Article

Synthesis and Biological Evaluation of Novel Furozan-Based Nitric Oxide-Releasing Derivatives of Oridonin as Potential Anti-Tumor Agents

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Abstract: To search for novel nitric oxide (NO) releasing anti-tumor agents, a series of novel furoxan/oridonin hybrids were designed and synthesized. Firstly, the nitrate/nitrite levels in the cell lysates were tested by a Griess assay and the results showed that these furoxan-based NO-releasing derivatives could produce high levels of NO *in vitro*. Then the anti-proliferative activity of these hybrids against four human cancer cell lines was also determined, among which, **9h** exhibited the most potential anti-tumor activity with IC_{50} values of 1.82 μM against K562, 1.81 μM against MGC-803 and 0.86 μM against Bel-7402, respectively. Preliminary structure-activity relationship was concluded based on the experimental data obtained. These results suggested that NO-donor/natural product hybrids may provide a promising approach for the discovery of novel anti-tumor agents.

**Keywords:** NO donor; oridonin; hybrid; anti-tumor agents; SAR

1. Introduction

Nitric oxide, a special gaseous molecular, is a key mediator involved in many physiological and pathological processes [1,2]. High concentrations of NO and its metabolic derivatives can modify functional proteins, leading to cell cycle arrest and apoptosis, particularly in tumor cells [3–5]. Indeed, some synthesized NO-releasing compounds have shown cytotoxic activity against human carcinoma cells *in vitro* and inhibit the growth and metastasis of cancers *in vivo* [6–8]. So, the NO-based anti-cancer agents have been investigating for cancer therapy at clinic [9,10]. Furoxans represent one class of NO donors that can produce high levels of NO and exhibit strong anti-cancer activity [11,12]. In the previous work by our group, several classes of NO-releasing compounds have been reported, which possess strong anti-proliferative activity against human carcinoma cells *in vitro*, inhibition of cancer cells growth *in vivo* and the ability to increase sensitivity of Pgp-mediated multidrug resistance (MDR) in solid tumors, separately [13–17]. These results motivated us to further design some novel NO-donor/natural product hybrids.

Oridonin (**1**, see Figure 1) is a commercially available natural *ent*-kaurene diterpenoid that has recently attracted much attention because of its anti-tumor activity with a mechanism of inhibition effect on nuclear factor κB (NF-κB) activation, induction of G_2/M phase arrest and apoptosis [18]. Oridonin has been safely used for the treatment of hepatoma and promyelocytic leukemia in China for many years. In previous studies, we found that a series of novel 1-*O*- and 14-*O*-derivatives of oridonin exhibited stronger cytotoxicity against six cancer cell lines *in vitro* and some of them had stronger anti-tumor activity than the parent compound **1** and the positive control cyclophosphamide in mice with H22 liver tumor *in vivo* [19–21]. Hence, it may be a desired lead compound using for further design of novel furoxan-based NO-releasing derivatives for the development of anti-tumor agents. Therefore, a series of novel furozan-based nitric oxide-releasing derivatives of oridonin were designed and synthesized.

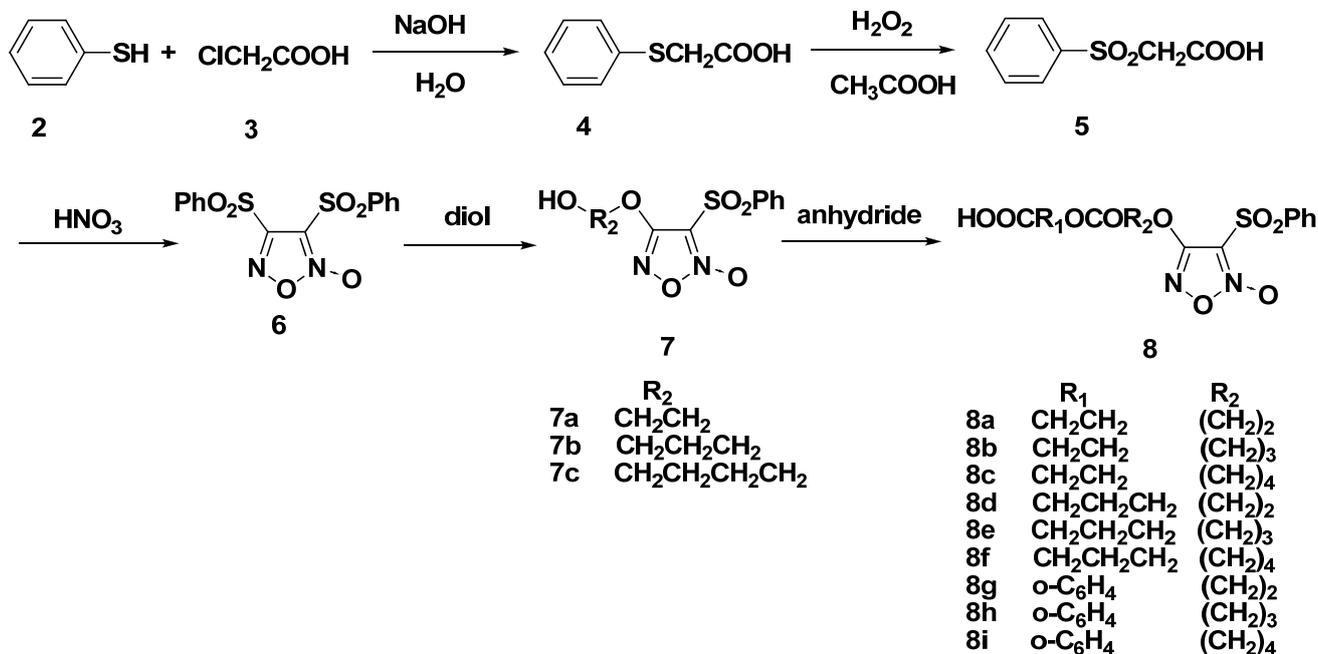**Figure 1.** The structure and atom numbering of oridonin.
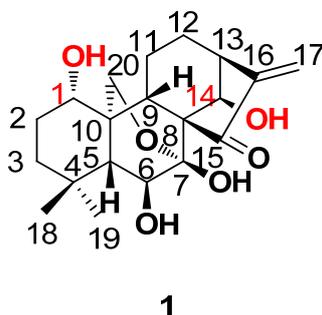
2. Results and Discussion

