JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Total Synthesis and Biological Activity of the Proposed Structure of Phaeosphaeride A

Anthoula Chatzimpaloglou,[†] Maria P. Yavropoulou,[‡] Karien E. Rooij,[§] Ralf Biedermann,[∥] Uwe Mueller,[⊥] Stefan Kaskel,[∥] and Vasiliki Sarli*,[†]

[†]Faculty of Chemistry, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece

[‡]Department of Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, Netherlands

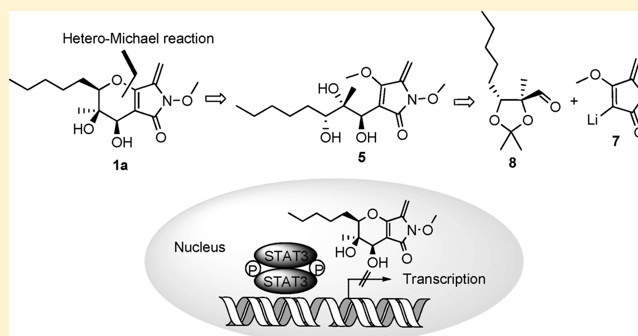
[§]Department of Radiology, Leiden University Medical Center, Leiden, Netherlands

[∥]Department of Inorganic Chemistry, Dresden University of Technology, Mommenstr. 6, 01069 Dresden, Germany

[⊥]Department BESSY-MX Group, F-12, Helmholtz Zentrum Berlin für Materialien und Energie, Albert-Einstein-Str. 15, 12489 Berlin, Germany

Supporting Information

ABSTRACT: The total synthesis of the structure assigned to the natural product phaeosphaeride A **1a** was accomplished. The key steps involve the addition of vinyllithium reagent **7** to the acetonide-protected aldehyde **8** to access the carbon backbone of **1a**, the introduction of the methoxylamino group followed by intramolecular hetero-Michael cyclization, and methanol elimination to form the dihydropyran ring. In this study, both enantiomers of **1a** were synthesized and tested for biological activity. Preliminary results showed that (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** inhibit STAT3-dependent transcriptional activity in a dose-dependent manner and exhibit antiproliferative properties in breast (MDA-MB-231) and pancreatic (PANC-1) cancer cells.



■ INTRODUCTION

The signal transducer and activator of transcription factors (STATs) regulate the expression of genes that mediate many physiological processes including cell growth, survival, differentiation, and motility.[1] Upon stimulation, STATs become transcriptionally active by phosphorylation on specific tyrosine residues, which leads to dimer formation, nuclear translocation, and gene transcription.[2] Among the STAT family members, STAT3 and STAT5 proteins are believed to contribute to the pathogenesis of a variety of human solid tumors and blood malignancies.[3] The possible downstream targets on which STAT3 promotes oncogenesis may be through the transcription of proliferation and antiapoptosis-associated genes,[4] such as BCL-2, BCL-XL, IL-17, IL-23, MCL1, survivin,[5] and tumor angiogenesis (VEGF, HIF-1).[6] Importantly, blocking STAT3 signaling in tumor cells by a dominant negative form of STAT3, antisense approaches, or siRNAs has been shown to induce apoptosis, inhibit cell proliferation, suppress angiogenesis, and stimulate immune responses.[7] Therefore, small molecule inhibitors of STAT3 signaling are of great therapeutic potential. So far, a number of STAT3 inhibitors have been identified by rational design or through the screening of chemical libraries. Examples include peptidomimetics,[8] small molecules (STA21, stattic, S3I-201, STX-0119),[9] and natural products (curcumin, galiellalactone, cucurbitacins).[10] Despite these efforts, the design of potent and cell-permeable STAT3 inhibitors remains a highly challenging task. Currently, there are no STAT3 inhibitors on the market, although some have entered clinical trials for treatment of solid tumors and lymphomas.

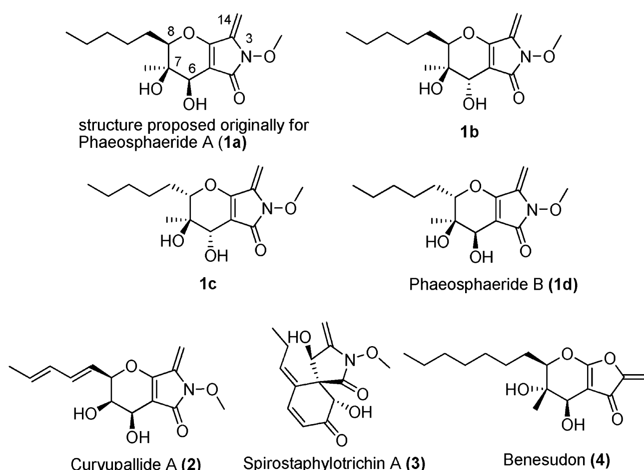
In 2006, Clardy and co-workers isolated phaeosphaeride A and its stereoisomer, phaeosphaeride B, from the endophytic fungus FA39 (*Phaeosphaeria avenaria*).[11] Phaeosphaeride A was reported to selectively inhibit STAT3/DNA binding with an IC_{50} of 0.61 mM and exhibit promising cell growth inhibition in STAT3-dependent U266 multiple myeloma cells with an IC_{50} of 6.7 μM. Interestingly, phaeosphaeride B was inactive against STAT3.

Phaeosphaerides A and B are structurally related to the fungal metabolites curvupallide A,[12] the spirocyclic lactams spirostaphylotrichins/triticones,[13] and the antibiotic benesudon (Scheme 1).[14] Phaeosphaerides contain a dihydroxylated dihydropyran ring fused to a five-membered cyclic *O*-methyl hydroxamate with an exocyclic C3−C14 double bond. The interesting structural features coupled with their significant biological activity have made them attractive synthetic targets. Clardy and co-workers proposed structure **1a** for phaeosphaer-

Received: August 17, 2012
Published: October 10, 2012

 © 2012 American Chemical Society

Scheme 1. Originally Proposed Structure of Phaeosphaeride A, Its Isomers, and Three Structurally Related Natural Products



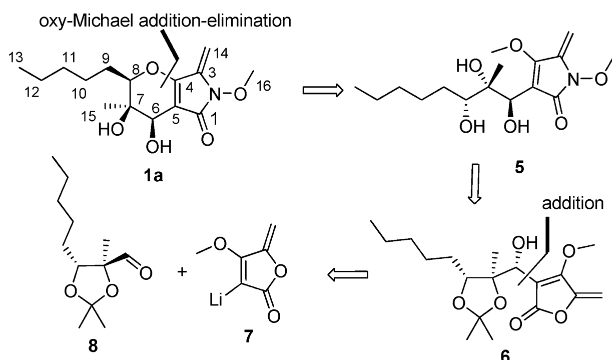
ide A and structure **1d** for phaeosphaeride B, on the basis of two-dimentional NMR data and mass spectrometric analysis. However, both the present work and the recent publication of Tamura suggest an incorrect assignment of the reported structure of phaeosphaeride A.[15]

Herein, we report our investigation leading to the stereoselective synthesis of the proposed structure of phaeosphaeride A **1a** and, additionally, the asymmetric synthesis of both enantiomers of **1a** that have been achieved by applying the Sharpless asymmetric dihydroxylation reaction. Preliminary biological evaluation of the synthetic phaeosphaerides revealed potent STAT3 inhibitors that exhibit antiproliferative activity in human breast and pancreatic cancer cells.

■ RESULTS AND DISCUSSION

According to our retrosynthetic strategy, the carbon backbone of **1a** was envisioned to arise from the addition of vinyllithium reagent **7** to the acetonide-protected aldehyde **8** (Scheme 2).

