Polyacetylenes from *Oplopanax horridus* and *Panax ginseng*: Relationship between Structure and PPARγ Activation

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**ABSTRACT:** *Oplopanax horridus* and *Panax ginseng* are members of the plant family Araliaceae, which is rich in structurally diverse polyacetylenes. In this work, we isolated and determined structures of 23 aliphatic C₁₇ and C₁₈ polyacetylenes, of which five are new compounds. Polyacetylenes have a suitable scaffold for binding to PPARγ, a ligand-activated transcription factor involved in metabolic regulation. Using a reporter gene assay, their potential PPARγ binding of their respective ligands. Ligands that activate transcription of their target genes, nuclear receptors require cells in vitro and in vivo. Additionally, polyacetylenes were investigated due to their inhibitory properties against fungi and bacteria, as well as against various types of cancer, for pathogen defense. Therefore, polyacetylenes have mostly been investigated due to their inhibitory properties against fungi and bacteria, as well as against various types of cancer cells in vitro and in vivo. Additionally, polyacetylenes were found to have allergic, neurotoxic, and anti-inflammatory properties.

PPARγ is a member of the nuclear receptor family of transcription factors that acts as regulators of lipid homeostasis, adipogenesis, and inflammation. In order to activate the transcription of their target genes, nuclear receptors require binding of their respective ligands. Ligands that activate PPARγ are beneficial for improving insulin sensitivity in conditions such as type II diabetes and for reducing inflammation. Since long-chain polyunsaturated fatty acids are effective PPARγ binders, and aliphatic polyacetylenes resemble their structure, our group investigated previously as to whether they could act as PPARγ activators. Indeed, the aliphatic C₁₇ polyacetylene falarcarindiol from *Notopterygium incisum* was shown to act as a partial PPARγ agonist.

In order to use a more systematic approach for the search for polyyne-derived PPARγ activators, a collection of polyyylene structural analogues was purified from Devil’s club (*Oplopanax horridus* (Sm.) Miq., Araliaceae) and Asian ginseng (*Panax ginseng C.A. Mey., Araliaceae*). Polyacetylenes are major bioactive constituents of the Devil’s club, with a variety of different structures. *O. horridus* is indigenous to the North American Pacific area and has been used traditionally by the indigenous peoples for treating respiratory infections and inflammatory conditions such as rheumatism and arthritis. *Panax ginseng* is a popular medicinal plant from East Asia, which, in addition to ginsenosides, also produces polyylenes.

In the present work, we isolated and determined the structures of 23 aliphatic C₁₇ and C₁₈ polyacetylenes, of which five (5–7, 14, and 15) were identified for the first time. Using a reporter gene assay, their PPARγ activation potential was investigated. By employing molecular docking and comparing the activities of structural analogues, structural features were identified that are involved in receptor binding and activation. In addition, structural characteristics correlating with their cytotoxicity are described.
RESULTS AND DISCUSSION

Compound Isolation and Structure Determination.

The dichloromethane extract of the root bark from O. horridus was subjected to successive column chromatographic procedures, yielding 18 native polyacetylenes and three deacetylation products. Their structures, as determined by spectroscopic methods, were oplopantriol B 18-acetate (1),10 oplopantriol B (2),11 oplopantriol A 18-acetate (3),10 oplopantriol A (4),11 1-hydroxyoplopantriol B 18-acetate (5), 1-hydroxyoplopantriol B (6), 11-hydroxyoplopolandiol (7), 11-hydroxyfalcarindiol (8),12 oplopandiol (9),10 3S,8S-falcarindiol (10),10,11,13,14 hederyne A (11),15 (3R,10S,8E)-8-octadecene-4,6-diyne-3,10,18-triol 18-acetate (12),16 (3R,10S,8E)-8-octadecene-4,6-diyne-3,10,18-triol (13),16 oplopantriol C 18-acetate (14), oplopantriol C (15), 1,2-dihydropanaxaryl (16),16 panaxydiol (17),17,18 dendostrifidiol 18-acetate (18),19 dendostrifidiol (19),19,20 panaxydiol (20),18,21,22 and (−)-falcarinol (21).23 Compounds 5, 6, 7, 14, and 15 have new structures and are discussed below. Compound 18 is reported as a natural product for the first time.