2.1. Synthesis of Furoxan-Based NO Donor

The substituted furoxans were prepared in five steps in the following way (Scheme 1). The starting material benzenethiol (**2**) was converted to 2-(phenylthio)acetic acid (**4**) by treatment with chloroacetic acid (**3**). Then, compound **4** was oxidized by 30% H_2O_2 aqueous solution to give 2-(phenylsulfonyl)

acetic acid (**5**) and fuming HNO$_3$ was added to obtain diphenylsulfonylfuroxan (**6**), which was then converted to various monophenylsulfonylfuroxans **7a–c** by treatment with the corresponding diol. Finally, anhydrides were added and furoxan-based NO donors **8a–i** were obtained.
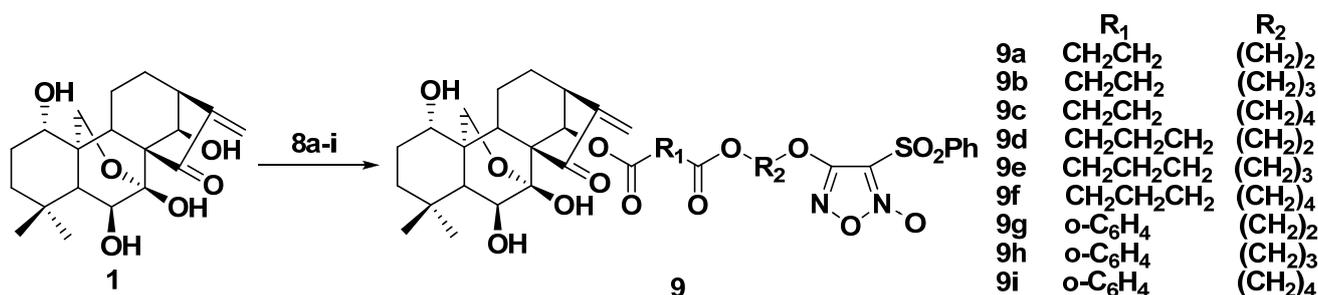
Scheme 1. Synthesis of compounds **8a–i**.

	R$_2$
7a	CH$_2$CH$_2$
7b	CH$_2$CH$_2$CH$_2$
7c	CH$_2$CH$_2$CH$_2$CH$_2$

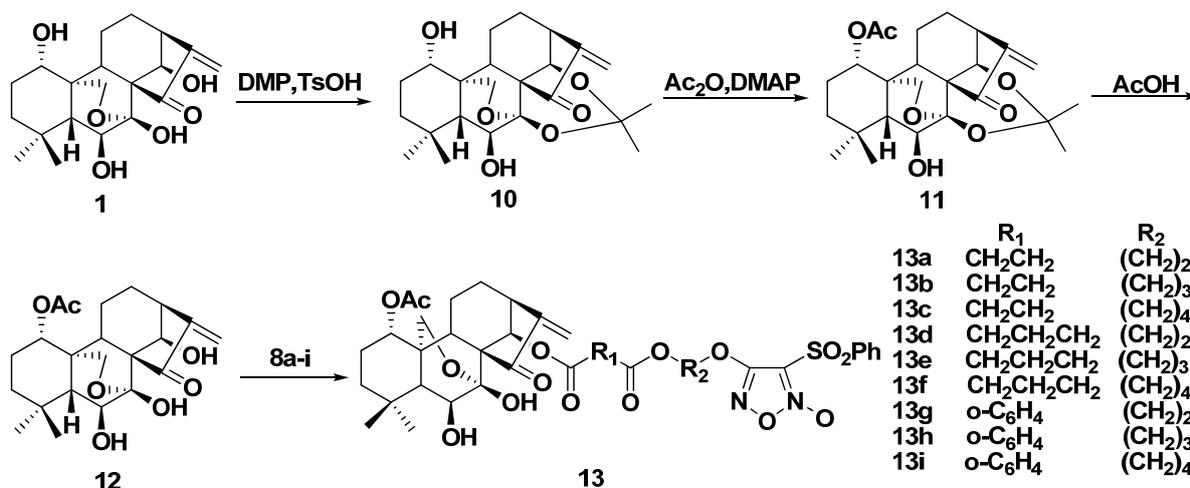|	R$_1$	R$_2$
8a	CH$_2$CH$_2$	(CH$_2$)$_2$
8b	CH$_2$CH$_2$	(CH$_2$)$_3$
8c	CH$_2$CH$_2$	(CH$_2$)$_4$
8d	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_2$
8e	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_3$
8f	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_4$
8g	o-C$_6$H$_4$	(CH$_2$)$_2$
8h	o-C$_6$H$_4$	(CH$_2$)$_3$
8i	o-C$_6$H$_4$	(CH$_2$)$_4$

2.2. Synthesis of Furoxan/Oridonin Hybrids

The resulting furoxans **8a–i** were treated with oridonin to give the target compounds **9a–i**, as shown in Scheme 2.

Scheme 2. Synthesis of compounds **9a–i**.

	R$_1$	R$_2$
9a	CH$_2$CH$_2$	(CH$_2$)$_2$
9b	CH$_2$CH$_2$	(CH$_2$)$_3$
9c	CH$_2$CH$_2$	(CH$_2$)$_4$
9d	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_2$
9e	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_3$
9f	CH$_2$CH$_2$CH$_2$	(CH$_2$)$_4$
9g	o-C$_6$H$_4$	(CH$_2$)$_2$
9h	o-C$_6$H$_4$	(CH$_2$)$_3$
9i	o-C$_6$H$_4$	(CH$_2$)$_4$

Treatment of oridonin with 2,2-dimethoxypropane (DMP) in the presence of *p*-toluenesulfonic acid (TsOH) in acetone afforded 7,14-(1-methylethylene)-dioxyoridonin derivative **10**. Compound **10** upon reaction with Ac$_2$O/pyridine yielded 1-*O*-acetyl derivative **11**. Deprotection of **11** with AcOH gave 1-*O*-acetyl-oridonin **12** in quantitative yield. Target compounds **13a–i** were prepared by reaction of **12** with furoxan-based NO donors **8a–i** in the presence of 4-dimethylaminopyridine/1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (DMAP/EDCI) in CH$_2$Cl$_2$, as shown in Scheme 3.

Scheme 3. Synthesis of compounds **13a–i**.

	R₁	R₂
13a	CH_2CH_2	$(CH_2)_2$
13b	CH_2CH_2	$(CH_2)_3$
13c	CH_2CH_2	$(CH_2)_4$
13d	$CH_2CH_2CH_2$	$(CH_2)_2$
13e	$CH_2CH_2CH_2$	$(CH_2)_3$
13f	$CH_2CH_2CH_2$	$(CH_2)_4$
13g	o-C_6H_4	$(CH_2)_2$
13h	o-C_6H_4	$(CH_2)_3$
13i	o-C_6H_4	$(CH_2)_4$

2.3. NO-Releasing Test of Hybrids **9a–i** and **13a–i** in Vitro

The levels of nitrate/nitrite in the lysates of target compounds **9a–i** and **13a–i** were determined at 100 μM by Griess assay over a duration of 0–60 min. As shown in Figure 2, variable levels of NO were produced by compounds **9a–i** and **13a–i**. The concentration of NO increased with time, and at the time point of 60 min, all tested compounds produced more than 15 μmol/L of NO. The amount of NO released by compounds **13a–i** (**13g** with the lowest level of 16.88 μmol/L at the 60 min time point) was less than that of **9a–i** (**9d** with the highest level of 45.44 μmol/L at the 60 min time point).