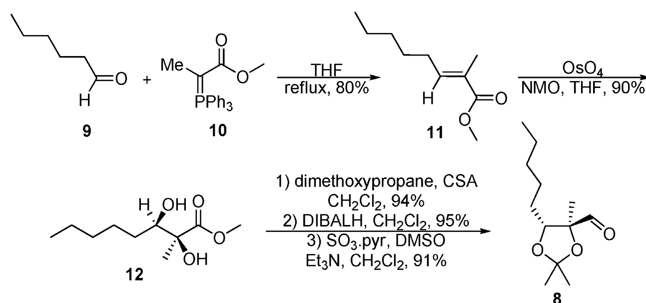
Scheme 2. Retrosynthetic Analysis of Compound 1a



Introduction of the methoxylamino group, followed by acetonide deprotection, was anticipated to provide precursor **5**, ideally functionalized for the preparation of the dihydropyran ring. Finally, the target molecule was planned to arise from an intramolecular hetero-Michael 6-endo-trig cyclization followed by an elimination of methanol.

The synthesis of aldehyde **8** was first investigated (Scheme 3). Hexanal was treated with the stabilized ylid methyl

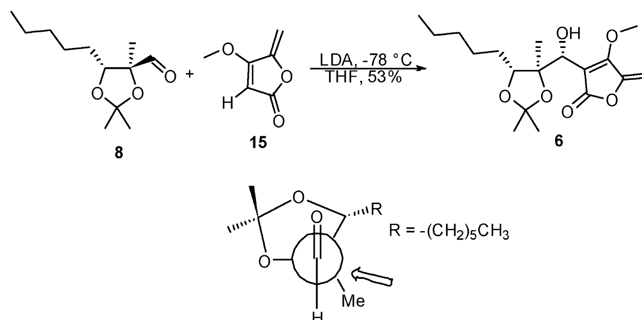
Scheme 3. Synthesis of Aldehyde 8



(triphenylphosphoranylidene) propionate **10** to give the *E*-ester in high isomeric purity (>97%). Wittig reagent **10** was prepared from methyl bromopropionate as described in the literature.[16] Consecutively, olefin **11** was dihydroxylated with OsO_4 and *N*-methylmorpholine-*N*-oxide (NMO) in THF to stereoselectively give the diol **12** in 90% yield as a white solid. After protection of the vicinal diol system in the form of an acetonide, the ester group was reduced by DIBALH, and the alcohol was successfully oxidized with the SO_3·py complex in DMSO and Et_3N to aldehyde **8** in 91% yield.

With aldehyde **8** in hand, we focused our attention to the key reaction of lithiated tetronate **7** to **8**. Tetronate derivative **15** was prepared from methyl tetronate in a three-step sequence according to Yoshii et al.[17] Deprotonation of **15** with LDA in THF at −78 °C as described by Yoshii[18] and addition of the resulting vinyllithium species to aldehyde **8** gave intermediate **6** as a single stereoisomer in 53% yield along with partial recovery of starting materials. The *anti*-selective addition to aldehyde **8** may be explained by using the Felkin−Ahn transition state shown below (Scheme 4).[19]

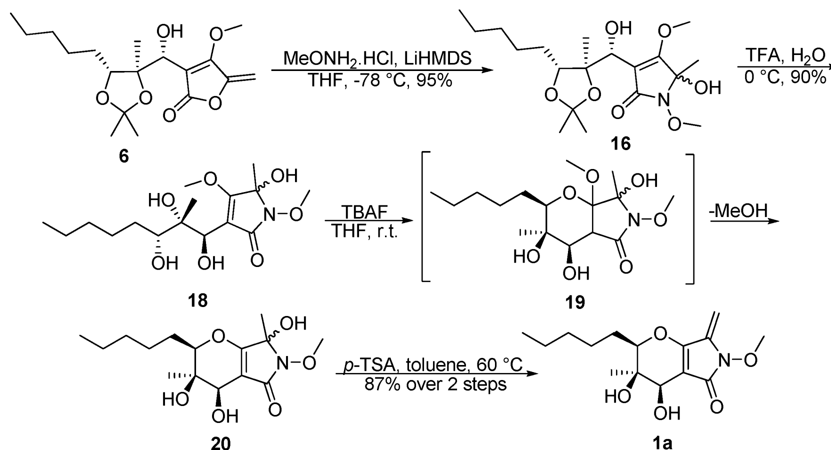
Scheme 4. Synthesis of Tetronate 6



The following steps involved the introduction of the methoxylamino group and the subsequent formation of the dihydropyran ring. Reaction of tetronate **6** with $MeONH_2$·HCl, using LiHMDS as a base in THF at −78 °C, easily afforded derivatives **16** in high yield (95%).[20] After acetonide deprotection of **16** with TFA/H_2O, the cyclization of the resulting triol was investigated. Initial attempts were focused on the acid-catalyzed ring closure of **18** to provide the desired dihydropyrans **20** through a transition state of the 6-endotrig type.[21]

Upon treatment of **18** with *p*-TsOH in toluene at 60 °C or TFA in dichloromethane, the hetero-Michael reaction took place, but a complicated reaction mixture was obtained including intermediate compounds **19** and products of acid-catalyzed alcohol dehydration. On the other hand, treatment of

Scheme 5. Completion of the Synthesis of 1a



18 with TBAF in THF gave rise to the dihydropyrans **20** at room temperature. The final transformation necessary to complete the total synthesis of **1a** was the regioselective dehydration of **20**. Rewardingly, exposure of intermediates **20** to 1 equiv of *p*-TsOH in toluene produced **1a** in 87% yield (over two steps) (Scheme 5).

The stereochemistry of C-6 of **1a** was determined by NOESY experiments (Figure 1). However, the spectral data of synthetic

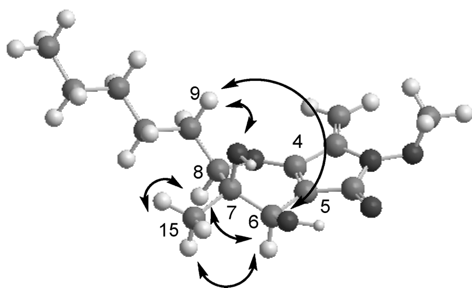


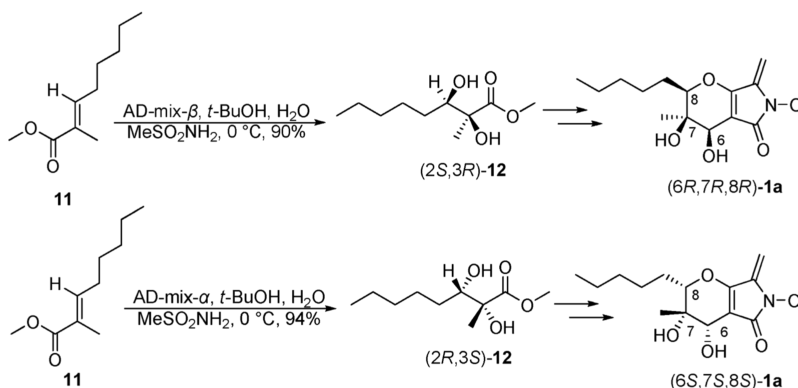
Figure 1. Selected NOESY correlations of compound **1a**.

1a did not match with the data reported for the natural phaeosphaeride A, leading to the conclusion that the structure of **1a** assigned for phaeosphaeride A is incorrect. Detailed structural analysis using 2D-NMR and NOESY fully supported our assignment of synthetic **1a** as the proposed structure of phaeoshaeride A. For example, H-6 correlated with H-8, and both of them correlated with H-15. Furthermore, the correlation between H-9 and OH-6 as well as OH-7 suggests that these protons are positioned on the same side of the ring. These findings are in agreement with the published data for the proposed structure of phaeosphaeride A by Tamura's group.[15]

Synthesis of (6*R*,7*R*,8*R*)-1a and (6*S*,7*S*,8*S*)-1a. The synthesis of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** was also successful by using the Sharpless asymmetric dihydroxylation of unsaturated ester **11**, providing sufficient quantities of material for biological evaluation of both enantiomers. Compound **11** was asymmetrically oxidized with commercial AD-mix-β reagent to form (2*S*,3*R*)-**12** in enantioexcess ≥91% ee and 90% yield or with AD-mix-*a* reagent to give (2*R*,3*S*)-**12** in enantioexcess ≥82% ee and 94% yield (Scheme 6).[22] The enantiomeric excess of (2*S*,3*R*)-**12** and (2*R*,3*S*)-**12** was indirectly determined by conversion of both enantiomers to the corresponding (*S*)-mandelate esters and ^{1}H NMR analysis of the resulting diastereomeric mixture. It should be noted that Tamura et al. reported the asymmetric dihydroxylation of ethyl (*E*)-2-methyl-oct-2-enoate under similar conditions (AD-mix-β, $MeSO_2NH_2$ in *t*-BuOH, H_2O), but with higher ee values (98% ee, 99% yield).[15] Intermediates (2*S*,3*R*)-**12** and (2*R*,3*S*)-**12** were then advanced through the previously described steps to complete the synthesis of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a**.

