1H, m, H-9), 1.66 (1H, m, H-15), 1.69 (1H, m, H-16), 1.97 (1H, dd, J = 3.3, 17.5 Hz, H-23α), 1.99 (1H, m, H-20), 2.01 (1H, m, H-12), 2.03 (1H, m, H-11), 2.07 (3H, s, Me-28), 2.17 (1H, dt, J = 3.0, 15.0 Hz, H-7β), 2.48 (1H, dd, J = 13.6, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.1 Hz, H-6), 4.41 (1H, dt, J = 3.4, 13.3 Hz, H-22), 4.36, 4.49 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.86 (1H, d, J = 10.3 Hz, H-2), 6.88 (1H, d, J = 10.3 Hz, H-3), ODMOS [0.13 (6H, s), 0.62 (2H, br t, J = 8.4 Hz), 0.88 (3H, t, J = 6.9 Hz), 1.23−1.34 (12H, m)]; ^{13}C NMR (CDCl$_3$, 125 MHz) δ 11.7 (CH$_3$, C-18), 13.3 (CH$_3$, C-21), 19.2 (CH$_3$, C-19), 20.4 (CH$_3$, C-28), 23.4 (CH$_2$, C-11), 24.2 (CH$_2$, C-15), 27.2 (CH$_2$, C-16), 30.5 (CH, C-8), 31.9 (CH$_2$, C-23), 33.4 (CH$_2$, C-7), 38.7 (CH, C-20), 39.4 (CH$_2$, C-12), 42.6 (C, C-13), 43.6 (CH, C-9), 49.8 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.4 (CH$_2$, C-27), 63.5 (CH, C-6), 63.9 (C, C-5), 78.0 (CH, C-22), 125.8 (C, C-25), 139.1 (CH, C-2), 141.6 (CH, C-3), 154.5 (C, C-24), 165.7 (C, C-26), 193.9 (C, C-4), 202.1 (C, C-1), ODMOS [−2.11 (2 × CH$_3$), 14.1 (CH$_3$), 16.3 (CH$_2$), 22.4 (CH$_2$), 23.2 (CH$_2$), 29.2 (CH$_2$), 29.3 (CH$_2$), 31.9 (CH$_2$), 33.4 (CH$_2$)]; ESIMS m/z 661 [M + Na]$^+$ (100); HRESIMS m/z 661.3909 [M + Na]$^+$ (calcd for C$_{38}$H$_{58}$O$_6$NaSi, 661.3900).

*Preparation of 27-O-(Dimethylvinylsilyl)-4-dehydroxy-4-oxowithaferin A (**32**).* A solution of **23** (10.0 mg, 0.02 mmol) and dimethylvinylsilyl chloride (10 μL, 0.07 mmol) was stirred at room temperature for 30 min. The residue was purified affording compound **32** (4.4 mg, 40%) as an amorphous solid. $[\alpha]_D^{20}$ +88.8 (c 0.25, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 1.00 (3H, d, J = 6.7 Hz, Me-21), 1.02 (1H, m, H-14), 1.13 (1H, m, H-17), 1.18 (1H, m, H-15), 1.27 (1H, m, H-12), 1.32 (1H, m, H-16), 1.37 (1H, m, H-7), 1.39 (3H, s, Me-19), 1.43 (1H, m, H-11), 1.46 (1H, m, H-9), 1.60 (1H, m, H-8), 1.64 (1H, m, H-15), 2.06 (3H, s, Me-28), 1.68 (1H, m, H-16), 1.96 (1H, dd, J = 3.1, 17.5 Hz, H-23α), 1.99 (1H, m, H-20), 2.02 (1H, m, H-12), 2.05 (1H, m, H-11), 2.17 (1H, dt, J = 3.2, 14.9 Hz, H-7β), 2.48 (1H, dd, J = 13.3, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.2 Hz, H-6),