Figure 2. Variable levels of NO produced by compounds (**a**) **9a–i** and (**b**) **13a–i**.
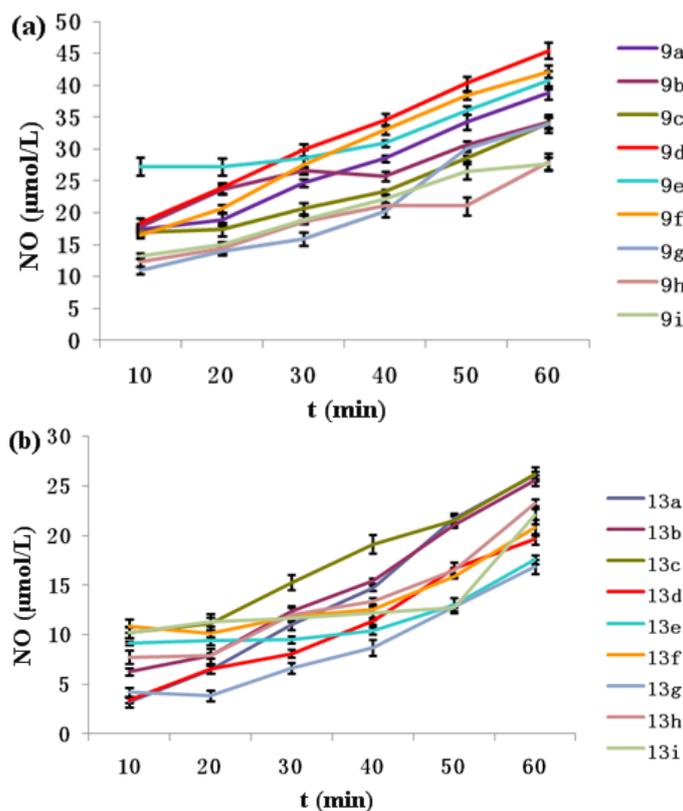
2.4. Anti-proliferative Activity in Vitro

The anti-proliferative activity of oridonin and its NO-donor hybrids **9a-**i as well as 1-*O*-derivatives of oridonin (**12**) and its NO-donor hybrids **13a–i** was evaluated against four human cancer cell lines (Bel-7402, K562, MGC-803, CaEs-17) by MTT assay. The results are shown in Table 1 [22]. All the target compounds **9a–i** and **13a–i** exhibited stronger anti-proliferative activity than their parent compounds **1** (oridonin) and **12** (1-oxo-oridonin), correspondingly. Among them, **13a–i** released less NO (Figure 2) and showed less potent anti-proliferative activity than **9a–i**. For example, **13e** with IC_{50} value of 2.13 µM compared to **9e** (1.33 µM) against Bel-7402 cells, **13g** with IC_{50} value of 2.45 µM compared to **9g** (1.33 µM) against MGC803 cells, and so on. These results and our previous studies [13–17] indicated that the releasing of NO contributed to anti-proliferative activity and higher levels of NO releasing could produce stronger activity.

Table 1. IC_{50} values of the furoxan/oridonin hybrids against four human tumor cell lines [a].

Compd.	Bel-7402	K562	MGC-803	CaEs-17
Taxol [b]	1.89 ± 0.09	0.41 ± 0.02 [c]	0.85 ± 0.06 [c]	0.43 ± 0.03 [d]
Oridonin	7.48 ± 0.53	4.76 ± 0.32	5.69 ± 0.39	11.03 ± 1.02
9a	2.37 ± 0.85	4.33 ± 0.14	3.22 ± 0.19	8.46 ± 0.05
9b	1.91 ± 0.09	3.46 ± 0.60	2.57 ± 0.07	6.98 ± 0.20
9c	2.23 ± 0.04	4.02 ± 0.05	3.46 ± 0.23	8.17 ± 1.01
9d	1.89 ± 0.22	3.78 ± 0.19	3.08 ± 0.47	8.04 ± 0.18
9e	1.33 ± 0.15	2.85 ± 0.03	2.21 ± 0.16	6.77 ± 0.32
9f	1.97 ± 0.04	3.72 ± 0.26	3.23 ± 0.25	8.09 ± 0.47
9g	0.95 ± 0.21 [c]	1.94 ± 0.14	1.98 ± 0.13	4.81 ± 0.10 [c]
9h	0.86 ± 0.08 [c]	1.82 ± 0.07	1.81 ± 0.20	4.56 ± 0.32 [c]
9i	0.97 ± 0.10 [c]	1.92 ± 0.34	1.90 ± 0.11	5.24 ± 0.18
12	3.21 ± 0.25	5.06 ± 0.18	4.05 ± 0.04	7.24 ± 0.41
13a	2.85 ± 0.14	4.65 ± 0.07	3.77 ± 0.31	5.30 ± 0.28
13b	2.19 ± 0.19	3.85 ± 0.06	2.90 ± 0.12	4.11 ± 0.07 [c]
13c	2.76 ± 0.42	4.11 ± 0.15	3.65 ± 0.40	5.22 ± 0.12
13d	2.70 ± 0.09	4.08 ± 0.30	3.64 ± 0.12	5.38 ± 0.24
13e	2.13 ± 0.17	3.04 ± 0.21	2.79 ± 0.10	4.00 ± 0.31 [c]
13f	2.66 ± 0.30	3.97 ± 0.16	3.42 ± 0.27	5.11 ± 0.39
13g	1.94 ± 0.13	2.22 ± 0.29	2.45 ± 0.51	3.28 ± 0.06 [c]
13h	1.72 ± 0.08	2.08 ± 0.34	2.22 ± 0.29	3.24 ± 0.23 [c]
13i	1.86 ± 0.15	2.65 ± 0.08	2.41 ± 0.16	3.13 ± 0.21 [c]

[a] Results are expressed as IC_{50} values in µM and the values are means ± SD; n = 3. [b] Taxol was used as a positive control. [c] $p < 0.05$ *versus* oridonin; [d] $p < 0.01$ *versus* oridonin.

