Published in final edited form as:
Tetrahedron Lett. 2013 August 7; 54(32): 4300–4302. doi:10.1016/j.tetlet.2013.06.008.

Waol A, *trans*-dihydrowaol A, and *cis*-dihydrowaol A: polyketide-derived γ-lactones from a *Volutella* species

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Abstract

An organic extract of a filamentous fungus (MSX 58801), identified as a *Volutella* sp. (Hypocreales, Ascomycota), displayed moderate cytotoxic activity against NCI-H460 human large cell lung carcinoma. Bioactivity-directed fractionation led to the isolation of three γ-lactones having the furo[3,4-*b*]pyran-5-one bicyclic ring system [waol A (**1**), *trans*-dihydrowaol A (**2**), and *cis*-dihydrowaol A (**3**)]. The structures were elucidated using a set of spectroscopic and spectrometric techniques; the absolute configuration of **2** was established via a modified Mosher's ester method. Compounds **1** and **2** were evaluated for cytotoxicity against a human cancer cell panel.

Keywords

Polyketide; Cytotoxicty; γ-Lactone; Filamentous Fungi; Waol A

In pursuit of structurally diverse anticancer leads from nature,[1,2] our group has been investigating filamentous fungi, particularly the Mycosynthetix library, representing over 55,000 accessions.[3–9] Fungi represent an exciting reservoir of bioactive natural products, as they are an underexplored and renewable resource.[10–12]

An organic extract of the filamentous fungus MSX 58801, which was isolated from leaf litter in 1991, displayed moderate cytotoxic activity against NCI-H460 human large cell lung carcinoma (~86% inhibition of cell growth when tested at 20 μg/mL).[3] Bioactivity-

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directed fractionation using flash chromatography followed by preparative RP-HPLC resulted in the isolation of three γ-lactones (**1**–**3**) containing a furo[3,4-*b*]pyran-5-one bicyclic ring system, with >95% purity for compounds **1** and **2** according to UPLC (Figure S1, Supplementary data). Compounds **1** and **2** were evaluated for cytotoxicity against a human cancer cell panel.

Compound **1** (2.46 mg), which was obtained as a colorless oil, had a molecular formula of $C_{13}H_{16}O_4$ as determined by HRESIMS. The NMR (Figure S2, Supplementary data), HRMS, and optical rotation data identified **1** as the known compound, waol A (FD-211; Figure 1). First isolated in 1995 from a fermentation of *Myceliophthora lutea* TF-0409,[13] the structure of **1** was revised in 2003.[14,15]

Compound **2** (9.67 mg) was also obtained as a colorless oil.[16] The molecular formula was determined as $C_{13}H_{18}O_4$ via HRESIMS, establishing an index of hydrogen deficiency of 5. The NMR data suggested structural similarity with compound **1**. However, compound **2** lacked the olefinic proton at δ_H 6.90, which was replaced by three aliphatic protons (δ_H 1.79, 2.43, and 2.91). These data suggested a difference between **1** and **2** of a double bond, as supported by a 2 amu difference in the HRMS data. The ^{1}H NMR data of **2** revealed the presence of four olefinic protons, corresponding to two *trans*-disubstituted olefins (δ_H 5.52, ddq, J = 15.5, 8.0, 1.7; 5.55, ddq, J = 15.5, 5.2, 1.7; 5.91, dqd, J = 15.5, 6.9, 1.7; and 5.99, dq, J = 15.5, 6.9, for H-1″, H-1′, H-2′, and H-2″, respectively), four oxymethines (δ_H 3.48, dd, J = 12.0, 8.6; 3.84, bq, J = 2.9; 4.03, ddd, J = 5.2, 2.9, 1.7; and 4.67, dd, J = 8.6, 8.0, for H-7a, H-3, H-2, and H-7, respectively), one methine (δ_H 2.91, ddd, J = 12.6, 12.0, 3.4, for H-4a), one methylene (δ_H 1.79, ddd, J = 13.2, 12.6, 2.9; and 2.43, ddd, J = 13.2, 3.4, 2.9, for H-4α and H-4β, respectively), two equivalent methyls (δ_H 1.77, dd, J = 6.9, 1.7, for H-3′ and H-3″), and one exchangeable proton (δ_H 1.84, for 3-OH). The ^{13}C NMR data revealed 13 carbons, consistent with the HRMS data and indicative of one carbonyl (δ_C 173.5 for C-5), four olefinic carbons (δ_C 125.7, 126.4, 130.6, and 134.3, for C-1″, C-1′, C-2′, and C-2″, respectively), five methines (δ_C 39.0, 66.3, 81.2, 82.1, and 82.4 for C-4a, C-3, C-2, C-7a, and C-7, respectively), one methylene (δ_C 30.0 for C-4), and two methyls (δ_C 18.1 and 18.2 for C-3′ and C-3″, respectively) (see Supplementary Figures S3 and S4 for the ^{1}H and ^{13}C NMR spectra and Table S1). The two double bonds and the carbonyl group accounted for three degrees of unsaturations, leaving the remaining two accommodated by the bicyclic ring system. COSY data identified one spin system as H_3-3′/H-2′/H-1′/H-2/H-3/H_2-4/H-4a/H-7a/H-7/H-1″/H-2″/H_3-3″ (Figure 2a). The following key HMBC correlations were observed: H_3-3′→C-1′, H_3-3″→C-1″, H-2→C-2′, H-7→C-2″, H-3→C-4a, H-7a→C-4, H-4a→C-7, and H-4a→C-5 (Figure 2a). NOESY correlations from H-1″ to H-7a, from H-7a to H-2, and from H-2 to H-3 and H-2′ indicated that H-1″, H-7a, H-2, H-3, and H-2′ were all on the same face. Alternatively, NOESY correlations observed from H-4a to H-7 indicated that these two protons were on the same side of the molecule but opposite to the previous set (Figure 2b). Comparing all of these data with those for **1** yielded the structure of **2** (Figure 1), which was ascribed the trivial name *trans*-dihydrowaol A. The absolute configuration of **2** was assigned via a modified Mosher's ester method,[17] establishing the configuration as 2*R*, 3*R*, 4a*R*, 7*S*, and 7a*R* (Figure 3).[18]

