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Novel Nitrogen-Enriched Oridonin Analogues with Thiazole-Fused A-Ring: Protecting Group-Free Synthesis, Enhanced Anticancer Profile, and Improved Aqueous Solubility

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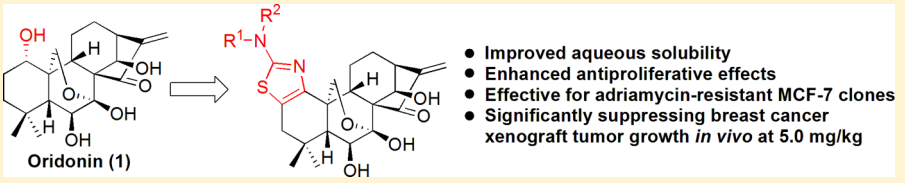
Supporting Information



ABSTRACT: Oridonin (**1**), a complex *ent*-kaurane diterpenoid isolated from the traditional Chinese herb *Isodon rubescens*, has demonstrated great potential in the treatment of various human cancers due to its unique and safe anticancer pharmacological profile. Nevertheless, the clinical development of oridonin for cancer therapy has been hampered by its relatively moderate potency, limited aqueous solubility, and poor bioavailability. Herein, we report the concise synthesis of a series of novel nitrogen-enriched oridonin derivatives with thiazole-fused A-ring through an efficient protecting group-free synthetic strategy. Most of them, including compounds **7**−**11**, **13**, and **14**, exhibited potent antiproliferative effects against breast, pancreatic, and prostate cancer cells with low micromolar to submicromolar IC_{50} values as well as markedly enhanced aqueous solubility. These new analogues obtained by rationally modifying the natural product have been demonstrated not only to significantly induce the apoptosis and suppress growth of triple-negative MDA-MB-231 breast cancer both in vitro and in vivo but also effective against drug-resistant ER-positive MCF-7 clones.

INTRODUCTION

Natural products have a profound impact upon both chemical biology and drug discovery, and the great structural diversity of natural products with various interesting biological characteristics has always provided medicinal chemists an important source of inspiration in their search for new molecular entities with pharmacological activity.[1−3] Among them, natural tetracyclic diterpenoids, especially *ent*-kaurane diterpenoids with *exo*-methylene cyclopentanone in the D-ring such as oridonin (**1**), ponicidin, and phyllostachysin F (Figure 1), constitute an important class of natural products which exhibit considerable pharmacological activities including antitumor, antibacterial, antituberculosis, and anti-inflammatory effects.[4−7]

Isodon rubescens (Chinese name "Donglingcao") is an important source of a traditional Chinese herbal medicine that has been approved by State Food and Drug Administration of China for the treatment of inflammation such as acute tonsillitis, esophagitis, stomatitis, and gingivitis.[7,8a] It has also been widely used as herbal medicine in China for the treatment of esophageal and cardia cancer for many years.[8b] **1**, an *ent*-kaurane diterpenoid isolated from this herb, has been attracting a rising attention in recent years due to its extensive biological activities.[9−11] Of particular interest is its unique, safe, and remarkable anticancer pharmacological profile.[12] Increasing clinical evidence has suggested that oridonin may greatly improve the survival rates of cancer patients through hampering the progression of tumor, mitigating tumor burden, and alleviating cancer syndrome.[13] Because of the relatively low toxicity of **1**, it can be used for a long period of time without significant abdominal discomfort.[5] For instance, **1** was reported not only to induce typical mitochondrial apoptosis in acute myeloid leukemia cells at low micromolar concentration but also to exhibit significant antileukemia activities with a low side-effect in murine models (15 mg/kg).[14] Accumulating mechanistic studies have revealed that **1** possesses unique but versatile antiproliferative capabilities including cell cycle arrest, apoptosis, and autophagy by regulating a series of transcription factors, protein kinases, as well as pro- and/or antiapoptotic proteins.[15−25] All these findings support that oridonin

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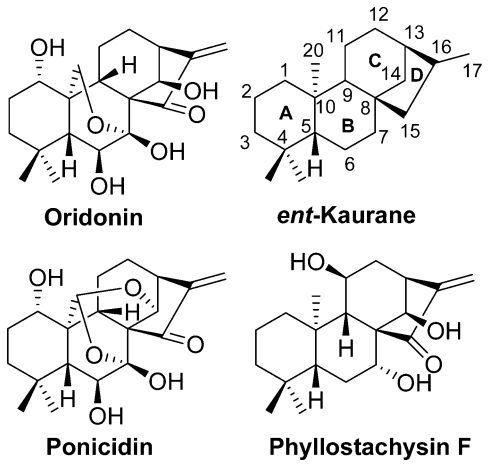


Figure 1. Structures of oridonin and other typical natural *ent*-kaurane diterpenoids.

represents a promising anticancer drug candidate with great potential for bench to bedside development. However, the clinical development of oridonin for cancer therapy has been hampered, to a large degree, by its relatively moderate potency, limited aqueous solubility, and bioavailability.[26,27] In addition, oridonin exerts only modest to poor inhibitory effects on highly aggressive cancer cells such as estrogen triple-negative breast cancer cell MDA-MB-231[28] and multiple drug resistant (MDR) breast cancer cells. All these observations combine to justify an urgent need for developing novel oridonin analogues by rationally modifying the natural product to improve the anticancer activity profile and drug-like properties including aqueous solubility.

The highly oxygenated oridonin, structurally belonging to 7,20-epoxy-*ent*-kaurane-type diterpenoid, is primarily characterized with its densely functionalized, stereochemistry-rich frameworks including an α,β-unsaturated ketone moiety in the D-ring and a 6-hydroxyl-7-hemiacetal group in the B-ring. Previous studies on the structure−activity relationship (SAR) of **1** indicated that the α-methylene cyclopentanone system in D-ring was an essential active center for the antitumor activity, and any destruction of this enone system (e.g., split ring or saturated methylene) could lead to a total loss of bioactivity.[9,29] Meanwhile, the presence of hydrogen-bonding between 6β-OH and ketone group at C-15 was beneficial for an enhanced antitumor activity of **1**.[30] To date, the reported structural modifications to generate new oridonin derivatives have been mainly focused on the 1-*O* and 14-*O* positions by introducing aqueous solubility-enhancing moieties via coupling ester appendages to its hydroxyl groups.[31−33] However, ester bonds usually suffer from poor metabolic stability in vivo due to the enzymatic hydrolysis[34,35] and such derivatives tend to act as prodrugs. Therefore, it is imperative to develop novel oridonin analogues with effective modifications to accelerate the search for promising anticancer drug candidates.

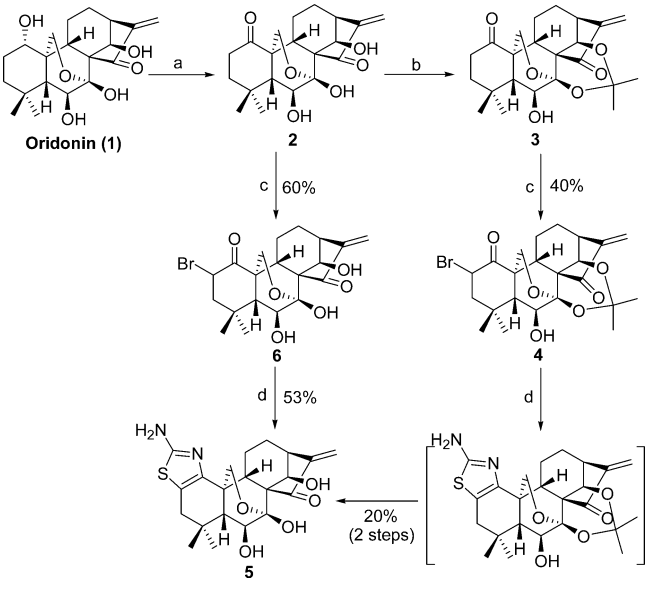
In the design of novel oridonin derivatives, our approach was guided by the idea of incorporating nitrogen into the core scaffold of oridonin that may enhance their aqueous solubility through the formation of salts with acids while retaining key pharmacophores of **1** such as the enone moiety. The thiazole ring system, a nitrogen-containing aromatic heterocycle, represents an important building block that exists in many biologically active natural products including clinically used anticancer drugs such as epothilones, ixabepilone, and bleomycin.[36−38] Modifications of the thiazole ring have proven highly effective with regards to improved potency and less toxicity.[39] Therefore, we have designed a series of nitrogen-enriched oridonin derivatives in which a thiazole ring is fused at C-1 and C-2 of the A-ring and envisioned these natural product-like molecules may possess better anticancer potency and aqueous solubility. Herein, we describe our efforts for the synthesis of these novel compounds through a protecting group-free semisynthetic approach and the discovery of promising anticancer agents in this endeavor. It is noteworthy that this work is the first attempt to generate new molecules with a nitrogen-containing heterocyclic scaffold that is fused with oridonin A-ring system.