Among the tested compounds, the series **9g–i** and **13g–i** with a *o*-C_6H_4 linker (R_1) (compounds **g–i**) showed lower IC_{50} values than the corresponding compounds **a–f**. Compared the IC_{50} values of the compounds of series **a–c** with **d–f** in different cell lines, there was a decline with the extension of the length of R_1. In general, when R_1 were aromatic groups (compounds **g–i**), the activity was stronger than those with alkyl groups. While R_1 were alkyl groups, IC_{50} values decreased with lengthening of carbon chain. In almost all cases (except **13h**), when the length of R_2 is three carbons, more potential

anti-proliferative activity was observed than those of two and four carbons, correspondingly (for instance, **9b** > **9a** and **9c**, **9e** > **9d** and **9f**, **9h** > **9g** and **9i**, **13b** > **13a** and **13c**, **13e** > **13d** and **13f**). This suggested that the length of the linker group R_2 with three carbons would be more suitable. In all the target synthetic hybrids, compound **9h** (R_1=*o*-C_6H_4; R_2=$CH_2CH_2CH_2$) exhibited the most potential anti-tumor activity against tested cell lines: IC_{50} values of 0.86 μM against Bel-7402 (stronger than parent compound oridonin of 7.48 μM and positive control taxol of 1.89 μM), 1.82 μM against K562, 1.81 μM against MGC-803 (stronger than oridonin of 5.69 μM) and 4.56 μM against CaEs-17 (stronger than oridonin of 11.03 μM). Subsequent design and synthesis of novel NO releasing anti-tumor agents based on present SAR and more intensive biological studies were undertaking.

3. Experimental

3.1. Chemistry

All commercially available solvents and reagents were used without further purification. Melting points were taken on XT-4 micro melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr pellets) were recorded on a Nicolet Impact 410 instrument (Madison, WI, USA). ^{1}H-NMR spectra were recorded at 300 MHz with a Bruker AV-300 spectrometer (Karlsruhe, Germany) in the indicated solvents (TMS as internal standard): The values of the chemical shifts are expressed in *δ* values (ppm) and the coupling constants (*J*) in Hz. Mass spectra were obtained using FTMS-2000 (Madison, WI, USA). HR-MS were obtained using an Agilent QTOF 6520 (Palo Alto, CA, USA). Compounds **2**–**4** were commercially available. Compounds **5**, **6**, **7a**–**c**, **10**, **11** and **12** were synthesized, as previously described [13,19,20].

3.1.1. General Procedure for the Preparation of **8a**–**i**

Compound **7a**–**c** (2 mmol) in pyridine (5 mL) was mixed with the corresponding anhydride (4 mmol) by stirring at room temperature for 6–12 h. The mixture was concentrated *in vacuo*, dissolved in H_2O (15 mL) and extracted with CH_2Cl_2 (15 mL × 3). The organic layers were combined, washed with water and saturated NaCl solution sequentially, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude products **8a**–**i** used for the next step without further purification.

3.1.2. General Procedure for the Preparation of **9a**–**i** and **13a**–**i**

Compounds **8a**–**i** (0.24 mmol) were dissolved in CH_2Cl_2 (10 mL) and stirred at room temperature. Oridonin or its derivative **12** (0.2 mmol), EDCI (93 mg, 0.6 mmol) and DMAP (catalytic amount) were added. After 8–12 h, the reaction mixture was washed with water and saturated NaCl solution sequentially, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude products were purified by column chromatography (MeOH/CH_2Cl_2 1:200 v/v) to give the title compounds.

ent-1α,6β,7β-Trihydroxy-(14β-O-(4-oxo-butyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4) oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**9a**). Yield 41%, m.p. 93–95 °C; ^{1}H-NMR (CDCl$_3$), *δ* (ppm) 1.12 (3H, s, –CH$_3$), 1.25 (3H, s, –CH$_3$), 3.16 (1H, d, *J* = 9.6 Hz, 13-CH), 3.49 (1H, m, 1-CH), 3.76 (1H, m, 6-CH), 4.02(1H, s, 1-OH), 4.06, 4.30 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.51 (2H, t,

J = 4.5 Hz, –CH$_2$), 4.62 (2H, t, J = 4.8 Hz, –CH$_2$), 5.53 (1H, s, 17-CH$_2$), 5.92 (1H, s, 14-CH), 6.04 (1H, d, J = 10.8 Hz, 6-OH), 6.15 (1H, s, 17-CH$_2$), 7.64 (2H, t, J = 7.2 Hz, Ar-H), 7.77 (1H, t, J = 7.5 Hz, Ar-H), 8.07 (2H, d, J = 8.1 Hz, Ar-H); MS(ESI) *m/z*: 755.4 [M+Na]$^+$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{34}$H$_{40}$N$_2$NaO$_{14}$S: 755.2092, found 755.2095.

ent-1α,6β,7β-Trihydroxy-(14β-O-(4-oxobutyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**9b**). Yield 34%, m.p. 86–88 °C; IR υ_{max} 3419, 2955, 2025, 1736, 1615, 1554, 1451, 1359, 733, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.11 (6H, s, –CH$_3$), 3.14 (1H, d, J = 9.6 Hz, 13-CH), 3.49 (1H, m, 1-CH), 3.74 (1H, m, 6-CH), 4.07 (1H, s, 1-OH), 4.28 (2H, m, –CH$_2$), 4.06, 4.30 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.50 (2H, t, J = 6.0 Hz, –CH$_2$), 5.52 (1H, s, 17-CH$_2$), 5.89 (1H, s, 14-CH), 6.07 (1H, d, J = 10.8 Hz, 6-OH), 6.15 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.8 Hz, Ar-H), 7.77 (1H, t, J = 7.2 Hz, Ar-H), 8.06 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 764.3 [M+NH$_4$]$^+$, 747.3 [M+H]$^+$, 781.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{35}$H$_{42}$N$_2$NaO$_{14}$S: 769.2249, found 769.2254.

ent-1α,6β,7β-Trihydroxy-(14β-O-(4-oxobutyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**9c**). Yield 48%, m.p. 83–85 °C; IR υ_{max} 3417, 2955, 2025, 1734, 1635, 1554, 1451, 1367, 733, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.11 (6H, s, –CH$_3$), 3.15 (1H, d, J = 9.6 Hz, 13-CH), 3.48 (1H, m, 1-CH), 3.75 (1H, m, 6-CH), 4.07 (1H, s, 1-OH), 4.17 (2H, m, –CH$_2$), 4.05, 4.29 (each 1H, dd, J_A = J_B = 11.1 Hz, 20-CH$_2$), 4.45 (2H, t, J = 6.0 Hz, –CH$_2$), 5.52 (1H, s, 17-CH$_2$), 5.90 (1H, s, 14-CH), 6.06 (1H, d, J = 10.8 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.5 Hz, Ar-H), 7.77 (1H, t, J = 7.2 Hz, Ar-H), 8.06 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 778.2 [M+NH$_4$]$^+$, 761.1 [M+H]$^+$, 795.2 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{36}$H$_{44}$N$_2$NaO$_{14}$S: 783.2405, found 783.2411.