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4.41 (1H, dt, J = 3.3, 13.1 Hz, H-22), 4.36, 4.50 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.86 (1H, d, J = 10.4 Hz, H-2), 6.88 (1H, d, J = 10.4 Hz, H-3), ODMVS [0.23 (6H, s), 5.81 (1H, dd, J = 3.9, 20.3 Hz), 6.04 (1H, dd, J = 3.9, 14.9 Hz), 6.17 (1H, dd, J = 14.9, 20.3 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.8 (CH_3, C-18), 13.4 (CH_3, C-21), 19.2 (CH_3, C-19), 20.5 (CH_3, C-28), 23.5 (CH_2, C-11), 24.3 (CH_2, C-15), 27.2 (CH_2, C-16), 29.6 (CH, C-8), 30.0 (CH_2, C-23), 30.6 (CH_2, C-7), 38.8 (CH, C-20), 39.4 (CH_2, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.6 (CH_2, C-27), 63.5 (CH, C-6), 64.0 (C, C-5), 78.1 (CH, C-22), 125.8 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.6 (C, C-24), 165.7 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), ODMVS [−2.1 (CH_3), −2.2 (CH_3), 133.4 (CH), 137.4 (CH_2)]; ESIMS m/z 575 $[M + Na]^+$ (100); HRESIMS m/z 575.2804 $[M + Na]^+$ (calcd for $C_{32}H_{44}O_6NaSi$, 575.2805).

*Preparation of 27-O-(Dimethylphenylsilyl)-4-dehydroxy-4-oxowithaferin A (**33**).* A solution of **23** (10.0 mg, 0.02 mmol) and dimethylphenylsilyl chloride (10 μL, 0.06 mmol) was stirred at room temperature for 20 min. The residue was purified affording compound **33** (3.8 mg, 32%) as an amorphous solid. $[\alpha]_D^{20}$ +83.8 (c 0.21, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.71 (3H, s, Me-18), 0.98 (3H, d, J = 6.7 Hz, Me-21), 1.02 (1H, m, H-14), 1.10 (1H, m, H-17), 1.18 (1H, m, H-15), 1.27 (1H, m, H-12), 1.32 (1H, m, H-16), 1.35 (1H, m, H-7), 1.38 (3H, s, Me-19), 1.44 (1H, m, H-12), 1.46 (1H, m, H-9), 1.59 (1H, m, H-8), 1.62 (1H, m, H-15), 1.65 (1H, m, H-16), 1.89 (1H, dd, J = 3.2, 17.7 Hz, H-23α), 1.96 (1H, m, H-20), 1.97 (3H, s, Me-28), 1.99 (1H, m, H-12), 2.01 (1H, m, H-11), 2.17 (1H, dt, J = 3.0, 15.0 Hz, H-7β), 2.39 (1H, dd, J = 13.3, 17.5 Hz, H-23β), 3.44 (1H, d, J = 2.2 Hz, H-6), 4.40 (1H, dt, J = 3.5, 13.1 Hz, H-22), 4.37, 4.50 (2H, d_{AB}, J = 11.5 Hz, H-27), 6.86 (1H, d, J = 10.3 Hz, H-2), 6.88 (1H, d, J = 10.3 Hz, H-3), ODMPS [0.42 (3H, s), 0.43 (3H, s), 7.37 (3H, m), 7.59 (2H, m)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.8 (CH_3, C-18), 13.3 (CH_3, C-21), 19.2 (CH_3, C-19), 20.4 (CH_3, C-28), 23.5 (CH_2, C-11), 24.3 (CH_2, C-15), 27.2 (CH_2, C-16), 29.6 (CH, C-8), 29.9 (CH_2, C-23), 30.6 (CH_2, C-7), 38.7 (CH, C-20), 39.4 (CH_2, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 56.8 (CH_2, C-27), 63.5 (CH, C-6), 64.0 (C, C-5), 77.9 (CH, C-22), 125.6 (C, C-25), 139.1 (CH, C-2), 141.6 (CH, C-3), 154.6 (C, C-24), 165.7 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), ODMPS [−2.0 (CH_3), −1.8 (CH_3), 127. 6 (2 × CH), 129.8 (CH), 133.6 (2 × CH), 137.8 (C)]; ESIMS m/z 625 $[M + Na]^+$ (100); HRESIMS m/z 625.2956 $[M + Na]^+$ (calcd for $C_{36}H_{46}O_6NaSi$, 625.2961).