RESULTS AND DISCUSSION

Chemistry. We took advantage of **1**, a naturally abundant and commercially available *ent*-kaurane diterpenoid, as a basic template to synthesize the thiazole-fused oridonin derivatives. To date, there is little evidence available for the pursuit of chemical transformations at the A-ring of **1**, probably due to its structural complexity. Given the prevalence of the successful application of Hantzsch synthesis of thiazoles[40] and amino-thiazoles[41] in other natural products, we were inspired to apply this reaction to the oridonin template for the synthesis of new derivatives with variously substituted thiazole-fused A-rings.

To explore a meaningful structure−activity relationship of substituents on the thiazole ring, we have designed a series of diversely substituted thiazole derivatives such as oridonin analogues **5**, **7**−**12**, and **14**−**15**, which were endowed with typical alkyl, amino, allylamino, alkyl-substituted amino, or amide groups. With the aim of further improving aqueous solubility, compounds **13** and **16** have been designed with additional hydrophilic moieties such as piperidinyl and amidinyl groups. As depicted in Scheme 1, following a literature procedure,[42] oxidation of **1** with Jones reagent in ice−water

Scheme 1. Synthesis of 2-Amino Thiazole-Fused Oridonin Derivative 5[a]



[a]Reagents and conditions: (a) Jones reagent, acetone, rt, 30 min, 82%; (b) $Me_2C(OMe)_2$, *p*-TsOH, acetone, rt, 2 h, 95%; (c) $PyHBr_3$, THF, 0 °C, 2 h; (d) thiourea, EtOH, reflux, 5 h.

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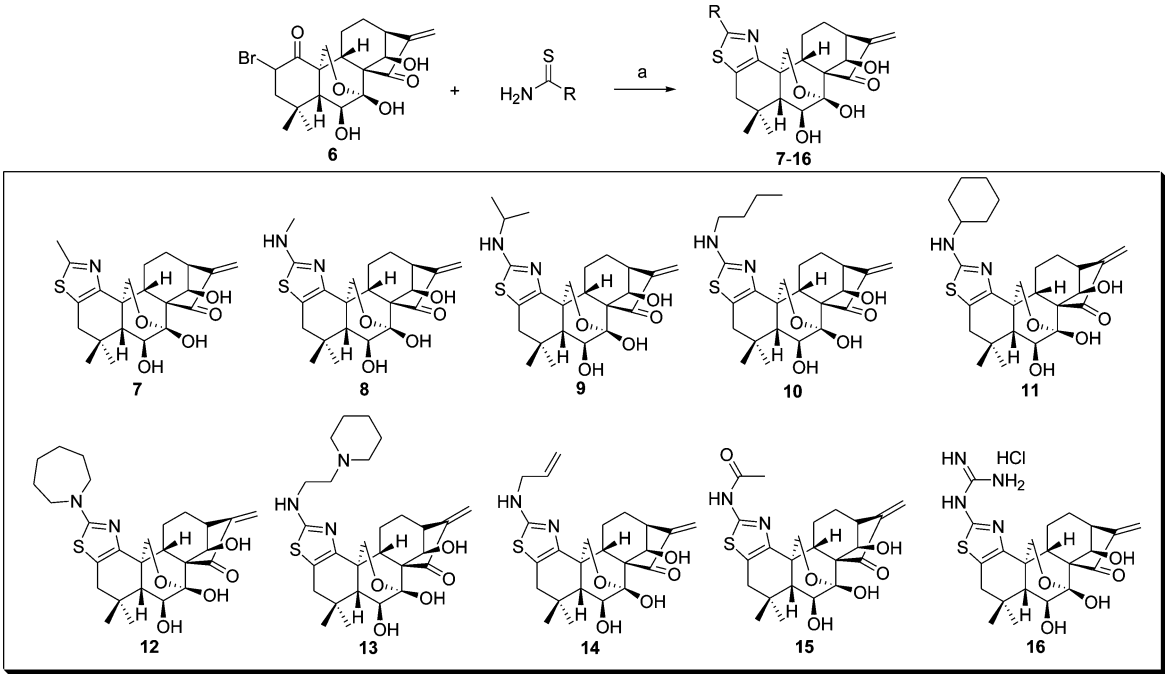
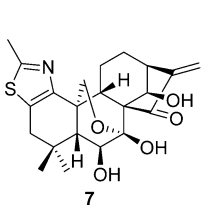
Scheme 2. Synthesis of 2-Substituted Thiazole-Fused Oridonin Derivatives 7−16[a]



[a]Reagents and conditions: (a) EtOH, reflux, 3−6 h, 35−65%.

Table 1. Effects of New Oridonin Analogues on Proliferation of Human Breast, Pancreatic Cancer, and Prostate Cancer Cells



5: $R^1 = R^2 = H$
8: $R^1 = H$, R^2 = methyl
9: $R^1 = H$, R^2 = isopropyl
10: $R^1 = H$, R^2 = butyl
11: $R^1 = H$, R^2 = cyclohexyl
12: R^1, R^2 = -$(CH_2)_6$-
13: $R^1 = H$, R^2 = 2-(piperidin-1-yl)ethyl
14: $R^1 = H$, R^2 = allyl
15: $R^1 = H$, R^2 = acetyl
16: $R^1 = H$, R^2 = amidinyl

	IC_{50} (μM)[a]				
	breast cancer		pancreatic cancer		prostate cancer
compd	MCF-7 (ER positive)	MDA-MB-231 (ER negative)	AsPC1	Panc-1	DU145
1	6.6	29.4	19.3	15.6	14.2
5	1.0	3.2	5.6	6.1	3.5
7	2.6	6.9	6.5	7.8	7.6
8	1.3	2.1	1.8	2.3	4.1
9	0.8	0.3	1.4	3.3	5.3
10	0.6	1.1	1.4	4.0	4.2
11	0.9	0.8	1.7	3.7	1.8
12	1.2	6.8	2.3	6.3	4.7
13	1.0	1.8	1.1	1.5	1.2
14	0.2	0.2	1.1	1.1	1.2
15	2.0	6.8	4.8	6.7	6.3
16	3.4	>10[b]	>10	>10	>10

[a]Breast cancer cell lines: MCF-7 and MDA-MB-231. Pancreatic cancer cell lines: AsPC1 and Panc-1. Prostate cancer cell line: DU145. Software: MasterPlex ReaderFit 2010, MiraiBio, Inc. The values are the mean ± SE of at least three independent experiments. [b]If a specific compound is given a value >10, it indicates that a specific IC_{50} cannot be calculated from the data points collected, meaning "no effect".

bath selectively afforded 1-oxo-oridonin derivative **2** in 82% yield. Initially, taking into account of the oridonin's delicate and complex template, particularly the reactive hydroxyl functional groups, it was thought important to elaborate a protecting group strategy that would avoid possible side reactions during the subsequent steps. Thus, protection of 7,14-dihydroxyl of **2** with 2,2-dimethoxypropane catalyzed by *p*-TsOH in acetone solely provided cyclic ketal **3** in 95% yield. However, bromination of **3** with NBS in the refluxing CCl_4 resulted in a complex mixture. Alternatively, $PyHBr_3$ was chosen as the bromination agent to react with **3** in THF at 0 °C to give intermediate **4** as a major product in 40% yield. Hantzsch

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reaction of **4** with thiourea in the refluxing ethanol directly afforded the target oridonin derivative **5** in 20% yield, in which cyclic ketal was simultaneously removed due to the presence of HBr generated from the thiazole formation. Nevertheless, the total yield of this synthetic route was only 6.2%, which was too low to gain sufficient amount of **5** for further pharmacological evaluation. Considering that cyclic ketal could be easily deprotected in Hantzsch thiazole synthesis, we attempted a protecting group-free synthetic strategy to improve the total yield. Thus, intermediate **2** without any protecting groups was directly utilized to react with $PyHBr_3$ at 0 °C. To our delight, the desired intermediate **6** was obtained in 60% yield, and the subsequent Hantzsch reaction of **6** with thiourea yielded the desired compound **5** in a much better yield of 53%, suggesting that the protection of 7,14-dihydroxyl of **2** was unnecessary. Therefore, a concise protecting group-free synthetic approach to readily access **5** has been established through three steps in 26% total yield. To examine the antitumor effects of substituents on a thiazole ring, a series of novel oridonin derivatives with diversely substituted thiazole scaffolds were generated in 35−65% yields using the Hantzsch reaction of common building block **6** with thioacetamide or *N*-substituted thioureas (Scheme 2).