ent-1α,6β,7β-Trihydroxy-(14β-O-(5-oxo-pentanoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**9d**). Yield 45%, m.p. 86–89 °C; IR υ_{max} 3405, 2952, 2025, 1739, 1618, 1554, 1451, 1360, 732, 676 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.10 (6H, s, –CH$_3$), 3.16 (1H, d, J = 9.9 Hz, 13-CH), 3.48 (1H, m, 1-CH), 3.73 (1H, m, 6-CH), 4.14 (1H, s, 1-OH), 4.05, 4.30 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.47 (2H, m, –CH$_2$), 4.61 (2H, m, –CH$_2$), 5.50 (1H, s, 17-CH$_2$), 5.87 (1H, s, 14-CH), 6.06 (1H, d, J = 10.5 Hz, 6-OH), 6.13 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.5 Hz, Ar-H), 7.77 (1H, t, J = 7.5 Hz, Ar-H), 8.06 (2H, d, J = 7.8 Hz, Ar-H); MS(ESI) *m/z*: 764.0 [M+NH$_4$]$^+$, 747.1 [M+H]$^+$, 781.2 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{35}$H$_{42}$N$_2$NaO$_{14}$S: 769.2249, found 769.225.

ent-1α,6β,7β-Trihydroxy-(14β-O-(5-oxopentanoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**9e**). Yield 42%, m.p. 80–82 °C; IR υ_{max} 3439, 2954, 2025, 1734, 1615, 1554, 1452, 1383, 733, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.11 (6H, s, –CH$_3$), 3.16 (1H, d, J = 9.6 Hz, 13-CH), 3.50 (1H, m, 1-CH), 3.75 (1H, m, 6-CH), 4.07, 4.30 (each 1H, dd, J_A = J_B = 10.8 Hz, 20-CH$_2$), 4.25 (2H, t, J = 6.0 Hz, –CH$_2$), 4.50 (2H, t, J = 6.0 Hz, –CH$_2$), 5.51 (1H, s, 17-CH$_2$), 5.87 (1H, s, 14-CH), 6.05 (1H, d, J = 10.5 Hz, 6–OH), 6.14 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.5 Hz, Ar-H), 7.77 (1H, t, J = 7.5 Hz, Ar-H), 8.05 (2H, d, J = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 778.3

[M+NH$_4$]$^+$, 761.3 [M+H]$^+$, 795.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{36}$H$_{44}$N$_2$NaO$_{14}$S: 783.2405, found 783.2419.

ent-1α,6β,7β-Trihydroxy-(14β-O-(5-oxo-pentanoic   acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**9f**). Yield 37%, m.p. 92–94 °C; IR υ_{max} 3440, 2955, 2025, 1733, 1615, 1554, 1451, 1367, 732, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.11 (6H, s, –CH$_3$), 3.39 (1H, d, *J* = 9.9 Hz, 13-CH), 3.50 (1H, m, 1-CH), 3.76 (1H, m, 6-CH), 4.06 (1H, s, 1-OH), 4.08, 4.34 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.46 (1H, m, –CH$_2$), 4.57 (1H, m, –CH$_2$), 4.58 (2H, m, –CH$_2$), 5.56 (1H, s, 17-CH$_2$), 6.05 (1H, d, *J* = 10.5 Hz, 6-OH), 6.07 (1H, s, 14-CH), 6.17 (1H, s, 17-CH$_2$), 7.52 (2H, m, Ar-H), 7.57 (3H, m, Ar-H), 7.75 (2H, m, Ar-H), 8.06 (2H, d, *J* = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 792.3 [M+NH$_4$]$^+$, 775.5 [M+H]$^+$, 809.6 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{37}$H$_{46}$N$_2$NaO$_{14}$S: 797.2562, found 797.2565.

ent-1α,6β,7β-Trihydroxy-(14β-O-(2-formyl benzoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**9g**). Yield 53%, m.p. 123–125 °C; IR υ_{max} 3392, 2951, 2025, 1714, 1618, 1553, 1451, 1363, 739, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.09 (6H, s, –CH$_3$), 3.37 (1H, d, *J* = 9.6 Hz, 13-CH), 3.50 (1H, m, 1-CH), 3.72 (1H, m, 6-CH), 4.05 (1H, s, 1–OH), 4.07, 4.36 (each 1H, dd, J_A = J_B = 10.2 HZ, 20-CH$_2$), 4.67 (2H, m, –CH$_2$), 4.76 (2H, m, –CH$_2$), 5.54 (1H, s, 17-CH$_2$), 6.04 (1H, d, *J* = 12.0 Hz, 6-OH), 6.07 (1H, s, 14-CH), 6.14 (1H, s, 17-CH$_2$), 7.46 (2H, t, *J* = 7.8 Hz, Ar-H), 7.58 (4H, m, Ar-H), 7.78 (1H, m, Ar-H), 8.01 (2H, d, *J* = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 798.3 [M+NH$_4$]$^+$, 781.2 [M+H]$^+$, 815.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{38}$H$_{40}$N$_2$NaO$_{14}$S: 803.2092, found 755.2093.

ent-1α,6β,7β-Trihydroxy-(14β-O-(2-formyl benzoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**9h**). Yield 47%, m.p. 113–115 °C; IR υ_{max} 3418, 2954, 2025, 1714, 1616, 1554, 1451, 1384, 736, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.11 (6H, s, –CH$_3$), 3.39 (1H, d, *J* = 9.9 Hz, 13-CH), 3.50 (1H, m, 1-CH), 3.76 (1H, m, 6-CH), 4.06 (1H, s, 1-OH), 4.08, 4.34 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.46 (1H, m, –CH$_2$), 4.57 (1H, m, –CH$_2$), 4.58 (2H, m, –CH$_2$), 5.56 (1H, s, 17-CH$_2$), 6.05 (1H, d, *J* = 10.5 Hz, 6-OH), 6.07 (1H, s, 14-CH), 6.17 (1H, s, 17-CH$_2$), 7.52 (2H, m, Ar-H), 7.57 (3H, m, Ar-H), 7.75 (2H, m, Ar-H), 8.06 (2H, d, *J* = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 812.3 [M+NH$_4$]$^+$, 795.3 [M+H]$^+$, 829.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{39}$H$_{42}$N$_2$NaO$_{14}$S: 817.2249, found 755.2252.