Compound **3** (1.45 mg) was obtained as a colorless oil.[19] The molecular formula was determined as $C_{13}H_{18}O_4$ via HRESIMS, and was the same as compound **2**. The NMR data (Table S1 and Figures S5 and S6) suggested structural similarity with **2**. Key differences were a coupling constant of 0.6 Hz between H-4a (δ_H 2.58, ddd, J = 7.5, 2.3, 0.6) and H-7a (δ_H 4.17, dd, J = 4.6, 0.6) in **3** vs 12 Hz in **2**, and a NOESY correlation from H-4a to H-7a in **3** vs H-4a to H-7 in **2** (Figure 2d). These data implied a pseudoaxial/pseudoequatorial cis orientation of H-4a/H-7a. NOESY correlations were also observed from H-2 to H-7a and H-4a, and from H-4a to H-3, indicating that those protons were on the same face (Figure

2d). These data suggested an inversion in the configuration at C-4a in **3** relative to **2**, establishing the structure of **3** as an epimer of **2** (Figure 1). The trivial name, *cis*-dihydrowaol A (**3**), was ascribed to this compound. The relative configuration of **3** was assigned by comparison with **2** as 2*R*, 3*R*, 4a*S*, 7*S*, and 7a*R*. An attempt to establish the absolute configuration via a modified Mosher's ester method[17] was unsuccessful.

Compounds **1** and **2** were tested against two cancer cell lines, MDA-MB-435 (human melanoma) and SW-620 (human colon cancer), using methods described previously;[20,3] due to paucity of sample, compound **3** was not tested. While compound **1** showed moderate cytotoxic activity against the SW-620 cancer cell line, compound **2** was inactive against both cancer cell lines (Table 1), suggesting the importance of the double bond for cytotoxicity. Compound **1** was reported by Nozawa et al[13] to have broad spectrum activity against cultured tumor cell lines, including adriamycin-resistant HL-60 cells. Several compounds having the furo[3,4-*b*]pyran-5-one bicyclic ring system have been reported from fungi with diverse biological activities, including antibacterial and cytotoxic activities.[21–26]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by program project grant P01 CA125066 from the National Cancer Institute/National Institutes of Health, Bethesda, MD, USA. The high resolution mass spectrometry data were acquired at the Triad Mass Spectrometry Laboratory at the University of North Carolina at Greensboro. Sequence data was generated at the Mycology laboratory of Dr. Andrew N. Miller, Illinois Natural History Survey, University of Illinois at Urbana-Champaign.