In Vitro Antiproliferative Activity. The growth inhibitory potency of synthesized novel oridonin derivatives was evaluated in two breast cancer cell lines MCF-7 (ER-positive) and MDA-MB-231 (ER-negative and triple-negative), two pancreatic cancer cell lines AsPC1 and Panc-1, as well as one prostate cancer cell line DU145 using MTT assays as described in the in vitro screening protocol (Experimental Section). The ability of these new analogues to inhibit the growth of cancer cells was summarized in Table 1. **1** was tested for comparison. The results showed that most of newly synthesized thiazole-fused oridonin derivatives (**5**, **7**−**15**) not only exhibited significantly improved antiproliferative activity against breast cancer cell MCF-7 relative to **1** but also displayed marked growth inhibitory effects on other tested cancer cell lines including highly invasive breast cancer MDA-MB-231 cells, for which **1** had only modest activity with an average IC_{50} value greater than 19 μM. As shown in Table 1, 2-aminothiazole derivative **5** exerted 2−9-fold more potent antitumor activity than **1** against all tested cancer cells, indicating that introduction of thiazole at C-1/C-2 of oridonin A-ring is tolerable. Analogue **7** with a methyl instead of free amino group at C-2 of thiazole ring led to a 2-fold decreased activity against breast and prostate cancer cells compared with **5**, suggesting that subsequent optimization of the substituents on the thiazole ring may tune their antiproliferative effects. In general, further substitution on the primary amine with various alkyl groups was found to significantly increase antiproliferative activities against all tested cancer cell lines. For example, most of the *N*-alkyl substituted derivatives including **8**−**11** and **13**−**14** displayed potent activity against breast cancer cells, with IC_{50} values varying from the low micromolar to submicromolar range. Particularly, compound **14** with an *N*-allyl substituted thiazole moiety exhibited the most potent antiproliferative activity against both MCF-7 and MDA-MB-231 cells with the same IC_{50} values of 0.2 μM, which are approximately 33-fold and 147-fold more potent than **1**, respectively. It is noteworthy to mention that *N*-methyl analogue **8** with low micromolar potency against all the tested cancer lines was found to display an excellent dose−response relationship. Compound **15** with an acetamide group on the thiazole showed a slightly lower antiproliferative activity than **5**. Interestingly, analogue **16** with a guanidinyl group on the thiazole only displayed a potent activity against MCF-7 cell line but moderate to low activity against other tested cell lines.

In Vitro Growth Inhibitory Activity in Drug-Resistant Breast Cancer Cells. Resistance to chemotherapy is a major cause of the ultimate failure of breast cancer treatment. To investigate whether these thiazole-fused oridonin analogues are still effective on drug-resistant breast cancer cells, compounds **7**, **8**, and **14** with different substituted thiazole moieties, as well as **1**, were selected to evaluate their growth inhibitory activity against adriamycin (ADR)-resistant MCF-7 clone at the dosages of 1.0, 5.0, and 10.0 μM using MTT assays. As shown in Figure 2, compound **1** displayed no growth inhibitory activity at all concentrations, while new analogues **7** and **8** have been found to significantly inhibit the growth of drug-resistant MCF-7 clone, and their growth inhibitory rates were even greater than 50% at 5.0 and 10.0 μM, respectively. Particularly, compound **14** exhibited the most potent antiproliferative activity against MCF-7/ADR cells with an IC_{50} value less than 1 μM.

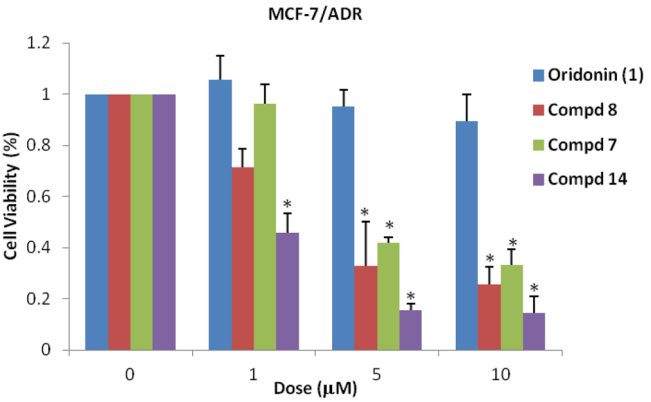

Figure 2. Growth inhibitory effect of **1**, **7**, **8**, and **14** against drug-resistant breast cancer cells. Adrimycin-resistant MCF-7/ADR clone was treated with variable concentrations of **1**, **7**, **8**, and **14** (1.0, 5.0, and 10.0 μM), respectively, for 48 h. The values are the mean ± SE of at least three independent experiments. * represents $p < 0.05$, comparing to the effect from oridonin at the same dosage.

Aqueous Solubility. To examine whether the synthesized thiazole-fused analogues have better aqueous solubility than **1**, a previously reported HPLC method[43] was employed to measure the solubility of several selected analogues such as **7** (CYD0619), **8** (CYD0554), and **14** (CYD0618). One-point calibration was performed against standards with known concentrations of the sample compounds to determine concentrations of the indicated compounds in samples. As expected, incorporating a substituted thiazole-fused moiety into oridonin not only enhanced the antiproliferative activity but also significantly improved their aqueous solubility. For instance, aqueous solubility of analogue **7** with 2-methyl thiazole moiety was determined to be 4.47 mg/mL, and the *N*-alkyl derivatives **8** and **14** in the form of HCl salt demonstrated an excellent solubility, with a saturated concentration of 42.4 and 81.2 mg/mL, respectively, indicating approximately 32-fold to 62-fold improvement in comparison with that of oridonin (1.29 mg/mL) (Figure 3). Some other analogues such as compounds **13** and **16** (in the HCl salt form) possess an even superior aqueous solubility greater than 100 mg/mL.

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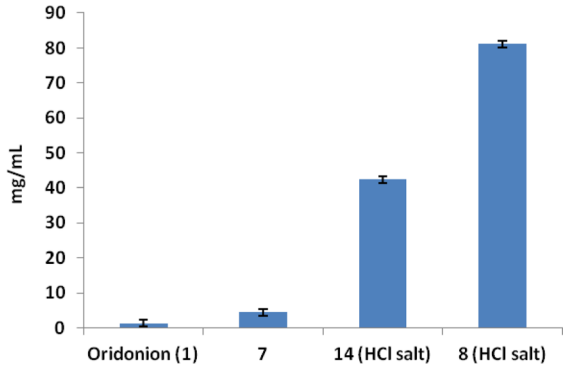

Figure 3. Aqueous solubility of novel oridonin analogues. Compounds **7** (CYD0619), **14** (CYD0618), and **8** (CYD0554) showed significantly improved solubility compared with **1**. Compounds **14** (HCl salt) and **8** (HCl salt) are soluble in water with saturated concentrations of 42.4 and 81.2 mg/mL, respectively, which are approximately 32- and 62-fold better than that of **1** (1.29 mg/mL). The values are the mean ± SE of at least three independent experiments.