ent-1α,6β,7β-Trihydroxy-(14β-O-(2-formylbenzoic   acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**9i**). Yield 50%, m.p. 108–110 °C; IR υ_{max} 3384, 2952, 2025, 1715, 1615, 1553, 1450, 1368, 734, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.10 (6H, s, –CH$_3$), 3.32 (1H, d, *J* = 9.9 Hz, 13-CH), 3.50 (1H, m, 1-CH), 3.76 (1H, m, 6-CH), 4.08, 4.34 (each 1H, dd, J_A = J_B = 8.4 HZ, 20-CH$_2$), 4.44 (2H, m, –CH$_2$), 4.50 (2H, t, *J* = 5.4 Hz, –CH$_2$), 5.30 (1H, s, 1-OH), 5.56 (1H, s, 17-CH$_2$), 6.04 (1H, d, *J* = 10.5 Hz, 6-OH), 6.09 (1H, s, 14-CH), 6.61 (1H, s, 17-CH$_2$), 7.53 (3H, m, Ar-H), 7.61 (2H, m, Ar-H), 7.76 (2H, m, Ar-H), 8.06 (2H, d, *J* = 7.8 Hz, Ar-H); MS(ESI) *m/z*: 826.1 [M+NH$_4$]$^+$, 809.0 [M+H]$^+$, 843.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{40}$H$_{44}$N$_2$NaO$_{14}$S: 831.2405, found 831.2411.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(4-oxobutyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**13a**). Yield 40%, m.p. 105–107 °C; IR v_{max} 3384, 2958, 2025, 1739, 1618, 1554, 1452, 1363, 732, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 2.17 (3H, s, –CH$_3$), 3.13 (1H, d, J = 9.6 Hz, 13-CH), 3.76 (1H, m, 6-CH), 4.17, 4.28 (each 1H, dd, J_A = J_B = 10, 5 Hz, 20-CH$_2$), 4.51 (2H, m, –CH$_2$), 4.61 (1H, m, 1-CH), 4.62 (2H, m, –CH$_2$), 5.52 (1H, s, 17-CH$_2$), 5.87 (1H, s, 14-CH), 6.09 (1H, d, J = 9.3 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.2 Hz, Ar-H), 7.91 (1H, t, J = 7.8 Hz, Ar-H), 8.07 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 775.3 [M+H]$^+$, 809.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{36}$H$_{42}$N$_2$NaO$_{15}$S: 797.2198, found 797.2207.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(4-oxobutyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**13b**). Yield 51%, m.p. 95–97 °C; IR v_{max} 3383, 2957, 2025, 1738, 1615, 1554, 1452, 1373, 733, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 1.99 (3H, s, –CH$_3$), 3.12 (1H, d, J = 9.6 Hz, 13-CH), 3.77 (1H, m, 6-CH), 4.31 (2H, m, –CH$_2$), 4.17, 4.34 (each 1H, dd, J_A = J_B = 10.2 Hz, 20-CH$_2$), 4.51 (2H, t, J = 6.0 Hz, –CH$_2$), 4.62 (1H, m, 1-CH), 5.52 (1H, s, 17-CH$_2$), 5.85 (1H, s, 14-CH), 6.12 (1H, d, J = 11.1 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.64 (2H, t, J = 7.8 Hz, Ar-H), 7.77 (1H, t, J = 7.5 Hz, Ar-H), 8.06 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 806.3 [M+NH$_4$]$^+$, 789.3 [M+H]$^+$, 823.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{37}$H$_{44}$N$_2$NaO$_{15}$S: 811.2355, found 811.2362.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(4-oxobutyric acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**13c**). Yield 48%, m.p. 109–111 °C; IR v_{max} 3385, 2957, 2025, 1737, 1616, 1554, 1451, 1371, 733, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 1.99 (3H, s, CH$_3$), 3.13 (1H, d, J = 9.6 Hz, 13-CH), 3.78 (1H, m, 6-CH), 4.19 (2H, m, –CH$_2$), 4.11, 4.27 (each 1H, dd, J_A = J_B = 10.5 Hz, 20-CH$_2$), 4.46 (2H, t, J = 7.5 Hz, –CH$_2$), 4.62 (1H, m, 1-CH), 5.53 (1H, s, 17-CH$_2$), 5.88 (1H, s, 14-CH), 6.11 (1H, d, J = 10.5 Hz, 6–OH), 6.16 (1H, s, 17-CH$_2$), 7.64 (2H, t, J = 7.5 Hz, Ar-H), 7.78 (1H, t, J = 7.2 Hz, Ar-H), 8.07 (2H, d, J = 7.8 Hz, Ar-H); MS(ESI) *m/z*: 820.4 [M+NH$_4$]$^+$, 803.3 [M+H]$^+$, 837.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{38}$H$_{46}$N$_2$NaO$_{15}$S: 825.2511, found 825.2525.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(4-oxopentanoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**13d**). Yield 42%, m.p. 92–94 °C; IR v_{max} 3530, 3386, 2956, 2025, 1738, 1618, 1553, 1451, 731, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, CH$_3$), 1.99 (3H, s, –CH$_3$), 3.16 (1H, d, J = 9.9 Hz, 13-CH), 3.75 (1H, m, 6-CH), 4.17, 4.27 (each 1H, dd, J_A = J_B = 10.5 Hz, 20-CH$_2$), 4.49 (2H, m, –CH$_2$), 4.61 (2H, m, –CH$_2$), 5.50 (1H, s, 17-CH$_2$), 5.83 (1H, s, 14-CH), 6.06 (1H, d, J = 9.6 Hz, 6–OH), 6.13 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.8 Hz, Ar-H), 7.76 (1H, t, J = 7.5 Hz, Ar-H), 8.06 (2H, d, J = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 806.4 [M+NH$_4$]$^+$, 789.2 [M+H]$^+$, 823.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{37}$H$_{44}$N$_2$NaO$_{15}$S: 811.2355, found 811.2367.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(5-oxopentanoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**13e**). Yield 36%, m.p. 86–88 °C; IR v_{max} 3421, 2958, 2025, 1737, 1616, 1554, 1452, 1374, 732, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s,