The petroleum ether extract of P. ginseng was subjected to successive column chromatography, yielding three native polyacetylenes. Their structures were determined by spectroscopic methods as (−)-falcarinol (21),23 (9R,10S)-epoxyheptadecan-4,6-diyne-3-one (22),24,25 and (9R,10S)-epoxy-16-heptadecene-4,6-diyne-3-one (23).24 Compound 5 was isolated as colorless solid. The HRESIMS data allowed for the assignment of a molecular formula of C20H30O5. The observed UV absorption maxima at 232, 245, and 258 nm and the four quaternary carbon signals (δ 81.8, 80.7, 69.1, and 68.9) in the 13C NMR spectrum were characteristic of a bisacetylene/diyne skeleton.14 The assignments of all proton and carbon resonances were achieved through interpretation of the 2D NMR data. When compared with the 1H NMR spectrum of oplopantriol B 1-acetate (1), the triplet of a terminal methyl group was replaced by a signal of a hydroxy methylene group at δH 3.69 (m) in 5. Inductive effects were therefore observed for the protons of its vicinal methylene (H-2; ΔδH −0.19) and the hydroxy methine on its β-position (H-3; ΔδH −0.25), with both sharing an HMBC correlation with C-4 of the diyne system. As also revealed by the HMBC and COSY spectra, the diyne system was connected through a hydroxy methine to a cis-configured olefinic bond, which, in turn, was connected to the C-11 of the aliphatic chain. On comparison of their 13C NMR spectra, the remaining signals belonging to an aliphatic chain of compounds 5 and 1 were highly superimposable.

Figure 1. Structures of isolated compounds and their respective specific rotations, with concentrations and solvents used, are given in the Experimental Section.
of C17H26O3. The UV absorption maxima at 231, 244, and 258 nm and four quaternary carbon signals (δ 72.5, 4.11 qd (6.1, 1.7), 72.5 4.11 qd (6.0, 1.6) were assigned as having the same 3S,8R,9Z)-octadec-9-ene-4,6-diyne-1,3,8,18-tetrol 18-acetate. Deacetylation occurred at room temperature. The deacetylation product was obtained and given the trivial name 1-hydroxyoplopandiol B.

Compound 7 was obtained as a colorless solid. The HRESIMS data allowed the deduction of a molecular formula of C20H28O4. The UV absorption maxima at 215, 230, 242, 254, 268, and 283 nm and the four quaternary carbon signals (δ 72.5, 4.11 qd (6.1, 1.7), 72.5 4.11 qd (6.0, 1.6) of the 13C NMR spectrum were characteristic of a diacetylene skeleton.13 Interpretation of the 2D NMR data led to the assignment of all proton and carbon resonances. The 1H NMR signals at δ 0.99 (t, J = 7.4 Hz, H-1), 1.68 (m, H-2), and 4.30 (t, J = 6.6 Hz, H-3) were assigned to a hydroxy propyl group, for which the connection to the polyacetylene skeleton was supported by HMBC correlations observed between H-3 and the acetylenic C-4 and C-5. Although highly overlapping NMR signals at δ 5.49 (m, H-9) and 5.47 (m, H-10) provided little coupling constant information, their corresponding carbon signals at δ 136.1 (C-10) and 130.2 (C-9) indicated clearly the presence of a cis-configured olefinic group.
another methylene pentet at 1.62 ($J = 6.7$ Hz, H-17), was observed in 14. Both of the first two proton signals showed HMBC correlations with a carbonyl signal at $\delta 173.1$ (C-19), indicating acetylation at a primary alcohol position. The third one was assigned as belonging to the methylene group vicinal to the acetylation position, as evidenced by the HMBC correlation with the carbon signal at $\delta 65.7$ (C-18). Its HMBC correlation with another methylene carbon at $\delta 30.0$ (C-16) supported the acetyl functionalization at the end of the aliphatic chain. Comparison of their $^{13}$C NMR spectra suggested compound 14 has a longer aliphatic chain, with one more methylene group, than compound 17. A second cluster of known polyacetylenes (12, 13, 16, 17, 20) isolated from *O. horridus* exhibited stereogenic centers at C-3 and C-10, which are separated by two triple bonds and an additional trans double bond; these compounds are derived from (3R,10S,8E)-8-octadecene-4,6-diyne-3,10,18-triol (13). Representatives of this type have been found to possess an $R$-configuration at C-3 and an S-configuration at C-10, and, with the exception of the epoxide (20), all of these compounds exhibit a small negative specific rotation (Figure 1). Due to their similar specific rotation values, compounds 14 and 15 were assumed to have the same 3R,10S configuration as the known compounds of this cluster. Therefore, the structure of compound 14 was determined as (3R,10S,8E)-octadec-1,8-diene-4,6-diyne-3,10,18-triol 18-acetate, and it was assigned the trivial name oplopantriol C 18-acetate. After being kept at room temperature, a methanol solution of compound 14 yielded its deacetylation product (15), which was given the trivial name oplopantriol C.