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16. *trans*-Dihydrowaol A (**2**): colorless oil; $[\alpha]_D^{26} = -56°$ ($c = 0.1$, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Supplementary Data); HRESIMS *m*/*z* 239.1278 $[M+H]^+$ (calcd for $C_{13}H_{19}O_4$ 239.1278).
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18. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of *trans*-dihydrowaol A (**2**): To 0.75 mg of compound **2** was added 400 μL of pyridine-d_5 and transferred into an NMR tube. To initiate the reaction, 10 μL of *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride was added into the NMR tube with careful shaking and then monitored immediately by ^{1}H NMR at the following time points 0, 15, 30, and 60 min. The reaction was found to be complete within 30 min, yielding the mono (*R*)-MTPA ester derivative (**2b**) of **2**. ^{1}H NMR data of **2b** (500 MHz, pyridine-d_5): 5.93 (1H, m, H-2′), 5.89 (1H, m, H-1′), 5.69 (1H, m, H-2″), 5.60 (1H, m, H-1″), 5.53 (1H, bq, *J* = 2.3, H-3), 4.81 (1H, dd, *J* = 8.6, 8.0, H-7), 4.48 (1H, d, *J* = 5.7, H-2), 3.94 (1H, dd, *J* = 9.2, 8.6, H-7a), 2.69 (1H, m, H-4a), 2.67 (1H, m, H-4β), 2.29 (1H, m, H-4α), 1.63 (3H, d, *J* = 6.9, H_3-3′), and 1.55 (3H, d, *J* = 6.3, H_3-3″). In an analogues manner, 0.75 mg of compound **2** dissolved in 400 μL pyridine-d_5 was reacted in a second NMR tube with 10 μL (*R*)-(−)-α-MTPA chloride for 30 min, to afford the mono (*S*)-MTPA ester (**2a**). ^{1}H NMR data of **2a** (500 MHz, pyridine-d_5): δ_H 5.88 (1H, m, H-2′), 5.77 (1H, m, H-1′), 5.70 (1H, m, H-2″), 5.60 (1H, m, H-1″), 5.56 (1H, bq, *J* = 3.4, H-3), 4.89 (1H, dd, *J* = 8.6, 8.0, H-7), 4.15 (1H, d, *J* = 6.9, H-2), 3.85 (1H, m, H-7a), 2.84 (1H, m, H-4a), 2.77 (1H, m, H-4β), 2.37 (1H, m, H-4α), 1.53 (3H, d, *J* = 6.3, H_3-3′), and 1.50 (3H, d, *J* = 6.3, H_3-3″).
19. *cis*-Dihydrowaol A (**3**): colorless oil; $[\alpha]_D^{26} = -32°$ ($c = 0.1$, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) (see Supplementary Data); HRESIMS *m*/*z* 239.1280 $[M+H]^+$ (calcd for $C_{13}H_{19}O_4$ 239.1278).
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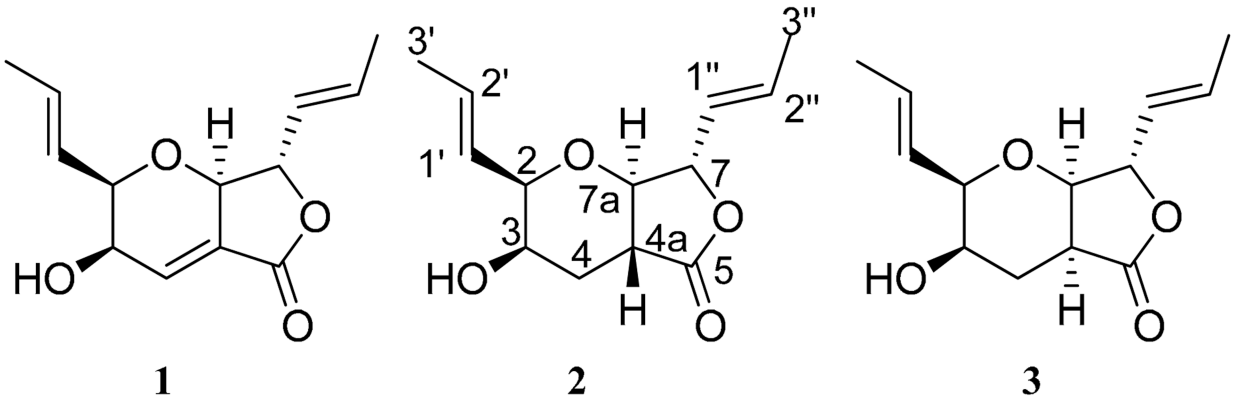

Figure 1.
Structures of compounds **1**–**3**.