Compound 14 Induced Apoptosis of Breast Cancer Cells. On the basis of the antiproliferative data, the most potent compound **14** was selected for further mechanistic studies to determine whether the growth inhibition induced by **14** in human breast cells was attributed to apoptosis. MDA-MB-231 and MCF-7/ADR cells were not only treated with vehicle alone as controls but also dealt with **14** at different concentrations (0.25, 0.5, or 1.0 μM) for 48 h and stained with FITC-Annexin V and propidium iodide (PI). The percentages of apoptotic MDA-MB-231 cells were determined by flow cytometry. As shown in Figure 4, compound **14** displayed marked effects to induce apoptosis of breast cancer cells in a dose-dependent manner. Treatment of the MDA-MB-231 cells with 0.25, 0.5, and 1.0 μM of compound **14** for 48 h resulted in 21.2% ± 1.1, 41.3% ± 16.3, and 61.7% ± 3.7 of apoptotic cells (early and late apoptosis), respectively, as compared to 6.1% ± 2.0 in an untreated vehicle control. Similarly, treatment of MCF-7/ADR cells with compound **14** also led to 10.4% ± 3.0, 23.1% ± 7.6, and 78.2% ± 2.5 of apoptotic cells at the same three concentrations as above, respectively. Apparently, compound **14** mediated apoptosis of MDA-MB-231 and MCF-7/ADR cells, at least in part, contributes to its antiproliferative effects.

Compound 14 Regulated Apoptotic Related Proteins. Previous studies have demonstrated that **1** induces apoptosis of cancer cells by regulating a series of transcription factors, protein kinases, as well as pro- and/or antiapoptotic proteins

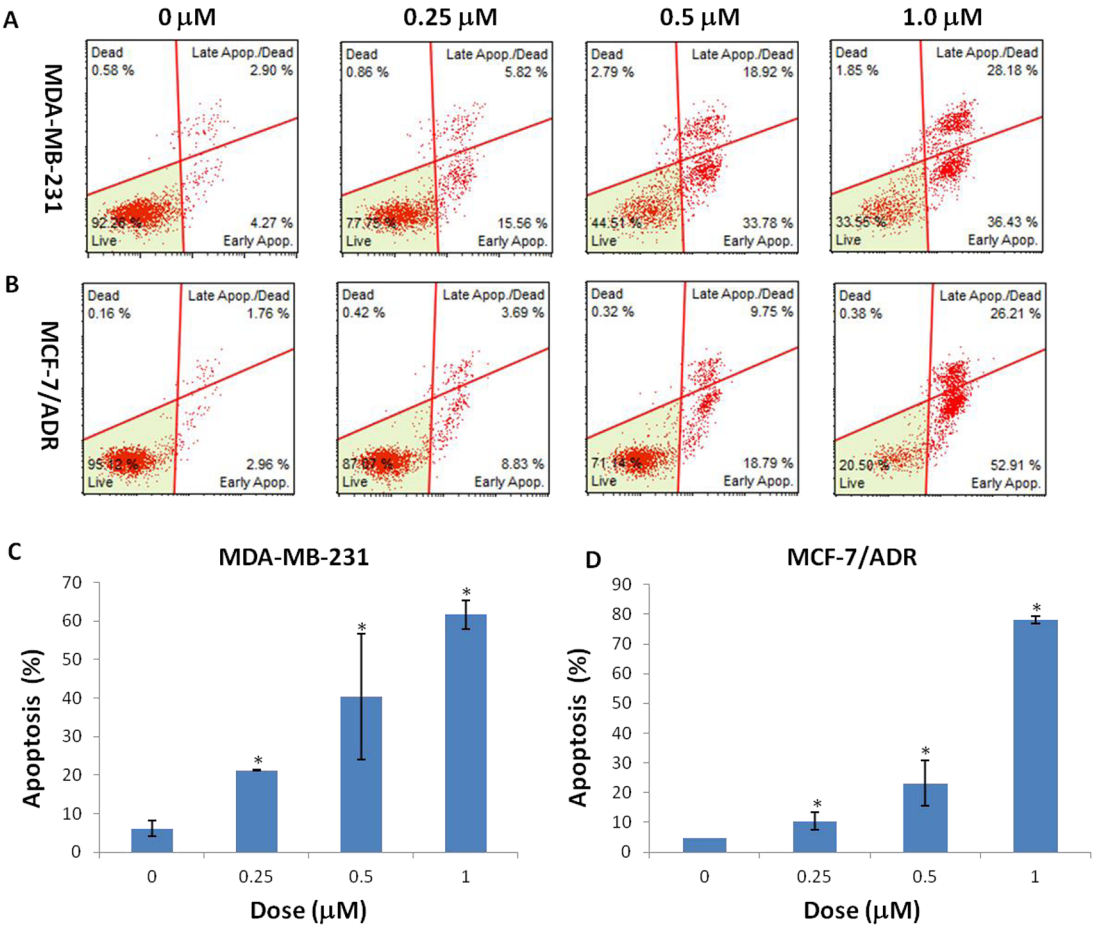

Figure 4. Induction of apoptosis on MDA-MB-231 and MCF-7/ADR cells by compound **14**. (A) Flow cytometry analysis of apoptotic MDA-MB-231 cells induced by **14** at different concentrations. (B) Flow cytometry analysis of apoptotic MCF-7/ADR cells induced by **14** at different concentrations. (C) Apoptotic ratio of different concentrations of **14** in MDA-MB-231 cells. (D) Apoptotic ratio of different concentrations of **14** in MCF-7/ADR cells. Cells were treated with vehicle or **14** at 0.25, 0.5, and 1.0 μM concentrations, respectively, for 48 h. The values are means ± SE of at least three independent experiments. * represents $p < 0.05$ comparing to vehicle-treated control.

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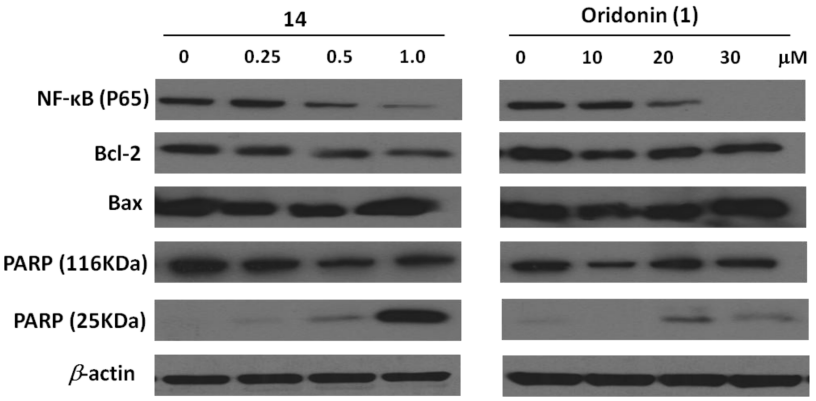

Figure 5. Western blot analysis of biological markers for apoptosis induction by compound **14** (CYD0618) and **1** in the MDA-MB-231 cells at different concentrations (48 h).

such as NF-κB,[23,25b,28] MAPK,[15,16] Bax, and Bcl-2.[17,19] To elucidate the potential mechanisms contributed to apoptosis induction by the new derivative **14**, several proteins related to apoptosis were determined by Western blotting. As shown in Figure 5, treatment of MDA-MB-231 cells with compound **14** at low concentrations (0.25−1.0 μM) dose dependently led to the down-regulation of antiapoptotic protein Bcl-2 levels and the up-regulation of the pro-apoptotic protein Bax. In addition, it also induced a significant decrease of NF-κB (p65) protein expression, suggesting that NF-κB inhibition might contribute to the reduction of Bcl-2/Bax ratio. Meanwhile, compound **14** also triggered PARP cleavage from its full-length form (116 kDa) to the cleaved form (25 kDa), as indicated by PARP fragments appearance in a dose-dependent manner, which could be viewed as a marker of apoptosis. Similarly, exposure to the high dosages of **1** (10−30 μM) also led to down-regulation of NF-κB (p65), Bcl-2 and PARP (116 kDa) and up-regulation of Bax and cleaved PARP (25 KDa). These preliminary data indicated that the thiazole derivative **14** might mediate the apoptosis in MDA-MB-231 cells at low concentrations through similar multiple apoptotic pathways to those of **1**. Other than apoptosis, oridonin has also been found to inhibit tumor cell proliferation and induces cancer cell death though cell cycle arrest,[17,20,28] autophagy,[23−25] and necrosis.[15] Therefore, more extensive mechanism studies on the new derivative **14** are ongoing, and the results will be reported in due course.