In addition, we were able to determine the crystal structure of synthetic (6*R*,7*R*,8*R*)-**1a** by synchrotron radiation, which confirmed our NMR structural and stereochemical assignments (Figure 2).[23]

Scheme 6



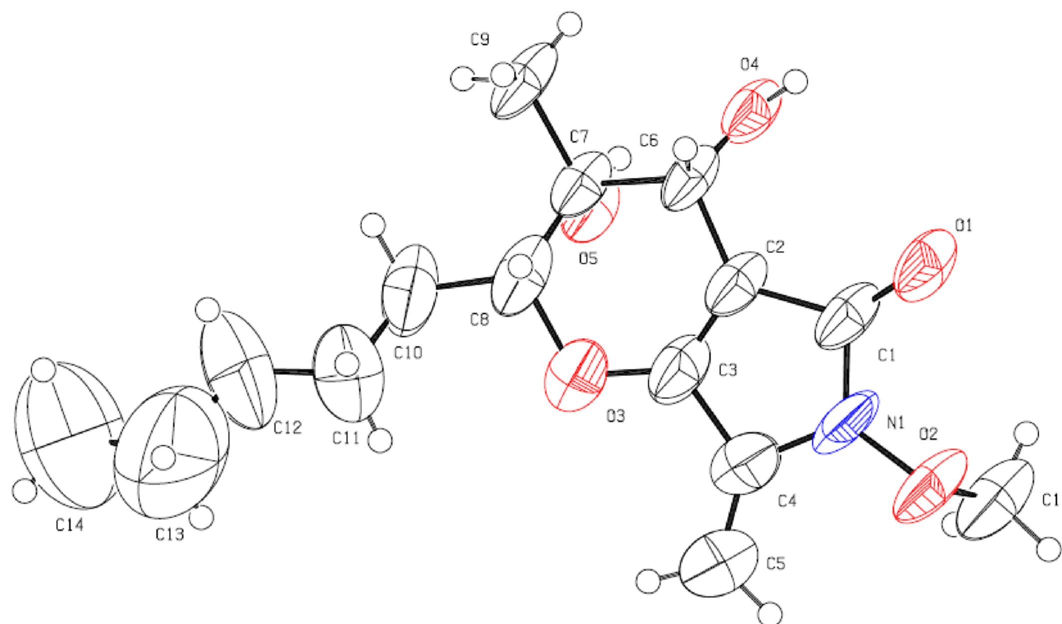


Figure 2. Crystal structure of (6*R*,7*R*,8*R*)-**1a**.

Biological Evaluation of (6*R*,7*R*,8*R*)-1a and (6*S*,7*S*,8*S*)-1a. *Inhibition of STAT3-Dependent Transcriptional Activity.* Compounds (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** were examined for their effects in rat preosteoblastic cells (UMR106), and (6*S*,7*S*,8*S*)-**1a** was further tested in breast (MDA-MB-231) and pancreatic (PANC-1) cancer cells. In all cases, cells were transiently transfected with STAT3-specific luciferase reporter construct, and STAT-3 phosphorylation was induced by human oncostatin M (hOSM) at a concentration of 10 ng/mL for 6 h.[24] Oncostatin M belongs to the family of gp130-signaling cytokines, which upon binding to the IL-6/GP130 receptors induce receptor dimerization, activation of the associated Jak kinases, and phosphorylation of the SH2-containing STAT-S.[25−27]Activation of STAT3 signaling cascade after treatment with hOSM has been previously reported for UMR106, PANC-1, and MDA-MB-231 cells.[27−29] Results from the luciferase assay showed both enantiomers to significantly reduce STAT3-dependent transcriptional activity in UMR106 cells with similar potency in a dose-dependent manner. The maximum dose tested was 20 μM (Figure 3a). Compound (6*S*,7*S*,8*S*)-**1a** displayed a comparable inhibitory effect in PANC-1 and MDA-MB-231 cancer cells, showing higher potency in the former cell line (Figure 3b).

To determine whether the ability of the enantiomers to suppress hOSM-induced STAT3 activity in rat preosteoblastic cells was due to inhibition of cell proliferation, a cell viability assay (MTS) was performed using the same time points and inhibitor concentrations as in the transcriptional activity experiments. No effect on cell proliferation was observed after 6 h incubation, indicating that the compounds were able to reduce STAT3 activity without affecting the survival of the treated cells (Figure 4).

Inhibition of Cell Proliferation in PANC-1 and MDA-MB-231 Cancer Cells. Constitutive activation of STAT3 has been associated with invasion, survival, and growth of a number of cancers.[30] Therefore, we examined the growth inhibitory activities of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** in PANC-1 and MDA-MB-231 cells that express high levels of STAT3. A luminescence cell viability assay (Cell Titer Glo) was used to generate dose−response curves and evaluate cell viability following 72 h of treatment with different concentrations of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a**.

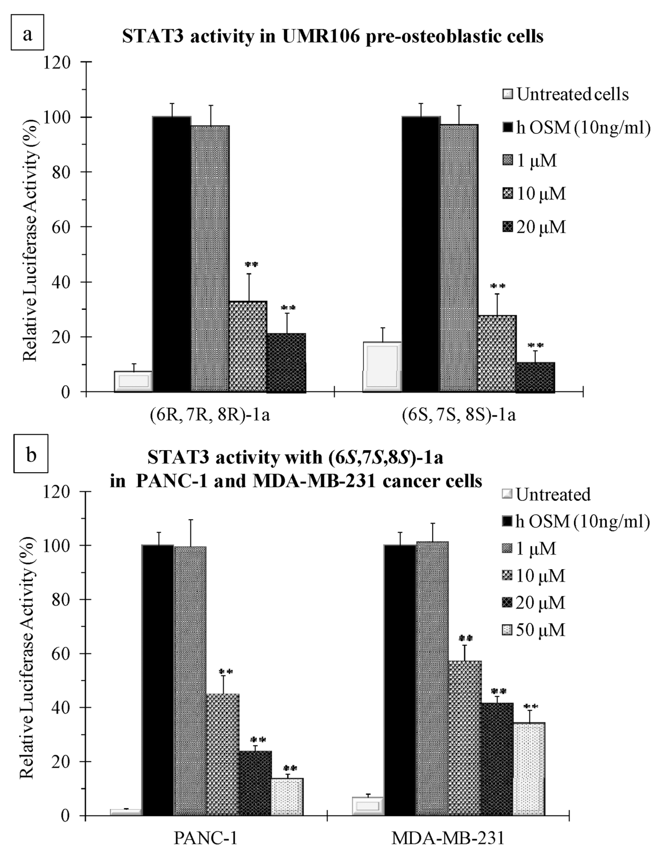


Figure 3. STAT3-dependent transcriptional activity. Cells were transiently transfected with a STAT3-specific luciferase reporter construct, followed by treatment with 10 ng/mL of hOSM and different concentrations of the inhibitors for 6 h. Results are reported relative to cells treated with 10 ng/mL of hOSM alone (**:$p < 0.001$).