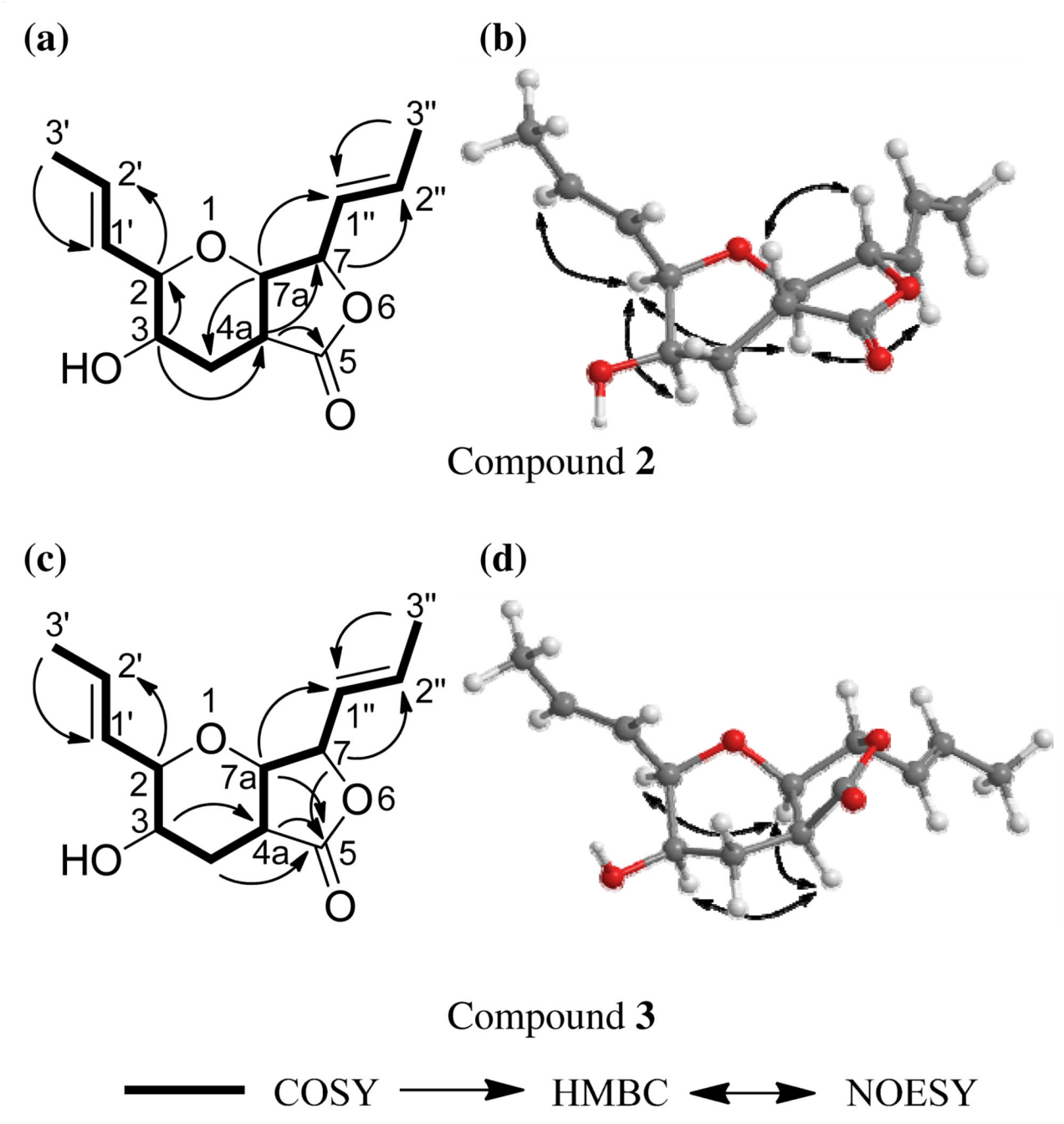

Figure 2.
Key HMBC, COSY, and NOESY correlations of **2** and **3**.