Compound 14 Suppressed Growth of Xenograft Tumors in Nude Mice. In our pilot in vivo studies, analogue **14** was further evaluated for its anticancer activity in suppression of tumor growth in the triple-negative breast cancer MDA-MB-231 xenograft model. As shown in Figure 6, mice treated with 5.0 mg/kg of compound **14** via ip showed a much better effect in inhibiting tumor growth as compared to the mice treated with the same dose of oridonin ($p < 0.0001$). Meanwhile, compound **14** was found to be tolerated during the experiments and showed no significant loss of body weight (data not shown). These findings suggest that compound **14** (CYD0618) is a promising anticancer drug candidate with potent antitumor activity and excellent aqueous solubility for further clinic development.

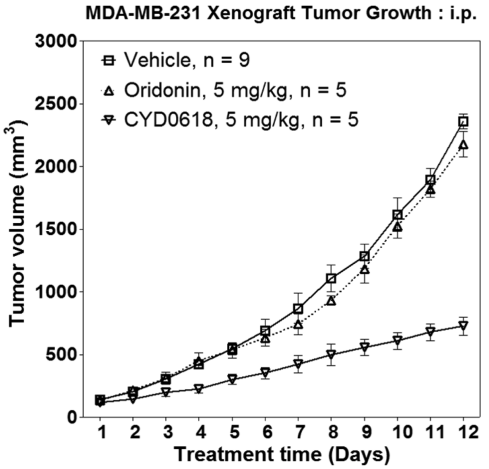

Figure 6. In vivo efficacy of **1** and compound **14** (CYD0618) in inhibiting growth of xenograft tumors (breast cancer MDA-MB-231) in mice (ip) at the dose of 5 mg/kg, respectively. Data are presented as the mean ± SE of tumor volume at each time point. Significant differences between compound **14** treatment group, oridonin treatment group, and control were determined using one way ANOVA. $p < 0.0001$.

CONCLUSIONS

For the first time, an efficient and concise protecting group-free synthetic approach has been established to readily access a series of novel thiazole-fused oridonin analogues starting from the natural product oridonin. In vitro pharmacological studies demonstrated that most of these new molecules not only exhibited significantly enhanced antiproliferative activity against breast cancer MCF-7 clone relative to **1** but also displayed marked growth inhibitory effects on the other oridonin-insensitive cancer cell lines including highly invasive triple-negative breast cancer MDA-MB-231 cells with low micromolar to submicromolar IC_{50} values. Particularly, compound **14** with an *N*-allyl substituted thiazole moiety exhibited the most potent antiproliferative activity against both MCF-7 and MDA-MB-231 cells with the IC_{50} values of 0.2 μM, which are approximately 33-fold and 147-fold more potent than **1**, respectively. Meanwhile, these new analogues such as **7**, **8**, and **14** with diversely substituted thiazole moieties remained to be effective against adriamycin-resistant MCF-7 clone, for which **1** displayed no effect at the same doses. Moreover, they also have significantly improved aqueous solubility in comparison with that of the natural product oridonin. In our pilot mechanism studies, compound **14** was found to significantly induce apoptosis of MDA-MB-231 and MCF-7/ADR cells at low concentrations in a dose-dependent manner and likely mediate apoptosis through similar multiple pathways to those of **1**. In nude mice bearing breast tumor xenografts, compound **14** at 5

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mg/kg significantly suppressed MDA-MB-231 xenograft tumor growth in vivo and was found more efficacious than oridonin. These new molecules with a nitrogen-containing heterocyclic scaffold that is fused with oridonin ring system open new avenues to explore the therapeutic potential of oridonin-based derivatives and develop promising natural product-like drug candidates for the treatment of cancer.

EXPERIMENTAL SECTION

General. All commercially available starting materials and solvents were reagent grade and used without further purification. Reactions were performed under a nitrogen atmosphere in dry glassware with magnetic stirring. Preparative column chromatography was performed using silica gel 60, particle size 0.063−0.200 mm (70−230 mesh, flash). Analytical TLC was carried out employing silica gel 60 F254 plates (Merck, Darmstadt). Visualization of the developed chromatograms was performed with detection by UV (254 nm). NMR spectra were recorded on a Bruker-600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with TMS as an internal reference. Chemical shifts downfield from TMS were expressed in ppm, and *J* values were given in Hz. High-resolution mass spectra (HRMS) were obtained from Thermo Fisher LTQ Orbitrap Elite mass spectrometer. Parameters include the following: nano ESI spray voltage was 1.8 kV, capillary temperature was 275 °C, and the resolution was 60000; ionization was achieved by positive mode. Melting points were measured on a Thermo Scientific Electrothermal digital melting point apparatus and uncorrected. Purity of final compounds was determined by analytical HPLC, which was carried out on a Shimadzu HPLC system (model: CBM-20A LC-20AD SPD-20A UV/vis). HPLC analysis conditions: Waters μBondapak C18 (300 mm × 3.9 mm), flow rate 0.5 mL/min, UV detection at 270 and 254 nm, linear gradient from 30% acetonitrile in water (0.1% TFA) to 100% acetonitrile (0.1% TFA) in 20 min, followed by 30 min of the last-named solvent. All biologically evaluated compounds are >95% pure.

(4a*R*,5*S*,6*S*,6a*R*,9*S*,11a*S*,11b*S*,14*R*)-5,6,14-Trihydroxy-4,4-dimethyl-8-methylenedecahydro-1*H*-6,11b-(epoxymethano)-6a,9-methanocyclohepta[*a*]naphthalene-1,7(8*H*)-dione (2). To a stirring solution of oridonin (500 mg, 1.37 mmol) in acetone (40 mL) was added Jones reagent (0.6 mL) dropwise at ice−water bath. The resulting mixture was stirred at 0 °C for 20 min, and isopropyl alcohol was added to quench excess Jones reagent. Then the mixture was diluted with water and extracted with dichloromethane. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated to give a solid crude product. The crude residue was recrystallized from acetone−hexane to give **2** as a white solid (410 mg, 82%); mp 219−220 °C (Lit.[42] mp 219−221 °C). ^{1}H NMR (600 MHz, $(CD_3)_2CO$): δ 6.52 (br s, 1H), 6.10 (s, 1H), 5.62 (s, 1H), 5.41 (d, 1H, *J* = 10.8 Hz), 5.24 (s, 1H), 4.91 (s, 1H), 4.22 (d, 1H, *J* = 10.2 Hz), 3.92 (d, 1H, *J* = 10.8 Hz), 3.69 (m, 1H), 3.31 (br s, 1H), 3.01 (d, 1H, *J* = 9.6 Hz), 2.76 (m, 5H), 2.46 (m, 1H), 2.36 (m, 1H), 2.19 (m, 1H), 1.92 (m, 3H), 1.68 (m, 1H), 1.61 (m, 1H), 1.19 (m, 1H), 1.14 (s, 3H), 0.97 (s, 3H).

(4a*R*,5*S*,6*S*,6a*R*,9*S*,11a*S*,11b*S*,14*R*)-2-Bromo-5,6,14-trihydroxy-4,4-dimethyl-8-methylenedecahydro-1*H*-6,11b-(epoxymethano)-6a,9-methanocyclohepta[*a*]naphthalene-1,7(8*H*)-dione (6). To a solution of **2** (100 mg, 0.27 mmol) in THF (4 mL) was added $PyHBr_3$ (88 mg, 0.27 mmol) at rt. The reaction mixture was stirred at rt for 4 h and then poured into water and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give an oily residue. The residue was further purified by silica gel column; elution with 50% EtOAc in hexane afforded the desired product **6** (80 mg, 66%) as a colorless amorphous gel and a mixture of two isomers. Major isomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.26 (s, 1H), 6.09 (d, 1H, *J* = 11.4 Hz), 6.00 (br s, 1H), 5.65 (s, 1H), 4.91 (s, 1H), 4.72 (br s, 1H), 4.31 (m, 2H), 3.97 (d, 1H, *J* = 10.8 Hz), 3.80 (m, 1H), 3.08 (d, 1H, *J* = 9.0 Hz), 2.59 (dd, 1H, *J* = 4.8 Hz, 13.2 Hz), 2.24 (d, 1H, *J* = 8.4 Hz), 2.12 (m, 1H), 1.90 (m, 1H), 1.65 (m, 1H), 1.43 (m, 1H), 1.21 (s, 3H), 0.98 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.4, 202.6, 150.6, 122.3, 98.0, 72.9, 72.2, 65.6, 61.7, 58.1, 52.2, 49.8, 48.4, 45.1, 42.7, 33.8, 29.8, 29.4, 22.3, 18.1. Minor isomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.26 (s, 1H), 5.98 (d, 1H, *J* = 12.0 Hz), 6.00 (br s, 1H), 5.66 (s, 1H), 4.87 (s, 1H), 4.80 (m, 1H), 4.39 (d, 1H, *J* = 10.8 Hz4), 4.06 (d, 1H, *J* = 10.8 Hz), 3.80 (m, 1H), 3.08 (d, 1H, *J* = 9.0 Hz), 2.67 (m, 1H), 2.36 (d, 1H, *J* = 5.4 Hz), 2.33 (d, 1H, *J* = 5.4 Hz), 2.12 (m, 1H), 1.90 (m, 1H), 1.65 (m, 1H), 1.25 (m, 1H), 1.22 (s, 3H), 1.05 (m, 1H), 1.04 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.2, 202.6, 150.5, 122.6, 98.0, 72.9, 71.9, 64.5, 61.2, 58.7, 51.4, 49.8, 48.9, 45.1, 42.6, 34.8, 30.4, 29.3, 24.9, 18.9. HRMS (ESI) calcd for $C_{20}H_{26}BrO_6$ $[M + H]^+$ 441.0907; found 441.0909.