**Biological Evaluation.** Using a luciferase reporter gene assay, altogether 22 compounds were tested for their potential to activate PPARγ (compound 6 was not tested due to the insufficient amount available). Mean fold activation is reported in Table 2 and Figure S1 (Supporting Information), arranged according to activity, from highly to least active compounds. The most potent activators were oplopantriol B 18-acetate (1) (mean ± SD: 4.2 ± 0.7), oplopantriol B (2) (4.1 ± 0.7), followed by oplopantriol (9) (2.5 ± 1.5), and (3R,8E,10S)-8-octadecene-4,6-diyne-3,10−18-triol (13) (2.5 ± 0.5), although only the effects of 1 and 2 reached statistical significance. Concentration−response characteristics of compounds 1 and 2 in comparison to the full agonist pioglitazone classify them as partial agonists (Figure S2, Supporting Information). All four compounds were previously examined in various cell lines for their cytostatic effects, and compound 9 was also shown to have antimycobacterial properties. To the best of our knowledge, no other specific biological functions have been assigned to these four polyacetylenes. Extracts of *O. horridus* have been reported to have antidiabetic properties, albeit with inconclusive results. However, the ability of polyacetylenes to activate PPARγ may explain some of the previously obtained results and should warrant further research. Additionally, ligand binding to PPARγ inhibits inflammatory responses, which partially may contribute to the anti-inflammatory properties of polyacetylenes.

Interestingly, in the present assay falcarindiol (10) did not show any PPARγ activation, while a previous report on this compound isolated from *N. incisum* showed activation of PPARγ with an EC$_{50}$ of 3.2 µM and $E_{\max} = 3.26$-fold. The most likely reason for this is that the previous study used falcarindiol in the 3R,8S configuration, while the one isolated from *O. horridus* was the 3S,8S diastereomer, highlighting the importance of the absolute configuration.

Molecular docking was employed to investigate the polyacetylene binding mode to the receptor. Polyacetylenes are anchored in the PPARγ ligand binding pocket via interactions with residues of all three subpockets. A more detailed description of the binding pocket and the docking experiments is available in the Supporting Information (Figure S3). Hydrogen bond predictions for the most active (1, 2, 9, 13, 11) and least active (14, 10, 17, 8) polyacetylenes are reported in Table 2. The anchoring of the two most active polyacetylenes, 1 and 2, is shown in Figure 2. Both polyacetylenes were positioned similarly with the polyyne chain within arm I and the alkyl tail in arm II. These compounds each formed hydrogen bonds between their C-3 hydroxy group and Ser289 and Cys285 within arm I and between the C-8 hydroxy group and Leu340 in the entrance.

Next, the structure−activity relationships were explored between polyacetylenes and PPARγ activation. Within the compound collection used, three structural categories were identified that may influence PPARγ activation: the C-1 to C-2 bond, the terminal group, and the backbone flexibility (Figure 3). Polyacetylenes were grouped based on the type of C-1 to C-2 bond, which could be either single or double. The terminal
The strongest impact on activation referred to the type of C-1 to C-2 bond (p = 0.001 67), followed by the type of terminal group at the end of the alkyl chain (p = 0.033 99). The backbone flexibility showed only a moderate tendency to impact PPARγ activation (p = 0.072 801 6).

Within each category, a single bond between C-1 and C-2 strongly favored PPARγ activation over a double bond (mean ± SD: 2.3 ± 0.9 vs 1.3 ± 0.6, p = 0.001 67). Compounds with a terminal hydroxy group had a higher mean fold activation in comparison to those with acetoxy and methyl groups (2.4 ± 1.0 vs 1.9 ± 1.2 vs 1.5 ± 0.8, respectively), but the difference was significant only between hydroxy and methyl groups (p = 0.029 03). Although not significant, flexible structures showed a tendency toward higher PPARγ activation (2.0 ± 1 vs 1.5 ± 0.7).

The polyacetylene collection utilized contained structural analogues, with pairs of polyacetylenes having identical structures, except for the examined feature. Therefore, in order to verify the observations made, the structural counterparts were compared further individually (Figure S4, Supporting Information). Although pairwise comparisons were not always statistically significant, the differences in activity were consistent and followed the trend observed in Figure 3.