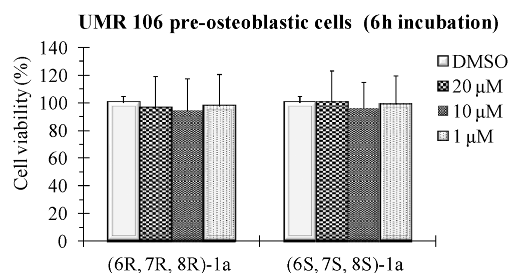


Figure 4. Cell toxicity of STAT3 inhibitors in rat preosteoblastic cells. Results are reported relative to untreated cells.

Gemcitabine, a chemotherapeutic agent for pancreatic and breast cancer, shown in a recent study to be more beneficial on pancreatic cancer patients compared to conventional medications,[31] has been used as a positive control. Both compounds decreased significantly malignant cell proliferation in a dose-dependent manner, in the low micromolar range (Figure 5), consistently with their capacity to inhibit STAT3 signaling.

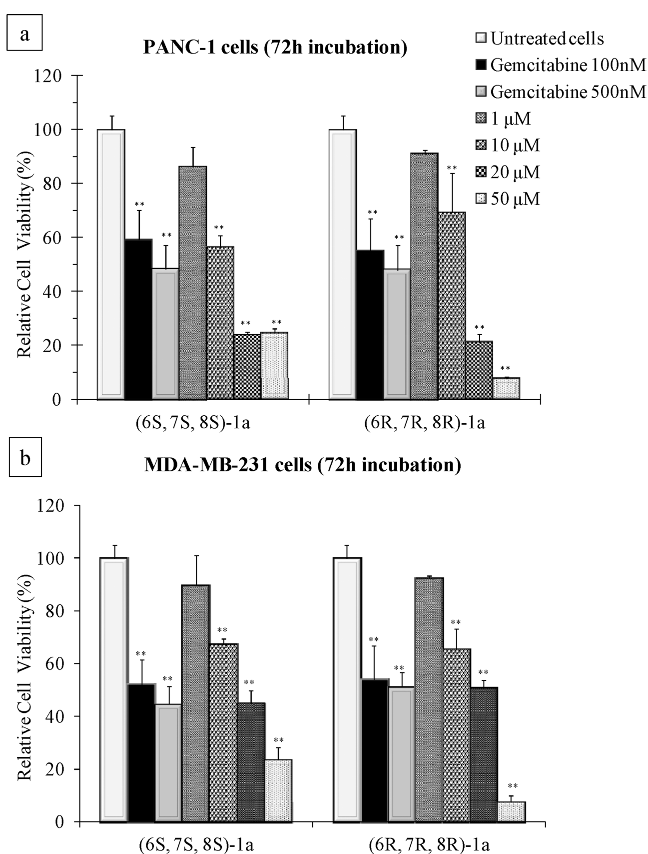


Figure 5. Cell toxicity of STAT3 inhibitors in cancer cells compared to chemotherapy drug gemcitabine. Results are reported relative to untreated cells (**:p < 0.001 compared to untreated cells).

CONCLUSIONS

In summary, we have synthesized the originally proposed structure of phaeosphaeride A **1a** in a highly diastereoselective manner from readily available starting materials. Both enantiomers of **1a** were synthesized and evaluated for their ability to inhibit STAT3-dependent transcriptional activity. Our results demonstrate that (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** decrease the growth of pancreatic (PANC-1) and human breast (MDA-MB-231) cancer cells in the low micromolar range. On the basis of our experience and considering the fact that the original isolation spectra of phaeosphaeride A indicate a NOESY correlation between H-6 and H-8, the most probable structure for phaeosphaeride A is **1c** or its enantiomer. Further investigation leading to the assignment of the natural phaeosphaeride A structure and detailed studies on the mechanism of phaeosphaeride-induced STAT3 inhibition are in progress and will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Details. All reactions were carried out under an atmosphere of Ar unless otherwise specified. Commercial reagents of high purity were purchased and used without further purification, unless otherwise noted. Reactions were monitored by TLC and using UV light as a visualizing agent and aqueous ceric sulfate/phosphomolybdic acid, ethanolic *p*-anisaldehyde solution, potassium permanganate solution, and heat as developing agents. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, and tetramethylsilane was used as an internal standard. Chemical shifts are indicated in δ values (ppm) from internal reference peaks (TMS ^{1}H 0.00; $CDCl_3$ ^{1}H 7.26, ^{13}C 77.00; DMSO-d_6 ^{1}H 2.50, ^{13}C 39.51). Melting points (m.p.) are uncorrected. High-resolution mass spectra (HRMS) were recorded on a mass spectrometer at a 4000 V emitter voltage.

(*E*)-Methyl 2-Methyloct-2-enoate, 11. To a solution of hexanal (10 g, 100 mmol) in THF (400 mL) was added methyl (triphenylphosphoranylidene) propionate (104 g, 300 mmol) at room temperature. Then, the reaction mixture was stirred for 8 h at the reflux temperature. The solvent was removed under reduced pressure. After the addition of Et_2O (80 mL), the mixture was filtered and concentrated. The crude product was purified by flash chromatography (eluent; hexane/ethyl acetate = 20/1) to afford (*E*)-methyl 2-methyloct-2-enoate (13.61 g, 80%) as yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 6.0 Hz, 3H), 1.26−1.33 (m, 4H), 1.39−1.46 (m, 2H), 1.83 (s, 3H), 2.17 (dd, J = 7.5, 14.5 Hz, 2H), 3.74 (s, 3H), 6.77 (dt, J = 9.0, 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 12.2, 13.8, 22.4, 28.1, 28.5, 31.4, 51.5, 127.2, 142.7, 168.6; FT-IR 1647, 1717, 2858, 2928, 2955; HRMS (ESI-TOF) m/z for $C_{10}H_{19}O_2$ $[M + H]^+$ calcd 171.1379, found 171.1380.

Methyl 2,3-Dihydroxy-2-methyloctanoate, (±)-12. To a solution of 3 g (17.6 mmol) of olefin **11** in 180 mL of THF were added 6.2 g (52.9 mmol) of *N*-methylmorpholine *N*-oxide and 1.7 mL of a 2.5% by weight solution of OsO_4 in *tert*-butanol, and the mixture was stirred for 24 h. Aqueous solution of sodium bisulfite (100 mL) was then added, and the resulting mixture was stirred for 30 min. The aqueous layer was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure. Purification by flash chromatography (eluent; hexane/ethyl acetate = 1/1) afforded the diol **12** (3.22 g, 15.8 mmol) in 90% yield as a white solid: mp = 40−42 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.0 Hz, 3H), 1.26−1.44 (m, 6H), 1.35 (s, 3H), 1.55−1.60 (m, 2H), 1.85 (br, 1H), 3.34 (br, 1H), 3.70 (d, J = 9.6 Hz, 1H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.9, 21.6, 22.5, 25.5, 30.2, 31.7, 52.9, 75.5, 77.5, 176.7; FT-IR 1735, 2858, 2954, 3448; HRMS (ESI-TOF) m/z for $C_{10}H_{21}O_4$ $[M + H]^+$ calcd 205.1434, found 205.1435.

(2*S*,3*R*)-Methyl 2,3-Dihydroxy-2-methyloctanoate, (+)-12. To a stirring solution of AD-mix-β (20.9 g, 1.4 g/mmol) in 58 mL of *t*-BuOH/H_2O (1:1) was added methanesulfonamide (2.86 g, 30.0 mmol, 2.00 equiv). The solution was stirred well for 15 min until one phase was present. The reaction mixture was cooled to 0 °C, and a solution of ester **11** (2.55 g, 15 mmol, 1.00 equiv) in 58 mL of *t*-BuOH/H_2O (1:1) was added. The reaction mixture was warmed to room temperature over 2 h and stirred overnight. The mixture was quenched with a saturated solution of Na_2SO_3 and stirred for 1 h. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under

reduced pressure. The product was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 5/1) to give 2.76 g of diol (+)-**12** as a white solid (90%, ee > 91%). $[\alpha]_D^{23}$ +36.2 (c= 0.714 g/mL, $CHCl_3$). The enantiomeric purity was determined by comparative analysis of ^{1}H NMR spectra of the corresponding mandelate ester.