(5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-2-Amino-6,7,15-trihydroxy-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta-[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (5). To a solution of **6** (50 mg, 0.11 mmol) in ethanol (4 mL) was added thiourea (12 mg, 0.16 mmol) at rt. The reaction mixture was heated under reflux for 3 h. After cooling and basifying with saturated $NaHCO_3$ aqueous solution, the mixture was concentrated in vacuo to give an oily residue. The residue was purified by silica gel column; elution with 50% EtOAc in hexane afforded the desired product **5** (25 mg, 53%) as an amorphous gel; $[\alpha]_D^{25}$ +190 (*c* 0.10, $CHCl_3$); HPLC purity 97.0% (t_R = 9.0 min). ^{1}H NMR (600 MHz, $CDCl_3/CD_3OD$ = 5:1): δ 6.16 (s, 1H), 5.59 (s, 1H), 5.01 (s, 1H), 4.37 (d, 1H, *J* = 10.2), 3.96 (d, 1H, *J* = 9.6 Hz), 3.80 (d, 1H, *J* = 3.0 Hz), 3.32 (s, 1H), 3.01 (d, 1H, *J* = 3.6 Hz), 2.52 (m, 2H), 2.31 (d, 1H, *J* = 15.6 Hz), 2.12 (m, 1H), 1.94 (dd, 1H, *J* = 4.8 Hz, 13.8 Hz), 1.83 (m, 1H), 1.69 (d, 1H, *J* = 9.0 Hz), 1.58 (m, 1H), 1.25 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3/CD_3OD$ = 5:1): δ 206.8, 166.0, 151.4, 141.7, 120.4, 120.2, 97.5, 72.7, 72.5, 64.9, 61.9, 59.1, 53.1, 43.2, 40.5, 38.6, 34.6, 30.2, 29.8, 20.2, 20.1. HRMS (ESI) calcd for $C_{21}H_{26}N_2O_5S$ $[M + H]^+$ 419.1635; found 419.1638.

(6*S*,7*S*,7a*R*,10*R*,12b*R*,15*R*)-6,7,15-Trihydroxy-2,5,5-trimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]-thiazol-8(9*H*)-one (7). Compound **7** (16 mg) was prepared in 35% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +184 (*c* 0.10, $CHCl_3$); HPLC purity 97.6% (t_R = 12.4 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.18 (s, 1H), 5.81 (d, 1H, *J* = 12.6 Hz), 5.57 (d, 1H, *J* = 0.6 Hz), 5.24 (br s, 1H), 5.16 (br s, 1H), 4.96 (d, 1H, *J* = 1.2 Hz), 4.72 (s, 1H), 4.36 (dd, 1H, *J* = 0.6 Hz, 10.2 Hz), 4.08 (dd, 1H, *J* = 10.2 Hz), 3.79 (m, 1H), 3.03 (d, 1H, *J* = 9.6 Hz), 2.45 (m, 1H), 2.25 (d, 1H, *J* = 15.0 Hz), 2.04 (d, 1H, *J* = 14.4 Hz), 1.99 (m, 1H), 1.92 (s, 3H), 1.66 (d, 1H, *J* = 3.6 Hz), 1.59 (m, 2H), 1.21 (s, 3H), 1.00 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 192.7, 162.0, 151.6, 121.3, 100.6, 98.1, 73.2, 72.0, 66.5, 62.4, 58.4, 51.6, 46.4 (2C), 42.5, 32.9, 30.5, 30.2, 21.7, 20.7, 20.1. HRMS (ESI) calcd for $C_{22}H_{27}NO_5S$ $[M + H]^+$ 418.1683; found 418.1685.

(5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-6,7,15-Trihydroxy-5,5-dimethyl-2-(methylamino)-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (8). Compound **8** (30 mg) was prepared in 65% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +146 (*c* 0.10, $CHCl_3$); HPLC purity 95.6% (t_R = 15.7 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.80 (br s, 1H), 6.17 (s, 1H), 5.97 (d, 1H, *J* = 12.0 Hz), 5.80 (br s, 1H), 5.56 (s, 1H), 5.03 (br s, 2H), 4.65 (d, 1H, *J* = 10.2 Hz), 3.87 (d, 1H, *J* = 10.2 Hz), 3.77 (dd, 1H, *J* = 9.6 Hz, 12.0 Hz), 3.05 (d, 1H, *J* = 9.0 Hz), 2.86 (s, 3H), 2.50 (m, 2H), 2.32 (d, 1H, *J* = 16.2 Hz), 2.15 (m, 1H), 2.04 (s, 3H), 1.88 (m, 1H), 1.82 (m, 1H), 1.69 (d, 1H, *J* = 9.0 Hz), 1.53 (m, 1H), 1.22 (s, 3H), 0.93 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 168.5, 151.9, 143.1, 120.9, 118.8, 97.9, 73.5, 72.1, 65.1, 62.6, 58.0, 53.2, 42.7, 41.2, 39.0, 35.0, 32.3, 30.5, 30.2, 20.9, 20.4. HRMS (ESI) calcd for $C_{22}H_{28}N_2O_5S$ $[M + H]^+$ 433.1792; found 433.1795.

(5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-6,7,15-Trihydroxy-2-(isopropylamino)-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-

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one (9). Compound **9** (20 mg) was prepared in 44% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +161 (*c* 0.10, $CHCl_3$); HPLC purity 98.0% (t_R = 12.3 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.64 (br s, 1H), 6.17 (s, 1H), 5.93 (d, 1H, *J* = 12.6 Hz), 5.56 (s, 2H), 5.02 (s, 2H), 4.64 (d, 1H, *J* = 10.2 Hz), 3.89 (d, 1H, *J* = 10.2 Hz), 3.79 (dd, 1H, *J* = 9.6 Hz), 3.47 (m, 1H), 3.06 (d, 1H, *J* = 9.6 Hz), 2.50 (m, 2H), 2.30 (d, 1H, *J* = 16.2 Hz), 2.15 (m, 1H), 1.88 (m, 1H), 1.83 (m, 1H), 1.69 (d, 1H, *J* = 9.6 Hz), 1.53 (m, 1H), 1.25 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H), 0.95 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 166.3, 151.9, 142.6, 120.9, 118.4, 97.9, 73.5, 72.1, 65.1, 62.6, 58.0, 53.3, 48.2, 42.7, 41.2, 39.0, 35.0, 30.6, 30.2, 22.8, 22.6, 21.0, 20.5. HRMS (ESI) calcd for $C_{24}H_{32}N_2O_5S$ $[M + H]^+$ 461.2105; found 461.2111.