–CH$_3$), 2.19 (3H, s, –CH$_3$), 3.16 (1H, d, J = 10.2 Hz, 13-CH), 3.77 (1H, m, 6-CH), 4.20, 4.38 (each 1H, dd, $J_A = J_B$ = 10.5 Hz, 20-CH$_2$), 4.26 (2H, t, J = 6.0 Hz, –CH$_2$), 4.50 (2H, t, J = 6.0 Hz, –CH$_2$), 4.62 (1H, m, 1-CH), 5.52 (1H, s, 14-CH), 5.83 (1H, s, 17-CH$_2$), 6.06 (1H, d, J = 10.5 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.5 Hz, Ar-H), 7.77 (1H, t, J = 7.2 Hz, Ar-H), 8.06 (2H, d, J = 7.5 Hz, Ar-H); MS(ESI) *m/z*: 803.3 [M+H]$^+$, 837.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{38}$H$_{46}$N$_2$NaO$_{15}$S: 825.2511, found 825.2523.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(5-oxopentanoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**13f**). Yield 40%, m.p. 98–101 °C; IR v_{max} 3394, 2957, 2025, 1737, 1617, 1554, 1451, 1373, 732, 686 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 2.35 (3H, s, –CH$_3$), 3.17 (1H, d, J = 9.3 Hz, 13-CH), 3.76 (1H, m, 6-CH), 4.20 (2H, t, J = 6.0 Hz, –CH$_2$), 4.18, 4.27 (each 1H, dd, $J_A = J_B$ = 10.2 Hz, 20-CH$_2$), 4.46 (2H, t, J = 5.7 Hz, –CH$_2$), 4.61 (1H, m, 1-CH), 5.52 (1H, s, 17-CH$_2$), 5.83 (1H, s, 14-CH), 6.07 (1H, d, J = 10.2 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.63 (2H, t, J = 7.2 Hz, Ar-H), 7.74 (1H, t, J = 7.8 Hz, Ar-H), 8.05 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 834.4 [M+NH$_4$]$^+$, 817.3 [M+H]$^+$, 851.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{39}$H$_{48}$N$_2$NaO$_{15}$S: 839.2668, found 839.2679.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(3-formylbenzoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxyethyl))-15-oxo-7,20-epoxy-16-kaurene (**13g**). Yield 46%, m.p. 154–156 °C; IR v_{max} 3383, 2957, 2025, 1736, 1617, 1554, 1451, 1364, 739, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 2.01 (3H, s, CH$_3$), 3.36 (1H, d, J = 9.6 Hz, 13-CH), 3.75 (1H, m, 6-CH), 4.19, 4.36 (each 1H, dd, $J_A = J_B$ = 10.5 Hz, 20-CH$_2$), 4.64 (2H, m, –CH$_2$), 4.79 (2H, m, –CH$_2$), 4.82 (1H, m, 1-CH), 5.54 (1H, s, 17-CH$_2$), 6.02 (1H, s, 14-CH), 6.03 (1H, d, J = 10.5 Hz, 6–OH), 6.15 (1H, s, 17-CH$_2$), 7.44 (2H, t, J = 7.5 Hz, Ar-H), 7.79 (1H, m, Ar-H), 7.58 (4H, m, Ar-H), 8.03 (2H, d, J = 7.8 Hz, Ar-H); MS(ESI) *m/z*: 840.2 [M+NH$_4$]$^+$, 823.2 [M+H]$^+$, 857.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{40}$H$_{42}$N$_2$NaO$_{15}$S: 845.2198, found 845.2208.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(3-formylbenzoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxypropyl))-15-oxo-7,20-epoxy-16-kaurene (**13h**). Yield 52%, m.p. 136–138 °C; IR v_{max} 3379, 2957, 2025, 1735, 1616, 1554, 1451, 1374, 738, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 2.02 (3H, s, –CH$_3$), 3.33 (1H, d, J = 9.6 Hz, 13-CH), 3.78 (1H, m, 6-CH), 4.22, 4.34 (each 1H, dd, $J_A = J_B$ = 8.7 Hz, 20-CH$_2$), 4.45 (1H, m, –CH$_2$), 4.57 (1H, m, –CH$_2$), 4.61 (2H, m, –CH$_2$), 4.64 (1H, m, 1-CH), 5.56 (1H, s, 17-CH$_2$), 6.02 (1H, s, 14-CH), 6.10 (1H, d, J = 10.5 Hz, 6–OH), 6.17 (1H, s, 17-CH$_2$), 7.53 (2H, m, Ar-H), 7.62 (3H, m, Ar-H), 7.72 (2H, m, Ar-H), 8.09 (2H, d, J = 7.8 Hz, Ar-H); MS(ESI) *m/z*: 854.3 [M+NH$_4$]$^+$, 837.2 [M+H]$^+$, 871.3 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{41}$H$_{44}$N$_2$NaO$_{15}$S: 859.2355, found 859.2368.

ent-(1α-O-Acetyl)-6β,7β-dihydroxy-(14β-O-(2-formylbenzoic acid-(3-phenylsulfonyl-1,2,5-oxadiazole-2-oxide-4)-oxybutyl))-15-oxo-7,20-epoxy-16-kaurene (**13i**). Yield 45%, m.p. 116–118 °C; IR v_{max} 3382, 2958, 2025, 1726, 1616, 1553, 1451, 1373, 733, 685 cm^{-1}; ^{1}H-NMR (CDCl$_3$), δ (ppm) 1.12 (6H, s, –CH$_3$), 1.97 (3H, s, –CH$_3$), 3.45 (1H, d, J = 9.9 Hz, 13-CH), 3.77 (1H, m, 6-CH), 4.21, 4.33 (each 1H, dd, $J_A = J_B$ = 10.5 Hz, 20-CH$_2$), 4.43 (2H, m, –CH$_2$), 4.50 (2H, t, J = 5.7 Hz, –CH$_2$), 4.64 (1H, m, 1-CH), 5.57 (1H, s, 17-CH$_2$), 6.07 (1H, s, 14-CH), 6.07 (1H, d, J = 10.2 Hz, 6–OH), 6.16 (1H, s,

17-CH$_2$), 7.54 (3H, m, Ar-H), 7.61 (2H, t, J = 7.8 Hz, Ar-H), 7.77 (2H, m, Ar-H), 8.06 (2H, d, J = 7.2 Hz, Ar-H); MS(ESI) *m/z*: 868.3 [M+NH$_4$]$^+$, 885.4 [M+Cl]$^-$; HR-MS (ESI, M+Na) *m/z*: calcd for C$_{42}$H$_{46}$N$_2$NaO$_{15}$S: 873.2511, found 873.2527.

3.2. In Vitro *MTT Assay*

The MTT assay was employed as an *in vitro* cytotoxicity assay, which was performed in 96-well plates. Test cells at the log phase of their growth cycle (5 × 10^4 cell/mL) were added to each well (100 µL/well), then treated in three replicates at various concentrations of the samples (0.39–100 µg/mL), and incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO$_2$. After 72 h, 20 µL of MTT solution (5 mg/mL) per well was added to each cultured medium, which was incubated for further 4 h. Then, DMSO was added to each well (150 µL/well). After 10 min at room temperature, the OD of each well was measured on a Microplate Reader (BIO-RAD instruments Inc. No. 550, Hercules, CA, USA) at a wavelength of 490 nm. In these experiments, the negative reference was 0.1% DMSO, and taxol was used as the positive reference with the concentration of 10 µg/mL.

3.3. NO-Releasing Test: Nitrate/Nitrite Measurement in Vitro

The levels of nitrate/nitrite formed from individual compounds were determined by the colorimetric assay using the nitrate/nitrite colorimetric assay kit, in triplicate with 100 μM of individual compounds for 0–60 min according to the manufacturer's instructions (Beyotime, Shanghai, China). The lysates were mixed with Griess for 40 min and centrifugalized for 10 min, and then followed by measuring at 540 nm, similar as previously reported [13–17].

4. Conclusions

In summary, a series of novel furoxan/oridonin hybrids were synthesized and tested for anti-proliferative activity against four human cancer cell lines by an *in vitro* MTT assay. Among them, compound **9h** exhibited the most potential anti-tumor activity against all test cell lines. The preliminary SAR of the target compounds was discussed based on the experimental data obtained. Furthermore, more than 15 µmol/L NO were produced by all target compounds at the 60 min time point, and the results showed that higher levels of NO releasing produced stronger anti-proliferative activity, so high levels of NO release by these hybrids could play a role in growth inhibition activity. These results suggested that NO-donor/natural product hybrids may provide a promising approach for the discovery of novel anti-tumor agents. Further studies on the structure modification of these hybrids and the mechanism of the derivatives are currently in progress and will be reported in due course.