(2*S*,3*R*)-Methyl 3-((*S*)-2-Acetoxy-2-phenylacetoxy)-2-hydroxy-2-methyloctanoate, S-1. To a solution of diol (+)-**12** (35 mg, 0.171 mmol) in 2.2 mL of CH_2Cl_2 were added (*S*)-(+)-α-acetoxyphenylacetic acid (35 mg, 0.18 mmol), dicyclohexylcarbodiimide (39 mg, 0.188 mmol), and a catalytic amount of DMAP (≈3 mg) successively. After 45 min, the CH_2Cl_2 was removed, and the residue was diluted with ether and washed with water. The aqueous layer was extracted with ether, and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 2/1) to give 50 mg of (*S*)-mandelate ester as a white solid (77%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.74 (t, *J* = 7.2 Hz, 3H), 0.85−1.26 (m, 6H), 1.35 (s, 3H), 1.50 (m, 2H), 2.17 (s, 3H), 3.31 (br, 1H), 3.72 (s, 3H), 5.14 (dd, *J* = 4.7, 7.5 Hz, 1H), 5.81 (s, 1H), 7.34−7.38 (m, 3H), 7.44−7.47 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.7, 20.5, 22.0, 22.1, 24.4, 28.1, 31.4, 53.1, 74.4, 76.1, 78.2, 127.6, 128.6, 129.2, 133.6, 168.1, 169.9, 175.2; HRMS (ESI-TOF) *m*/*z* for $C_{20}H_{28}O_7Na$ $[M + Na]^+$ calcd 403.1727, found 403.1729.

(2*R*,3*S*)-Methyl 2,3-Dihydroxy-2-methyloctanoate, (−)-12. To a stirring solution of AD-mix-α (25 g, 1.4 g/mmol) in 68 mL of *t*-BuOH/H_2O (1:1) was added methanesulfonamide (3.34 g, 35.2 mmol, 2.00 equiv). The solution was stirred well for 15 min until one phase was present. The reaction mixture was cooled to 0 °C, and a solution of ester **11** (3 g, 17.6 mmol, 1.00 equiv) in 35 mL of *t*-BuOH/H_2O (1:1) was added. The reaction mixture was warmed to room temperature over 2 h and stirred overnight. The mixture was quenched with a saturated solution of Na_2SO_3 and stirred for 1 h. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 5/1) to give 3.38 g of (2*R*,3*S*)-methyl 2,3-dihydroxy-2-methyloctanoate as a white solid (94%, ee ≥ 82%). $[\alpha]_D^{23}$ −30.7 (*c* = 0.724 g/mL, $CHCl_3$). The enantiomeric purity was determined by comparative analysis of ^{1}H NMR spectra of the corresponding mandelate ester.

(2*R*,3*S*)-Methyl 3-((*S*)-2-Acetoxy-2-phenylacetoxy)-2-hydroxy-2-methyloctanoate, S-2. To a solution of (2*R*,3*S*)-methyl 2,3-dihydroxy-2-methyloctanoate (20 mg, 0.097 mmol) in 1.3 mL of CH_2Cl_2 were added (*S*)-(+)-α-acetoxyphenylacetic acid (20 mg, 0.102 mmol), dicyclohexylcarbodiimide (22 mg, 0.106 mmol), and a catalytic amount of DMAP (≈1.7 mg) successively. After 45 min, the CH_2Cl_2 was removed, and the residue was diluted with ether and washed with water. The aqueous layer was extracted with ether, and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 2/1) to give 30 mg of (*S*)-mandelate ester as a white solid (81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, *J* = 6.5 Hz, 3H), 1.08−1.35 (m, 6H), 1.23 (s, 3H), 1.65 (m, 2H), 2.18 (s, 3H), 3.14 (s, 1H), 3.35 (s, 3H), 5.11 (t, *J* = 6.5 Hz, 1H), 5.84 (s, 1H), 7.34−7.40 (m, 3H), 7.45−7.49 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 13.8, 20.5, 22.2, 22.3, 24.8, 28.4, 31.5, 52.5, 74.6, 75.9, 78.2, 127.5, 128.6, 129.1, 133.6, 168.1, 170.1, 174.8; HRMS (ESI-TOF) *m*/*z* for $C_{20}H_{28}O_7Na$ $[M + Na]^+$ calcd 403.1727, found 403.1737.

Methyl 2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carboxylate, (±)-S-3. To a stirring solution of diol (±)-**12** (2 g, 9.79 mmol) in 20 mL of CH_2Cl_2 and dimethoxypropane (1.48 mL, 11.75 mmol) was added CSA (100 mg) at 0 °C. The mixture was stirred at room temperature overnight. Then, the mixture was quenched with a saturated solution of $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The product was purified by flash chromatography on silica gel (eluent; hexane/ethyl acetate = 5/1) to give 2.25 g of compound (±)-**S-3** (94%). (±)-**S-3**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 6.0 Hz, 3H), 1.32 (s, 3H), 1.32−1.34 (m, 6H), 1.39 (s, 3H), 1.46 (s, 3H), 1.49−1.59 (m, 2H), 3.76 (s, 3H), 4.17 (t, *J* = 6.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.9, 19.7, 22.4, 25.4, 26.3, 28.2, 29.5, 31.7, 52.3, 80.2, 82.5, 108.7, 173.7; FT-IR 1736, 2936, 2954, 2989; HRMS (ESI-TOF) *m*/*z* for $C_{13}H_{25}O_4$ $[M + H]^+$ calcd 245.1747, found 245.1742.

*(4S,5R)-Methyl 2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carboxylate (+)-**S-3**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**S-3**, starting from (+)-**12**. $[\alpha]_D^{20}$ +4.38 (*c* = 3.33 g/mL, $CHCl_3$).

*(4R,5S)-Methyl 2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carboxylate (−)-**S-3**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**S-3**, starting from (−)-**12**. $[\alpha]_D^{20}$ −4.0 (*c* = 3.00 g/mL, $CHCl_3$).

2,2,4-Trimethyl-5-pentyl-1,3-dioxolan-4-yl Methanol, (±)-S-4. To a solution of ester (±)-**S-3** (2 g, 8.19 mmol) in CH_2Cl_2 (45 mL) was added a solution of DIBALH (1 M in dichloromethane, 24.6 mL, 24.6 mmol) at 0 °C. After the reaction mixture had been stirred for 30 min at the same temperature, methanol was added. The mixture was allowed to warm to room temperature. Then, saturated aqueous potassium sodium tartrate was added to the solution. The mixture was extracted with CH_2Cl_2; the organic layer was washed with water and brine and dried over $MgSO_4$; and the solvent was evaporated. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 5/1) to give 1.68 g of alcohol (±)-**S-4** as colorless oil in 95% yield. (±)-**S-4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.0 Hz, 3H), 1.01 (s, 3H), 1.29−1.33 (m, 6H), 1.30 (s, 3H), 1.41 (s, 3H), 1.49−1.54 (m, 2H), 2.26 (br, 1H), 3.35 (dd, *J* = 9.0, 11.7 Hz, 1H), 3.49 (dd, *J* = 3.9, 11.7 Hz, 1H), 3.98 (dd, *J* = 3.0, 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.9, 18.6, 22.4, 26.5, 26.7, 28.6, 29.4, 31.9, 65.6, 78.0, 82.6, 106.9; FT-IR 2861, 2934, 2986, 3472; HRMS (ESI-TOF) *m*/*z* for $C_{12}H_{24}O_3Na$ $[M + Na]^+$ calcd 239.1618, found 239.1610.

*((4R,5R)-2,2,4-Trimethyl-5-pentyl-1,3-dioxolan-4-yl)methanol, (+)-**S-4**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**S-4**, starting from (+)-**S-3**. $[\alpha]_D^{20}$ +8.67 (*c* = 2.814 g/mL, $CHCl_3$).