(5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-2-(Butylamino)-6,7,15-trihydroxy-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (10). Compound **10** (22 mg) was prepared in 48% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +142 (*c* 0.10, $CHCl_3$); HPLC purity 98.0% (t_R = 13.7 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.80 (br s, 1H), 6.17 (s, 1H), 5.94 (d, 1H, *J* = 12.0 Hz), 5.74 (br s, 1H), 5.56 (s, 1H), 5.02 (s, 1H), 4.64 (d, 1H, *J* = 10.2 Hz), 3.88 (d, 1H, *J* = 10.2 Hz), 3.78 (m, 1H), 3.10 (d, 1H, *J* = 4.2 Hz), 3.05 (d, 1H, *J* = 9.0 Hz), 2.50 (m, 2H), 2.31 (d, 1H, *J* = 16.2 Hz), 2.15 (m, 1H), 1.88 (dd, 1H, *J* = 4.8 Hz, 13.8 Hz), 1.81 (m, 1H), 1.69 (d, 1H, *J* = 9.6 Hz), 1.61 (m, 2H), 1.54 (m, 1H), 1.39 (m, 2H), 1.24 (s, 3H), 0.93 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.8, 167.6, 151.9, 142.8, 120.9, 118.4, 97.9, 73.5, 72.1, 65.1, 62.6, 58.1, 53.3, 46.2, 42.7, 41.2, 39.0, 35.0, 31.3, 30.5, 30.2, 20.9, 20.5, 20.1, 13.8. HRMS (ESI) calcd for $C_{25}H_{34}N_2O_5S$ $[M + H]^+$ 475.2261; found 475.2264.

(6*S*,7*S*,7a*R*,10*R*,12b*R*,15*R*)-2-(Cyclohexylamino)-6,7,15-trihydroxy-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta-[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (11). Compound **11** (24 mg) was prepared in 52% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]^{25}_D$ +132 (*c* 0.10, $CHCl_3$); HPLC purity 98.9% (t_R = 22.2 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.84 (br s, 1H), 6.19 (s, 1H), 5.95 (d, 1H, *J* = 12.0 Hz), 5.74 (br s, 1H), 5.58 (s, 1H), 5.04 (s, 2H), 4.67 (m, 1H), 3.89 (d, 1H, *J* = 10.2 Hz), 3.80 (m, 1H), 3.08 (d, 2H, *J* = 9.0 Hz), 2.52 (m, 2H), 2.31 (d, 1H, *J* = 9.6 Hz), 2.19 (m, 1H), 2.07 (m, 1H), 2.02 (m, 1H), 1.89 (dd, 1H, *J* = 4.8 Hz, 13.8 Hz), 1.80 (m, 2H), 1.71 (d, 1H, *J* = 9.0 Hz), 1.65 (d, 1H, *J* = 12.6 Hz), 1.56 (m, 1H), 1.28 (m, 6H), 1.27 (s, 3H), 0.97 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 166.5, 152.0, 142.6, 120.8, 118.1, 97.9, 73.5, 72.1, 65.0, 62.6, 58.1, 55.7, 53.3, 42.7, 41.2, 39.1, 35.0, 32.9 (2C), 30.5, 30.2, 29.6, 25.5, 25.0, 21.0, 20.4. HRMS (ESI) calcd for $C_{27}H_{36}N_2O_5S$ $[M + H]^+$ 501.2418; found 501.2423.

(6*S*,7*S*,7a*R*,10*R*,12b*R*,15*R*)-2-(Azepan-1-yl)-6,7,15-trihydroxy-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta-[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (12). Compound **12** (19 mg) was prepared in 41% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +148 (*c* 0.10, $CHCl_3$); HPLC purity 97.8% (t_R = 24.3 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.17 (s, 1H), 6.05 (d, 1H, *J* = 12.0 Hz), 5.56 (s, 1H), 5.18 (br s, 1H), 5.01 (d, 1H, *J* = 1.2 Hz), 4.71 (s, 1H), 4.44 (dd, 1H, *J* = 1.2 Hz, 10.2 Hz), 4.00 (dd, 1H, *J* = 10.2 Hz), 3.86 (dd, 1H, *J* = 9.0 Hz, 12.0 Hz), 3.49 (m, 2H), 3.39 (m, 2H), 3.04 (d, 1H, *J* = 9.6 Hz), 3.48 (m, 2H), 2.32 (d, 1H, *J* = 15.6 Hz), 2.05 (m, 2H), 1.91 (m, 1H), 1.72 (m, 6H), 1.55 (m, 5H), 1.25 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 166.3, 151.7, 143.3, 121.1, 117.6, 97.8, 73.6, 72.2, 65.8, 62.7, 57.6, 53.2, 50.2 (2C), 42.7, 40.9, 38.8, 35.0, 30.4, 29.6, 27.9 (2C), 27.7 (2C), 21.1, 20.2. HRMS (ESI) calcd for $C_{27}H_{36}N_2O_5S$ $[M + H]^+$ 501.2418; found 501.2422.

(6*S*,7*S*,7a*R*,10*R*,12b*R*,15*R*)-6,7,15-Trihydroxy-5,5-dimethyl-9-methylene-2-((2-(piperidin-1-yl)ethyl)amino)-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (13). Compound **13** (24 mg) was prepared in 51% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +138 (*c* 0.10, $CHCl_3$); HPLC purity 98.5% (t_R = 10.5 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.18 (s, 1H), 6.06 (d, 1H, *J* = 12.0 Hz), 5.74 (br s, 1H), 5.57 (s, 1H), 5.03 (s, 1H), 4.47 (d, 1H, *J* = 10.2 Hz), 3.99 (d, 1H, *J* = 10.2 Hz), 3.85 (dd, 1H, *J* = 9.0 Hz, 12.0 Hz), 3.26 (t, 2H, *J* = 5.4 Hz), 3.05 (d, 1H, *J* = 9.6 Hz), 2.57 (m, 2H), 2.48 (m, 6H), 2.33 (d, 1H, *J* = 15.6 Hz), 2.14 (m, 1H), 1.92 (m, 2H), 1.71 (d, 1H, *J* = 8.4 Hz), 1.59 (m, 5H), 1.46 (m, 2H), 1.27 (s, 3H), 0.99 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 166.1, 151.8, 143.0, 120.9, 119.1, 97.8, 73.5, 72.2, 65.6, 62.6, 58.1, 57.2, 54.4 (2C), 53.2, 42.8, 42.1, 41.0, 39.0, 35.0, 30.5, 30.3, 25.6 (2C), 24.2, 21.0, 20.3. HRMS (ESI) calcd for $C_{28}H_{39}N_3O_5S$ $[M + H]^+$ 530.2683; found 530.2687.

(5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-2-(Allylamino)-6,7,15-trihydroxy-5,5-dimethyl-9-methylene-5,5a,6,7,10,11,12,12a-octahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-8(9*H*)-one (14). Compound **14** (21 mg) was prepared in 45% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +85 (*c* 0.10, $CHCl_3$); HPLC purity 98.5% (t_R = 11.8 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 6.30 (br s, 1H), 6.17 (s, 1H), 5.98 (d, 1H, *J* = 11.4 Hz), 5.88 (m, 1H), 5.67 (br s, 1H), 5.56 (s, 1H), 5.27 (d, 1H, *J* = 16.8 Hz), 5.18 (d, 1H, *J* = 9.6 Hz), 5.02 (s, 1H), 4.91 (br s, 1H), 4.57 (d, 1H, *J* = 10.2 Hz), 3.91 (d, 1H, *J* = 10.2 Hz), 3.80 (m, 3H), 3.05 (d, 1H, *J* = 9.6 Hz), 2.48 (m, 2H), 2.31 (d, 1H, *J* = 15.6 Hz), 2.13 (m, 1H), 1.86 (m, 2H), 1.69 (d, 1H, *J* = 9.0 Hz), 1.54 (m, 1H), 1.24 (s, 3H), 0.95 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.7, 166.7, 151.8, 142.8, 133.7, 121.0, 119.3, 117.2, 97.9, 73.5, 72.1, 65.2, 62.6, 58.0, 53.2, 48.4, 42.7, 41.1, 39.0, 35.0, 30.5, 30.2, 21.0, 20.4. HRMS (ESI) calcd for $C_{24}H_{30}N_2O_5S$ $[M + H]^+$ 459.1948; found 459.1952.