*Preparation of 27-O-(Methyldiphenylsilyl)-4-dehydroxy-4-oxowithaferin A (**34**).* A solution of **23** (10.0 mg, 0.02 mmol) and methyldiphenylsilyl chloride (10 μL, 0.05 mmol) was stirred at room temperature for 1 h. The residue was purified affording compound **34** (12.3 mg, 93%) as an amorphous solid. $[\alpha]_D^{20}$ +73.8 (c 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s, Me-18), 0.97 (3H, d, J = 6.6 Hz, Me-21), 1.02 (1H, m, H-14), 1.10 (1H, m, H-17), 1.18 (1H, m, H-15), 1.27 (1H, m, H-12), 1.35 (1H, m, H-7), 1.35 (1H, m, H-7), 1.36 (1H, m, H-16), 1.39 (3H, s, Me-19), 1.47 (2H, m, H-9, H-11), 1.61 (1H, m, H-8), 1.65 (1H, m, H-15), 1.66 (1H, m, H-16), 1.90 (1H, dd, J = 3.2, 17.8 Hz, H-23α), 1.97 (3H, s, Me-28), 1.98 (1H, m, H-20), 1.99 (1H, m, H-12), 2.02 (1H, m, H-11), 2.17 (1H, dt, J = 3.0, 14.9 Hz, H-7β), 2.37 (1H, dd, J = 13.2, 17.7 Hz, H-23β), 3.44 (1H, d, J = 2.3 Hz, H-6), 4.26 (1H, dt, J = 3.4, 13.2 Hz, H-22), 4.48, 4.61 (2H, d_{AB}, J = 11.8 Hz, H-27), 6.86 (1H, d, J = 10.3 Hz, H-2), 6.88 (1H, dd, J = 10.3 Hz, H-3), OMDPS [0.70 (3H, s), 7.38 (4H, t, J = 7.5 Hz), 7.40 (2H, t, J = 7.6 Hz), 7.61 (4H, d, J = 7.5 Hz)]; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.8 (CH_3, C-18), 13.3 (CH_3, C-21), 19.2 (CH_3, C-19), 20.5 (CH_3, C-28), 23.5 (CH_2, C-11), 24.3 (CH_2, C-15), 27.3 (CH_2, C-16), 29.6 (CH, C-8), 29.9 (CH_2, C-23), 30.5 (CH_2, C-7), 38.7 (CH, C-20), 39.4 (CH_2, C-12), 42.7 (C, C-13), 43.7 (CH, C-9), 49.9 (C, C-10), 52.1 (CH, C-17), 55.6 (CH, C-14), 57.3 (CH_2, C-27), 63.6 (CH, C-6), 64.0 (C, C-5), 77.9 (CH, C-22), 125.6 (C, C-25), 139.2 (CH, C-2), 141.6 (CH, C-3), 154.7 (C, C-24), 165.6 (C, C-26), 193.9 (C, C-4), 202.2 (C, C-1), OMDPS [−3.2 (CH_3), 127.8 (4 × CH), 129.8 (2 × CH), 134.4 (4 × CH), 135.9 (C), 136.0 (C)]; ESIMS m/z 687 $[M + Na]^+$ (100); HRESIMS m/z 687.3118 $[M + Na]^+$ (calcd for $C_{41}H_{48}O_6NaSi$, 687.3118).

Materials for Biological Studies. 3-(4,5-Dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and media for growing cell lines and all supplements were purchased from Sigma-Aldrich, U.K., and cell lines were purchased from ATCC. Noncancerous cells were kindly donated by Prof. Roger Phillips and Dr. Simon Allison at the University of Huddersfield. Phosphate-buffered saline, 50 μg/mL Annexin V−CF488A conjugate, Annexin V binding buffer (10× concentrate), solution 15 (500 μg/mL Hoechst 33342), solution 16 (500 μg/mL propidium iodide), solution 10 (Lysis buffer), solution 11 (stabilization buffer), solution 12 (500 μg/mL DAPI), NC-Slide A8, NC-Slide A2 glass slides, and via-1 cassettes were bought from ChemoMetec, Denmark. NC-3000 image cytometer was used to perform the assays.