References and Notes

1. Mocellin, S. Nitric oxide: Cancer target or anticancer agent? *Curr. Cancer Drug Targets* **2009**, *9*, 214–236.
2. Ignarro, L.J. Signal transduction mechanisms involving nitric oxide. *Biochem. Pharmacol.* **1991**, *41*, 485–490.
3. Fukuto, J.M.; Wink, D.A. Nitric oxide (NO): Formation and biological roles in mammalian systems. *Met. Ions Biol. Syst.* **1999**, *36*, 547–595.
4. Wink, D.A.; Ridnour, L.A.; Hussain, S.P.; Harris, C.C. The reemergence of nitric oxide and cancer. *Nitric Oxide* **2008**, *19*, 65–67.
5. Hirst, D.; Robson, T. Nitric oxide in cancer therapeutics: Interaction with cytotoxic chemotherapy. *Curr. Pharm. Des.* **2010**, *16*, 411–420.
6. Millet, A.; Bettaieb, A.; Renaud, F.; Prevotat, L.; Hammann, A.; Solary, E.; Mignotte, B.; Jeannin, J.F. Influence of the nitric oxide donor glyceryl trinitrate on apoptotic pathways in human colon cancer cells. *Gastroenterology* **2002**, *123*, 235–246.
7. Postovit, L.M.; Adams, M.A.; Lash, G.E.; Heaton, J.P.; Graham, C.H. Nitric oxide-mediated regulation of hypoxia-induced B16F10 melanoma metastasis. *Int. J. Cancer* **2004**, *108*, 47–53.
8. Findlay, V.J.; Townsend, D.M.; Saavedra, J.E.; Buzard, G.S.; Citro, M.L.; Keefer, L.K.; Ji, X.; Tew, K.D. Tumor cell responses to a novel glutathione S-transferase-activated nitric oxide-releasing prodrug. *Mol. Pharmacol.* **2004**, *65*, 1070–1079.
9. Yasuda, H.; Yamaya, M.; Nakayama, K.; Sasaki, T.; Ebihara, H.; Kanda, A.; Asada, M.; Inoue, D.; Suzuki, T.; Okazaki, T.; *et al*. Randomized phase II trial comparing nitroglycerin plus vinorelbine and cisplatin with vinorelbine and cisplatin alone in previously untreated stage IIIB/IV non-small-cell lung cancer. *J. Clin. Oncol.* **2006**, *24*, 688–694.
10. Siemens, D.R.; Heaton, J.P.; Adams, M.A.; Kawakami, J.; Graham, C.H. Phase II study of nitric oxide donor for men with increasing prostate-specific antigen level after surgery or radiotherapy for prostate cancer. *Urology* **2009**, *74*, 878–883.
11. Moharram, S.; Zhou, A.; Wiebe, L.I.; Knaus, E.E. Design and synthesis of 3'- and 5'-O-(3-benzenesulfonylfuroxan-4-yl)-2'-deoxyuridines: Biological evaluation as hybrid nitric oxide donor-nucleoside anticancer agents. *J. Med. Chem.* **2004**, *47*, 1840–1846.
12. Maksimovic-Ivanic, D.; Mijatovic, S.; Harhaji, L.; Miljkovic, D.; Dabideen, D.; Fan Cheng, K.; Mangano, K.; Malaponte, G.; Al-Abed, Y.; Libra, M.; *et al*. Anticancer properties of the novel nitric oxide-donating compound (S,R)-3-phenyl-4,5-dihydro-5-isoxazole acetic acid-nitric oxide *in vitro* and *in vivo*. *Mol. Cancer Ther.* **2008**, *7*, 510–520.
13. Chen, L.; Zhang, Y.; Kong, X.; Lan, E.; Huang, Z.; Peng, S.; Kaufman, D.L.; Tian, J. Design, synthesis, and antihepatocellular carcinoma activity of nitric oxide releasing derivatives of oleanolic acid. *J. Med. Chem.* **2008**, *51*, 4834–4838.
14. Lai, Y.; Shen, L.; Zhang, Z.; Liu, W.; Zhang, Y.; Ji, H.; Tian, J. Synthesis and biological evaluation of furoxan-based nitric oxide-releasing derivatives of glycyrrhetinic acid as anti-hepatocellular carcinoma agents. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6416–6420.

15. Ling, Y.; Ye, X.; Ji, H.; Zhang, Y.; Lai, Y.; Peng, S.; Tian, J. Synthesis and evaluation of nitric oxide-releasing derivatives of farnesylthiosalicylic acid as anti-tumor agents. *Bioorg. Med. Chem.* **2010**, *18*, 3448–3456.

16. Ling, Y.; Ye, X.; Zhang, Z.; Zhang, Y.; Lai, Y.; Ji, H.; Peng, S.; Tian, J. Novel nitric oxide-releasing derivatives of farnesylthiosalicylic acid: Synthesis and evaluation of antihepatocellular carcinoma activity. *J. Med. Chem.* **2011**, *54*, 3251–3259.

17. Tang, X.; Gu, X.; Ai, H.; Wang, G.; Peng, H.; Lai, Y.; Zhang, Y. Synthesis and evaluation of nitric oxide-releasing DDB derivatives as potential Pgp-mediated MDR reversal agents in MCF-7/Adr cells. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 801–805.

18. Kang, N.; Zhang, J.H.; Qiu, F.; Chen, S.; Tashiro, S.; Onodera, S.; Ikejima, T. Induction of G(2)/M phase arrest and apoptosis by oridonin in human laryngeal carcinoma cells. *J. Nat. Prod.* **2010**, *73*, 1058–1063.

19. Xu, J.; Yang, J.; Ran, Q.; Wang, L.; Liu, J.; Wang, Z.; Wu, X.; Hua, W.; Yuan, S.; Zhang, L.; *et al.* Synthesis and biological evaluation of novel 1-*O*- and 14-*O*-derivatives of oridonin as potential anticancer drug candidates. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4741–4744.

20. Wang, L.; Ran, Q.; Li, D.; Yao, H.; Zhang, Y.; Yuan, S.; Zhang, L.; Shen, M.; Xu, J. Synthesis and anti-tumor activity of 14-*O*-derivatives of natural oridonin. *Chin. J. Nat. Med.* **2011**, *9*, 194–198.

21. Wang, L.; Li, D.; Wang, C.; Zhang, Y.; Xu, J. Recent progress in the development of natural ent-kaurane diterpenoids with anti-tumor activity. *Mini-Rev. Med. Chem.* **2011**, *11*, 910–919.

22. Wang, L.; Li, D.; Xu, S.; Cai, H.; Yao, H.; Zhang, Y.; Jiang, J.; Xu, J. The conversion of oridonin to spirolactone-type or enmein-type diterpenoid: Synthesis and biological evaluation of ent-6,7-seco-oridonin derivatives as novel potential anticancer agents. *Eur. J. Med. Chem.* **2012**, *52*, 242–250.

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