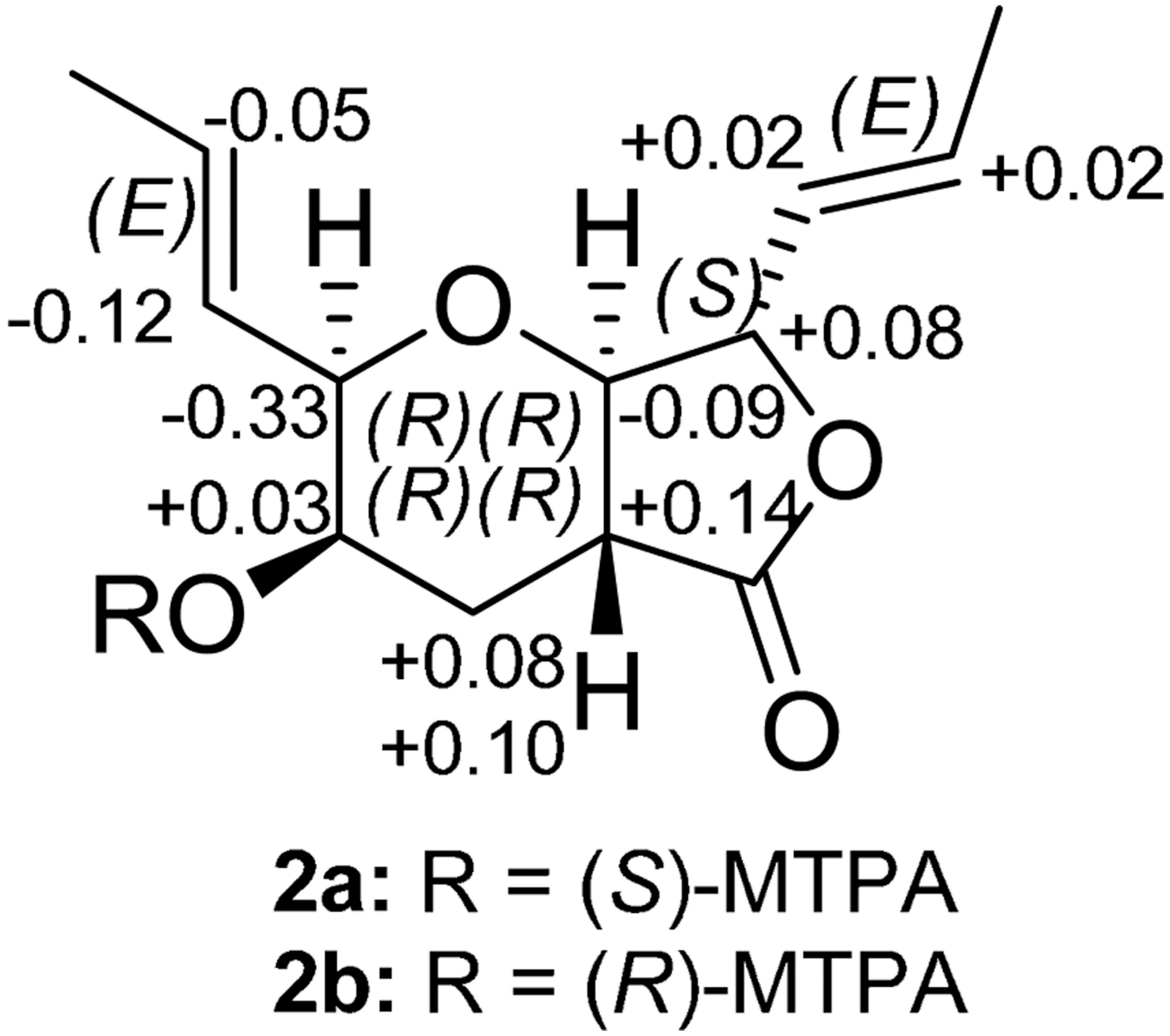

Figure 3.

$\Delta\delta_H$ values [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**2a** and **2b**, respectively) of *trans*-dihydrowaol A (**2**) in pyridine-d_5.

Table 1

Cytotoxicity of compounds **1** and **2** against two human tumor cell lines[a]

Compound	IC_{50} values in μM	
	MDA-MB-435[b]	SW-620[c]
Waol A (**1**)	39.6	13.8
trans-Dihydrowaol A (**2**)	>40	>40

[a] Positive controls were vinblastine and bortezomib. Vinblastine was tested at concentrations of 3 ng/mL and 1 ng/mL: MDA-MB-435 cells had 37% and 99% viable cells; SW620 cells had 76% and 90% viable cells; respectively. Bortezomib was tested at concentrations of 5 nM and 2.5 nM: MDA-MB-435 cells had 90% and 91% viable cells; SW620 cells had 79% and 71% viable cells; respectively.

[b] melanoma

[c] colon tumor cell lines were tested using published protocols.[3,20]