Cell Culture and Viability Assay. Cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) or Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum, 5% penicillin−streptomycin at 37 °C, and 5% CO_2/95% air, as instructed by the suppliers. The cells were plated in 96-well culture plates at a density of 1 × 10^4 cells/mL and allowed to adhere at 37 °C for 24 h. The following day, different doses of the compounds or vehicle were added to the cells in varying concentrations of compounds and were further incubated for 96 h. Following the aforementioned incubation time, the supernatant was removed and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added for 4 h. The ability of cells to form formazan crystals by active mitochondrial respiration was determined using a Microplate reader after dissolving the crystals in dimethyl sulfoxide. Cytotoxicity was expressed as a relative percentage of the absorbance measured at 540 nm in the control and extract-treated cells. Data were presented as the mean ± SEM.

Two-Step Cell Cycle Assay. Cells were seeded into T25 flasks containing 5 mL of complete media and were incubated for 24 h at 37 °C. After 24 h, elapsed cells were treated with vehicle control or compounds at different concentrations and left in the incubator for a further 48 h. Cells were then subjected to two-step cell cycle assay according to the manufacture's instructions. Briefly, 1 mL of cells (1 × 10^6 cells/mL) was transferred to Eppendorf tubes. In a separate Eppendorf, a mixture of 1960 μL of Lysis buffer (solution 10) plus 40 μL of 500 μg/mL DAPI (solution 12) was prepared. Eppendorfs containing cells were centrifuged at 400 g for 5 min, supernatant was removed, and cells were resuspended in 250 μL of the above mixture, mixed well, and incubated at 37 °C for 5 min. Two hundred and fifty microliters of stabilization buffer (solution 11) was then added to the cells and mixed well. Ten microliters of each sample was then loaded on A8 slide and subjected to the two-step cell cycle assay using NC-3000.

Statistical Analysis. All results were expressed as mean ± SEM. Significant differences between groups were determined using unpaired Student's t-test. Significance was set at $p < 0.05$.

ADME Property Predictions of WA Analogues. Prediction of descriptors related to adsorption, distribution, metabolism, and excretion (ADME) properties of the compounds was predicted using the QikProp program (QikProp, version 5.3)[36] in Fast mode and based on the method of Jorgensen.[37,38] Preparation of compounds and the 2D-to-3D conversion was performed using LigPrep tool, a module of the Small-Molecule Drug Discovery Suite in Schrödinger software package, followed by a MacroModel 12.01 Monte Carlo conformational search to locate the lowest energy conformation of each ligand. The program computes pharmacokinetic relevant properties, such as octanol/water partitioning coefficient, aqueous solubility, brain/blood partition coefficient, Caco-2 cell permeability, serum protein binding, number of likely metabolic reactions, and others. Drug likeness (#stars), the number of property descriptors from the full list of descriptors computed by the QikProp that fall outside the range of values determined for 95% of known drugs, was used as the additional compound selection filter.

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■ ASSOCIATED CONTENT

Supporting Information

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^{1}H and ^{13}C NMR spectra of novel withaferin A-silyl derivatives **2−20** and **24−34**; cytotoxic activity of WA-silyl analogues on human ovarian carcinoma cisplatin-sensible and cisplatin-resistant cell lines, and on a noncarcinoma cancer cell line; representative scatter plot indicating the percentage of cells in G0/G1 (M1), S (M2), and G2 (M3) and sub-G1 (M4) phases in human ovarian carcinoma cells treated with compounds **21** and **22**; in silico ADME profile of WA (**1**) and selected WA analogues (PDF)

Molecular formula strings with biochemical and biological data (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

SAR, structure−activity relationship; NPs, natural products; WA, withaferin A; Et_3N, triethylamina; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; rt, room temperature; DMIPSiCl, dimethylisopropylsilyl chloride; py, pyridine; TBDMSiCl, *tert*-butyldimethylsilyl chloride; MTT, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide

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