*((4S,5S)-2,2,4-Trimethyl-5-pentyl-1,3-dioxolan-4-yl)methanol, (−)-**S-4**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**S-4**, starting from (−)-**S-3**. $[\alpha]_D^{20}$ −7.5 (*c* = 1.385 g/mL, $CHCl_3$).

2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carbaldehyde, (±)-8. Alcohol (±)-**S-4** (1 g, 4.62 mmol) was dissolved in dichloromethane (40 mL). Triethylamine (3.9 mL) was added, and the mixture was cooled to 0 °C. A solution of SO_3-pyridine (2.94 g, 18.49 mmol) in anhydrous dimethylsulfoxide (7.4 mL) was added, and the reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 10/1) to afford 901 mg of aldehyde in 91% yield. (±)-**8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.0 Hz, 3H), 1.17 (s, 3H), 1.30−1.35 (m, 6H), 1.42 (s, 3H), 1.43 (m, 1H), 1.45 (s, 3H), 1.56 (m, 1H), 4.01 (dd, *J* = 3.3, 8.8 Hz, 1H), 9.59 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.8, 15.9, 22.3, 26.0, 26.2, 28.2, 29.0, 31.6, 77.4, 85.6, 109.1, 201.6; HRMS (ESI-TOF) *m*/*z* for $C_{12}H_{23}O_3$ $[M + H]^+$ calcd 215.1642, found 215.1642.

*(4S,5R)-2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carbaldehyde, (+)-**8**, and (4R,5S)-2,2,4-Trimethyl-5-pentyl-1,3-dioxolane-4-carbaldehyde, (−)-**8**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**8**, starting from (+)-**S-4** and (−)-**S-4**, respectively.

3-(Hydroxy-2,2,4-(trimethyl-5-pentyl-1,3-dioxolan-4-yl)-methyl)-4-methoxy-5-methylenefuran-2(5*H*)-one, (±)-6. Diisopropylamine (0.5 mL, 3.66 mmol) was dissolved in THF (2.2 mL) and cooled to −78 °C in a dry ice/acetone bath for 15 min. To this solution was *n*-BuLi (2.3 mL, 1.6 M in hexanes) dropwise over 2 min, and the deprotonation was allowed to proceed for 1 h. Tetronate (420 mg, 3.33 mmol) dissolved in a mixture of THF (2.8 mL) was added dropwise to the LDA solution over 6 min during which the solution turned lemon yellow. The reaction was stirred for exactly 5 min after the addition was complete during which the color of the reaction darkened to light brown. Aldehyde (±)-**8** (724 mg, 3.38 mmol)

dissolved in THF (3.5 mL) was added slowly to the lithiated tetronate over 5 min, and the reaction was allowed to proceed for 2 h. The reaction was quenched at −78 °C with a saturated aqueous NH_4Cl solution (30 mL) and allowed to reach room temperature. The mixture was extracted with AcOEt (20 mL), and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 7/1) to afford 432 mg (1.27 mmol, 53% yield) of (±)-**6** accompanied with recovered starting materials [213 mg of aldehyde (1 mmol) and 76 mg of ester (0.6 mmol)]. (±)-**6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.0 Hz, 3H), 1.14 (s, 3H), 1.16−1.39 (m, 5H), 1.32 (s, 3H), 1.41 (s, 3H), 1.43−1.69 (m, 3H), 3.91 (dd, J = 9.6, 1.8 Hz, 1H), 4.24 (1H, overlapping CH and OCH signals), 4.27 (s, 3H), 4.78 (d, J = 9.9 Hz, 1H), 5.11 (d, J = 2.7 Hz, 1H), 5.13 (d, J = 2.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 13.9, 16.6, 22.5, 26.6, 27.0, 28.7, 30.3, 31.8, 60.1, 72.3, 83.0, 84.3, 93.7, 103.1, 107.4, 149.4, 162.5, 170.7; FT-IR:1623, 1751, 2860, 2933, 2956, 2983, 3474; HRMS (ESI-TOF) m/z for $C_{18}H_{29}O_6$ [M + H]$^+$ calcd 341.1959, found 341.1960.

*3-((R)-Hydroxy((4R,5R)-2,2,4-trimethyl-5-pentyl-1,3-dioxolan-4-yl)methyl)-4-methoxy-5-methylenefuran-2(5H)-one, (+)-**6**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**6**, starting from (+)-**8**. $[\alpha]_D^{20}$ +7.2 (c = 1.461g/mL, $CHCl_3$).

*3-((S)-Hydroxy((4S,5S)-2,2,4-trimethyl-5-pentyl-1,3-dioxolan-4-yl)methyl)-4-methoxy-5-methylenefuran-2(5H)-one, (−)-**6**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**6**, starting from (−)-**8**. $[\alpha]_D^{20}$ −7.0 (c = 2.00 g/mL, $CHCl_3$).

5-Hydroxy-3-(hydroxy(2,2,4-trimethyl-5-pentyl-1,3-dioxolan-4-yl)methyl)-1,4-dimethoxy-5-methyl-1*H*-pyrrol-2(5*H*)-one, (±)-16. A stirred suspension of *O*-Me-hydroxylamine hydrochloride (37 mg, 0.44 mmol, 1.5 equiv) in dry THF (4 mL) at −78 °C and under argon atmosphere was treated with a 1 M solution of LiHMDS (2.7 mmol, 2.7 mL). After 10 min, a solution of the ester (±)-**6** (100 mg, 0.29 mmol, 1 equiv) in a minimum amount of dry THF was added. After 2 h stirring at −78 °C, the reaction was quenched with a saturated aqueous solution of NH_4Cl, warmed to room temperature, and extracted with AcOEt. The combined organic layers were dried, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (eluent; hexane/ethyl acetate = 1/1) to give 107 mg of compound (95%) (±)-**16**: FT-IR 1646, 2863, 2933, 3448; HRMS (ESI-TOF) m/z for $C_{19}H_{34}NO_7$ [M + H]$^+$ calcd 388.233, found 388.2337.

*5-Hydroxy-3-((R)-hydroxy((4R,5R)-2,2,4-trimethyl-5-pentyl-1,3-dioxolan-4-yl)methyl)-1,4-dimethoxy-5-methyl-1H-pyrrol-2(5H)one, (+)-**16**, and 5-Hydroxy-3-((S)-hydroxy((4S,5S)-2,2,4-trimethyl-5-pentyl-1,3-dioxolan-4-yl)methyl)-1,4-dimethoxy-5-methyl-1H-pyrrol-2(5H)-one, (−)-**16**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**16**, starting from (+)-**6** and (−)-**6**, respectively.

5-Hydroxy-1,4-dimethoxy-5-methyl-3-(1,2,3-trihydroxy-2-methyloctyl)-1*H*-pyrrol-2(5*H*)-one, (±)-18. A mixture of trifluoroacetic acid and water (3 mL of a 1:1 vol/vol solution) at 0 °C was added to the acetonide derivative (±)-**16** (62 mg, 0.16 mmol). The reaction mixture was stirred at 0 °C for 1 h and was then decanted into aqueous $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (eluent; ethyl acetate) to afford 50 mg of derivatives (±)-**18** in 90% yield. (±)-**18**: FT-IR 1646, 1685, 2858, 2929, 3421; HRMS (ESI-TOF) m/z for $C_{16}H_{30}NO_7$ [M + H]$^+$ calcd 348.2017, found 348.2009.

*5-Hydroxy-1,4-dimethoxy-5-methyl-3-((1R,2S,3R)-1,2,3-trihydroxy-2-methyloctyl)-1H-pyrrol-2(5H)-one, (+)-**18**, and 5-Hydroxy-1,4-dimethoxy-5-methyl-3-((1S,2R,3S)-1,2,3-trihydroxy-2-methyloctyl)-1H-pyrrol-2(5H)-one, (−)-**18**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**18**, starting from (+)-**16** and (−)-**16**, respectively.