***N*-((6*S*,7*S*,7a*R*,10*R*,12b*R*,15*R*)-6,7,15-Trihydroxy-5,5-dimethyl-9-methylene-8-oxo-5,5a,6,7,8,9,10,11,12,12a-decahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]-naphtho[1,2-*d*]thiazol-2-yl)acetamide (15).** Compound **15** (17 mg) was prepared in 36% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless amorphous gel; $[\alpha]_D^{25}$ +152 (*c* 0.10, $CHCl_3$); HPLC purity 99.2% (t_R = 13.5 min). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.29 (s, 1H), 7.96 (br s, 1H), 6.23 (d, 1H, *J* = 12.6 Hz), 6.22 (s, 1H), 5.63 (s, 1H), 5.17 (s, 1H), 5.09 (s, 1H), 4.96 (d, 1H, *J* = 10.2 Hz), 3.76 (m, 2H), 3.12 (d, 1H, *J* = 9.6 Hz), 2.56 (m, 2H), 2.45 (d, 1H, *J* = 15.6 Hz), 2.30 (s, 3H), 2.24 (m, 1H), 1.93 (dd, 1H, *J* = 3.6 Hz, 13.8 Hz), 1.79 (d, 1H, *J* = 9.0 Hz), 1.73 (br s, 1H), 1.57 (m, 2H), 1.26 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 206.8, 168.9, 156.6, 151.5, 140.8, 125.9, 121.7, 98.0, 73.8, 72.0, 64.4, 62.4, 58.1, 52.9, 42.6, 41.0, 38.6, 34.9, 30.4, 29.9, 23.0, 20.7, 20.6. HRMS (ESI) calcd for $C_{23}H_{28}N_2O_6S$ $[M + H]^+$ 461.1741; found 461.1747.

1-((5a*R*,6*S*,7*S*,7a*R*,10*S*,12a*S*,12b*R*,15*R*)-6,7,15-Trihydroxy-5,5-dimethyl-9-methylene-8-oxo-5,5a,6,7,8,9,10,11,12,12a-decahydro-4*H*-7,12b-(epoxymethano)-7a,10-methanocyclohepta[7,8]naphtho[1,2-*d*]thiazol-2-yl)guanidine (16). Compound **16** (23 mg) was prepared in 50% yield by a procedure similar to that used to prepare compound **5**. The title compound was obtained as a colorless solid; $[\alpha]_D^{25}$ +108 (*c* 0.10, $CHCl_3/CH_3OH$ = 4:1); HPLC purity 95.1% (t_R = 5.7 min). ^{1}H NMR (600 MHz,$CDCl_3/CD_3OD$ = 5:1): δ 6.19 (s, 1H), 5.62 (s, 1H), 5.06 (s, 1H), 4.44 (d, 1H, *J* = 9.6 Hz), 4.00 (d, 1H, *J* = 9.0 Hz), 3.85 (d, 1H, *J* = 8.4 Hz), 3.05 (d, 1H, *J* = 9.0 Hz), 2.66 (d, 1H, *J* = 16.2 Hz), 2.55 (m, 2H), 2.24 (m, 1H), 2.09 (dd, 1H, *J* = 4.8 Hz, 13.2 Hz), 1.81 (d, 1H, *J* = 8.4 Hz), 1.72 (m, 1H), 1.62 (m, 1H), 1.30 (s, 3H), 1.01 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3/CD_3OD$ = 5:1): δ 206.7, 156.5, 154.4, 151.1, 142.9, 126.6, 120.9, 97.5, 72.5, 64.8, 61.7, 58.8, 52.5, 43.1, 40.2, 38.0, 34.7, 30.0, 29.7, 29.3, 20.1, 19.6. HRMS (ESI) calcd for $C_{22}H_{28}N_4O_5S$ $[M + H]^+$ 461.1853; found 461.1856.

In Vitro Determination of Effects of Synthesized Compounds on Cancer Cell Proliferation. Cancer cells (breast cancer cell lines MCF-7 and MDA-MB-231, pancreatic cancer cell lines AsPC-1 and Panc-1, as well as the prostate cancer cell line DU145)

were seeded in 96-well plates at a density of 1×10^4 cells/well and treated with DMSO, 0.01, 0.1, 1, 5, 10, and 100 μM of individual compound for 48 h. Proliferation was measured by treating cells with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in a CellTiter 96t AQueous Non-Radioactive Cell Proliferation Assay kit (Promega, Madison, WI, USA). Absorbance of all wells was determined by measuring OD at 550 nm after 1 h incubation at 37 °C on a 96-well iMark microplate absorbance reader (BioRad, Hercules, CA). Each individual compound was tested in quadruplicate wells for each concentration.

Determination of Aqueous Solubility. Aqueous solubility for **7**, **8** (HCl salt), **14** (HCl salt), and **1** was determined by HPLC analysis according to a previously published protocol.[43] First, 2–4 mg of **7**, **8** (HCl salt), **14** (HCl salt), and oridonin were weighed and added to 1 mL of water, respectively. The suspensions were shaken at 25 °C for 24 h and then centrifuged, and the supernatants were filtered. Aliquots (10 μL) of the supernatants were injected into the HPLC system equipped with a C18 reverse-phase column under the same condition which was described in the general Experimental Section. One-point calibration[44] was done by injecting 10 μL aliquots of the corresponding buffer solutions of **7**, **8** (free base), **14** (free base), or **1** with known concentrations.

Cell Apoptosis Assay. Breast cancer MDA-MB-231 cells were incubated in 6-well plates (2.5×10^5 cells/well). Cells were then treated with DMSO, oridonin, or new compounds at different concentrations for 48 h, and then both adherent and floating cells were collected, washed once with PBS. Resuspended cells were incubated with 100 μL of PBS containing 1% BSA and 100 μL of Annexin V and dead cell detection reagent at room temperature for 20 min. Apoptosis was measured immediately using the Muse cell analyzer with the Muse apoptosis kit (catalogue no. MCH100105).

Western Blot Analysis. Breast cancer MDA-MB-231 cells were treated with DMSO, oridonin, or compound **14**, respectively. After 48 h of treatment, cells were harvested and lysed. Protein concentrations were quantified by the method of Bradford with bovine serum albumin as the standard. Equal amounts of total cellular protein extract (30 μg) was separated by electrophoresis on SDS-polyacrylamide gels and transferred to PVDF membranes. After blocking with 5% nonfat milk, the membrane was incubated with the desired primary antibody overnight at the following dilution: anti-Bcl-2 (1:200), anti-Bax (1:1000), anti-PARP (1:10000), anti-NF-κB (1:2000), and β-actin (1:20000). Subsequently, the membrane was incubated with appropriate secondary antibody. The immunoreactive bands were visualized by enhanced chemiluminescence as recommended by the manufacturer.

In Vivo Antitumor Activity Determination. All procedures including mice and in vivo experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of UT M.D. Anderson Cancer Center (MDACC). Nineteen female nude mice were obtained from MDACC and were used for orthotopic tumor studies at 4–6 weeks of age. The mice were maintained in a barrier unit with 12 h light–dark switch. Freshly harvested MDA-MB-231 cells (2.5×10^6 cells per mouse, resuspended in 100 μL of PBS) were injected into the third mammary fat pad of the mice and then randomly assigned into three groups. The mice were treated daily with 5 mg/kg of compound **14**, oridonin, or vehicle through intraperitoneal injection, when the tumor volume reached 200 mm^3. All drugs were dissolved in 50% DMSO with 50% polyethylene glycol for in vivo administration. Body weights and tumors volume were measured daily, and tumor volume was calculated according to the formula $V = 0.5 \times L \times W^2$, where L = length (mm) and W = width (mm).

Statistical Analysis. Statistical significance was determined using student's t test in drug-resistant breast cancer cell viability assay and cell apoptosis assay or one-way ANOVA in in vivo experiments. * represents a p value less than 0.05.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Growth inhibitory effects of adriamycin (ADR) on MCF-7 and ADR-resistant MCF-7 cells. ^{1}H and ^{13}C NMR spectra for the compounds described in this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

C.D. and Y.Z. contributed equally to this work. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

SFDA, State Food and Drug Administration; SAR, structure–activity relationships; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; IC_{50}, half-maximal inhibitory concentration; PI, propidium iodide; HRMS, high-resolution mass spectrometry; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; DMSO, dimethyl sulfoxide; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; THF, tetrahydrofuran; EtOAc, ethyl acetate; p-Ts, 4-toluenesulfonyl; Py, pyridine; PBS, phosphate-buffered saline; BCA, bicinchoninic acid; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PVDF, polyvinylidene difluoride

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