Proposed Structure of Phaeosphaeride A, (±)-1a. Compounds (±)-**18** (15 mg, 0.043 mmol) were dissolved in THF (1 mL), and TBAF (0.065 mmol, 0.1 M solution in THF) was added. The mixture was stirred at room temperature for 12 h. After the addition of saturated $NaHCO_3$ solution (2 mL), the reaction mixture was extracted with ethyl acetate. The organic layers were dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (eluent; ethyl acetate). NMR spectra showed that the product was contaminated with a significant amount of TBAF. The mixture was used to the next step without further purification. (±)-**20**: HRMS (ESI-TOF) m/z for $C_{15}H_{26}NO_6$ [M + H]$^+$ calcd 316.1755, found 316.1755.

Compounds (±)-**20** (0.043 mmol) were dissolved in toluene (1 mL). Then p-TSA monohydrate (10 mg, 0.052 mmol) was added, and the mixture was stirred at 60 °C for 30 min (control by TLC). The reaction was allowed to cool to room temperature and treated with 0.05 mL of Et_3N. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (eluent; hexane/ethyl acetate = 1/1) to afford 11 mg of compound (±)-**1a** as a white solid in 87% yield over two steps. (±)-**1a**: mp = 136−138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, J = 6.6 Hz, 3H), 1.29 (s, 3H), 1.26−1.55 (m, 5H), 1.65−1.83 (m, 2H), 1.99 (m, 1H), 2.74 (s, 1H), 3.24 (s, 1H), 3.89 (1H, overlapping with OMe), 3.91 (s, 3H), 4.36 (s, 1H), 5.03 (d, J = 1.5 Hz, 1H), 5.09 (d, J = 1.5 Hz, 1H);^{13}C NMR (75 MHz, $CDCl_3$) 13.9, 22.5, 22.6, 25.5, 27.4, 31.5, 64.4, 65.7, 69.0, 85.1, 92.8, 103.0, 136.4, 156.6, 166.8; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87 (t, J = 6.5 Hz, 3H), 1.09 (s, 3H), 1.24−1.37 (m, 5H), 1.53 (m, 1H), 1.67−1.84 (m, 2H), 3.78 (s, 3H), 3.99 (1H, overlap with H-8), 4.03 (d, J = 6.9 Hz, overlap with H-6), 4.49 (s, 1H), 4.95 (d, J = 1.5 Hz, 1H), 4.96 (d, J = 1.5 Hz, 1H), 5.15 (d, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) 13.8, 21.9, 22.6, 25.1, 26.9, 31.0, 63.6, 64.6, 68.7, 84.9, 90.7, 104.9, 136.6, 156.3, 165.8; FT-IR 1087, 1433, 1638, 1703, 2855, 2920, 2954, 3446; LC-ESI MS 320.29; HRMS (ESI-TOF) m/z for $C_{15}H_{24}NO_5$ [M + H]$^+$ calcd 298.1649, found 298.1648.

*(6R,7R,8R)-**1a**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**1a**, starting from (+)-**18**. $[\alpha]_D^{20}$ +78.7 (c = 0.128 g/mL, CH_2Cl_2).

*(6S,7S,8S)-**1a**.* The synthesis was carried out using the same procedure used for the synthesis of (±)-**1a**, starting from (−)-**18**. $[\alpha]_D^{20}$ −75.8 (c = 0.441 g/mL, CH_2Cl_2).

Cell Lines. Human breast cancer cell line MDA-MB-231 was purchased from ATCC. The human pancreatic carcinoma cell line PANC-1 and rat osteosarcoma cells (UMR106) were a gift from Dr. H. W. Verspaget, Department of Gastroenterology, Leiden University Medical Center, The Netherlands. All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Breda, The Netherlands), containing 100 U/mL of penicillin (Invitrogen), 100 U/mL of streptomycin (Invitrogen), and 10% fetal bovine serum (FBS; Cambrex, Belgium) in a humidified atmosphere of 5% CO_2 at 37 °C.

STAT3 Inhibitors. All STAT3 inhibitors were dissolved in sterile DMSO to make a 10 mM stock solution. Aliquots of the stock solution were stored at −20 °C.

STAT3-Dependent Transcriptional Luciferase Activity. Cells were seeded at a density of 20 000 cells/well in 96-well plates. The second day, the cells were transiently cotransfected with 20 ng of the STAT3-specific luciferase reporter construct[25] and 1.25 ng of CMV-renilla construct using FuGENE HD transfection reagent (Promega Benelux BV, Leiden, The Netherlands), according to the manufacturer's protocol. Cells were treated with 10 ng/mL of Oncostatin M and a dose range of the synthesized compounds the next day. After 6 h, luciferase assays were performed using the Dual Luciferase Reporter Assay system (Promega), according to the manufacturer's protocol. Luciferase activity was measured using a luminescence counter. Firefly luciferase activity was corrected for renilla luciferase activity.

Cell Viability Assays. To test the potential cytotoxicity of the synthesized molecules, UMR106 cells were seeded at a density of 19 000 cells/well in 96-well plates. To simulate the transfection conditions, cells were treated with a dose range of the enantiomers (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** on the third day after seeding. A tetrazolium-based (MTS) assay (CellTiter 96 AQueous, Promega) was performed 6 h after stimulation, according to the manufacturer's

protocol. The reagent was added directly to the assay wells at the recommended ratio of 20 μL of reagent to 100 μL of culture medium. Cells were then incubated for approximately 30 min at 37 °C, and absorbance was measured at 490 nm in a microplate reader.

Human breast (MDA-MB-231) and pancreatic (PANC-1) cancer cell lines were seeded in opaque-walled 96-well plates, compatible with the luminometer used, at a density of 5000 cells/well. Different concentrations of (6*R*,7*R*,8*R*)-**1a** and (6*S*,7*S*,8*S*)-**1a** or gemcitabine (100 nM) were added in triplicate to the plates in the presence of 10% FBS. Cells were incubated at 37 °C for a period of 72 h, and the luminescent cell viability assay (CellTiter-Glo, Promega) was performed according to the manufacturer's protocol. After 72 h, cells were treated with a volume of CellTiter-Glo Reagent equal to the volume of cell culture medium present in each well and incubated for approximately 10 min at room temperature to stabilize the luminescent signal. Luminescence was then recorded using a luminescence counter.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Characterization data of the described compounds and X-ray crystal data for compound (6*R*,7*R*,8*R*)-**1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sarli@chem.auth.gr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are grateful to Professor Jon Clardy (Harvard Medical School) for providing an authentic sample of (−)-Phaeosphaeride A. The authors thank Ineke Oudshoorn (Dept. of Radiology, LUMC) for technical assistance, Dr. Hein Verspaget (Dept. of Gastroenterology, LUMC) for the PANC-1 cell line, and Professor Clemens Löwik (Dept of Radiology, LUMC) for critical reading of the manuscript.

■ REFERENCES

(1) Bowman, T.; Garcia, R.; Turkson, J.; Jove, R. *Oncogene* **2000**, *19*, 2474−2488.

(2) (a) Darnell, J. J.; Kerr, I.; Stark, G. *Science* **1994**, *264*, 1415−1421. (b) Zhong, Z.; Wen, Z.; Darnell, J. *Science* **1994**, *264*, 95−98.

(3) Yu, H.; Jove, R. *Nat. Rev. Cancer* **2004**, *4*, 97−105.

(4) Real, P. J. A.; Sierra, A.; De Juan, A.; Segovia, J. C.; Lopez-Vega, J. M.; Fernandez-Luna, J. L. *Oncogene* **2002**, *21*, 7611−7618.

(5) (a) Kortylewski, M.; Xin, H.; Kujawski, M.; Lee, H.; Liu, Y.; Harris, T.; Drake, C.; Pardoll, D.; Yu, H. *Cancer Cell* **2009**, *15*, 114−123. (b) Gritsko, T.; Williams, A.; Turkson, J.; Kaneko, S.; Bowman, T.; Huang, M.; Nam, S.; Eweis, I.; Diaz, N.; Sullivan, D.; Yoder, S.; Enkemann, S.; Eschrich, S.; Lee, J. H.; Beam, C. A.; Cheng, J.; Minton, S.; Muro-Cacho, C. A.; Jove, R. *Clin. Cancer Res.* **2006**, *12*, 11−19. (c) Epling-Burnette, P. K.; Liu, J. H.; Catlett-Falcone, R.; Turkson, J.; Oshiro, M; Kothapalli, R.; Li, Y.; Wang, J. M.; Yang-Yen, H. F.; Karras, J.; Jove, R.; Loughran, T. P., Jr. *J. Clin. Invest.* **2001**, *107*, 351−362.

(6) Wei, D.; Le, X.; Zheng, L.; Wang, L.; Frey, J. A.; Gao, A. C.; Peng, Z.; Huang, S.; Xiong, H. Q.; Abbruzzese, J. L.; Xie, K. *Oncogene* **2003**, *22*, 319−329.

(7) (a) Niu, G.; Heller, R.; Catlett-Falcone, R.; Coppola, D.; Jaroszeski, M.; Dalton, W.; Jove, R.; Yu, H. *Cancer Res.* **1999**, *59*, 5059−5063. (b) Redell, M. S.; Tweardy, D. J. *Curr. Pharm. Des.* **2005**, *11*, 2873−2887. (c) Ling, X.; Arlinghaus, R. B. *Cancer Res.* **2005**, *65*, 2532−2536.

(8) Coleman, D. R.; Ren, Z.; Mandal, P. K.; Cameron, A. G.; Dyer, G. A.; Muranjan, S.; Campbell, M.; Chen, X.; McMurray, J. S. *J. Med. Chem.* **2005**, *48*, 6661−6670.

(9) (a) Schust, J.; Sperl, B.; Hollis, A.; Mayer, T. U.; Berg, T. *Chem. Biol.* **2006**, *13*, 1235−1242. (b) Song, H.; Wang, R.; Wang, S.; Lin, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4700−4705. (c) Siddiquee, K.; Zhang, S.; Guida, W. C.; Blaskovich, M. A.; Greedy, B.; Lawrence, H. R.; Yip, M. L.; Jove, R.; McLaughlin, M. M.; Lawrence, N. J.; Sebti, S. M.; Turkson, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7391−7396. (d) Matsuno, K.; Masuda, Y.; Uehara, Y.; Sato, H.; Muroya, A.; Takahashi, O.; Yokotagawa, T.; Furuya, T.; Okawara, T.; Otsuka, M.; Ogo, N.; Ashizawa, T.; Oshita, C.; Tai, S.; Ishii, H.; Akiyama, Y.; Asai, A. *ACS Med. Chem. Lett.* **2010**, *1*, 371−375.

(10) (a) Shin, D. S.; Kim, H. N.; Shin, K. D.; Yoon, Y. J.; Kim, S. J.; Han, D. C.; Kwon, B. M. *Cancer Res.* **2009**, *69*, 193−202. (b) Bharti, A. C.; Donato, N.; Aggarwal, B. B. *J. Immunol.* **2003**, *171*, 3863−3871. (c) Weidler, M.; Rether, J.; Anke, T.; Erkel, G. *FEBS Lett.* **2000**, *484*, 1−6. (d) Chen, C.; Qiang, S.; Lou, L.; Zhao, W. *J. Nat. Prod.* **2009**, *72*, 824−829.

(11) Maloney, K. N.; Hao, W.; Xu, J.; Gibbons, J.; Hucul, J.; Roll, D.; Brady, S. F.; Schroeder, F. C.; Clardy, J. *Org. Lett.* **2006**, *8*, 4067−4070.

(12) Abraham, W. R.; Meyer, H.; Abate, D. *Tetrahedron* **1995**, *51*, 4947−4952.

(13) Sugawara, F.; Takahashi, N.; Strobel, G. A.; Strobel, S. A.; Lu, H. S. M.; Clardy, J. *J. Am. Chem. Soc.* **1988**, *110*, 4086−4087.

(14) Thines, E.; Arendholz, W. R.; Anke, H.; Sterner, O. *J. Antibiot.* **1997**, *50*, 13−17.

(15) Kobayashi, K.; Okamoto, I.; Morita, N.; Kiyotani, T.; Tamura, O. *Org. Biomol. Chem.* **2011**, *9*, 5825−5832.

(16) Handa, M.; Scheidt, K. A.; Bossart, M.; Zheng, N.; Roush, W. R. *J. Org. Chem.* **2008**, *73*, 1031−1035.

(17) Takeda, K.; Yano, S.; Sato, M.; Yoshii, E. *J. Org. Chem.* **1987**, *52*, 4135−4137.

(18) For examples in the synthesis of tetronate derivatives with lithiated tetronate **7**, see: (a) Hori, K.; Hikage, N.; Inagaki, A.; Mori, S.; Nomura, K.; Yoshii, E. *J. Org. Chem.* **1992**, *57*, 2888−2902. (b) Zapf, C. W.; Harrison, B. A.; Drahl, C.; Sorensen, E. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 6533−6537. (c) Snider, B. B.; Zou, Y. *Org. Lett.* **2005**, *7*, 4939−4941. (d) Niu, D.; Hoye, T. R. *Org. Lett.* **2012**, *14*, 828−831. (e) Couladouros, E. A.; Bouzas, E. A.; Magos, A. D. *Tetrahedron* **2006**, *62*, 5272−5279.

(19) Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, *18*, 2199−2204.

(20) Gissot, A.; Volonterio, A.; Zanda, M. *J. Org. Chem.* **2005**, *70*, 6925−6928.

(21) Baldwin, J. E.; Cutting, J.; Dupont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. *Chem. Soc. Chem. Commun.* **1976**, 736−738.

(22) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.

(23) Crystal data for **1a** (293 K): $C_{15}H_{23}NO_5$; fw 297.34; monoclinic; space group *P*21; a = 10.213(2) Å, b = 5.204(1) Å, c = 15.962(3) Å, β = 100.28(3)°; V = 834.7(3) Å^3; Z = 2; D_{calcd} = 1.183 mg/m^3, R_1 = 0.0778 [$I > 2\sigma(I)$], $wR2$ = 0.2191 (all data); GOF = 1.045. For details, see the Supporting Information.

(24) Ward, A. C.; Hermans, M. H.; Smith, L; van Aesch, Y. M.; Schelen, A. M.; Antonissen, C.; Touw, I. P. *Blood* **1999**, *93*, 113−124.

(25) Stahi, N.; Boulton, T. G.; Farruggella, T.; Ip, N. Y.; Davis, S.; Witthuhn, B. A.; Quelle, F. W.; Silvennoinen, O.; Barbieri, G.; Pellegrini, S.; Ihle, J. N.; Yancopoulos, G. D. *Science* **1994**, *263*, 92−95.

(26) Heinrich, P. C.; Behrmann, I.; Müller-Newen, G.; Schaper, F.; Graeve, L. *Biochem. J.* **1998**, *334*, 297−314.

(27) Bellido, T.; Borba, V. Z. C.; Roberson, P.; Manolagas, S. C. *Endocrinology* **1997**, *138*, 3666−3676.

(28) Li, C.; Ahlborn, T. E.; Kraemer, F. B.; Liu, J. *Breast Cancer Res. Treat.* **2001**, *66*, 111−121.

(29) Kamohara, H.; Ogawa, M.; Ishiko, T.; Sakamoto, K.; Baba, H. *Int. J. Oncol.* **2007**, *30*, 977−983.

(30) Li, L.; Hooi, D.; Chhabra, S. R.; Pritchard, D.; Shaw, P. E. *Oncogene* **2004**, *23*, 4894−4902.

(31) Tang, S. N.; Fu, J.; Shankar, S.; Srivastava, R. K. *PLoS One*. **2012**, *7*, e31067.