Molecular docking was employed also to compare the structural pairs of each category in their binding to the PPARγ ligand binding pocket. A comparison of the docking positions of the C-1 to C-2 single:double bond pair 1:3 is shown in Figure 4. Compound 1 was able to form two hydrogen bonds between the C-3 hydroxy group and the amino acids Ser289 and Cys285, while in compound 3, the C-3 hydroxy group was positioned differently, allowing the formation of a hydrogen bond with Ser289 only. Otherwise, the two compounds were positioned similarly in the binding pocket and both formed a hydrogen bond with Leu340.

Molecular docking did not reveal any structural basis for the observed differences in polyacetylene activity based on their terminal group, possibly because the differences in activity were too small and due to high flexibility of the alkyl chain in arm II. However, the increased PPARγ activation by the more polar polyacetylenes may reflect their increased bioavailability.
Structural analogues that were identified between polyacetylenes of the 3S,8S orientation and 3R,10S orientation differed in the presence of an extra C-8 to C-9 double bond after the polyyne chain, thus shifting the C-8 hydroxy group of the 3S,8S polyacetylenes to the C-10 position in 3R,10S polyacetylenes. The extended and rigid spacer between the C-3 and C-10 hydroxy groups was unfavorable for the interaction in arm I, and, since the distance cannot be shortened by bending, the compounds were flipping in orientation within the PPARγ ligand binding pocket. Figure 5 shows compound 2 (flexible) with its polyyne part in arm I, whereas compound 13 (rigid) had its alkyl chain in arm I and the polyyne chain in arm II. However, the amino acids involved in the interactions stayed the same in both groups.

Overall, the present observations made suggest that PPARγ activation by aliphatic polyacetylenes is supported by the presence of a single C-1 to C-2 bond, polar terminal groups at the end of the alkyl chain, and a flexible backbone that can bend.

Next, a group comparison was used to discern whether the structural features that impact PPARγ activation may also impact viability and thus confound the present results. As a measure for cell viability during the luciferase assay, EGFP expression (fluorescence) in comparison to vehicle control (0.1% DMSO) was quantified. No statistically significant association was obtained using the multiway ANOVA and post hoc TukeyHSD tests (Figure 6), but the trend was observable particularly for the C-1 to C-2 bond. The mean relative viability values were higher for polyacetylenes with a single C-1 to C-2 bond in comparison to those with the presence of a double bond (mean ± SD: 0.7021 ± 0.3014 vs 0.4392 ± 0.3873, respectively), which was in agreement with previously published observations on polyacetylenes from O. horridus.29–31 However, this did not hold true for the compounds 18–21, which all possess a double bond in this position, and showed viability higher than 70% (Figure S5, Supporting Information) and were not diols. Interestingly, falcarindiol (21) was not toxic in our assay, while falcarindiol (10) was cytotoxic, contradictory to previous reports.22,32 Although direct comparisons are difficult due to different cell lines and procedures used, it is important to consider the configuration, as previous reports compared (−)-falcarindiol to (3R,8S)-falcarindiol, typical for Apiaceae plants. Therefore, the most toxic polyacetylenes in the present group were diols with two hydroxy groups at each side of the polyyne chain and a C-1 to C-2 double bond.

Since all of the structural pairs of C-1 to C-2 bond type contained the second hydroxy group, the C-1 to C-2 double-bond analogues were shown to be more toxic by direct comparison to their single-bond counterparts (Figure S6A, Supporting Information). The present observations were verified with an independent resazurin cell viability assay. Since in this assay cells are not stressed with the transfection solution, polyacetylenes were not as cytotoxic even at 10 μM and after 24 h of incubation (Figure S7, Supporting Information). However, the relative toxicity between C-1 to C-2 single:double bond pairs remained the same (Figure S6B, Supporting Information). Even though the luminescence values used to estimate the PPARγ activation were normalized to fluorescence values, it cannot be excluded that the PPARγ activation data are not at least partly influenced by the cytotoxicity of these compounds.

In summary, 23 aliphatic C17 and C18 polyacetylenes were isolated from O. horridus and P. ginseng, with five of these being new compounds. Oplopantriol B 18-acetate (1) and oplopantriol B (2) were identified as nontoxic, partial PPARγ activators, thus corroborating the antidiabetic and anti-inflammatory potential of O. horridus extracts. In addition, the relationship was explored between the structural features of polyacetylenes and their potential to activate PPARγ, pointing to the significance of the type of bond between C-1 and C-2, the terminal group polarity, and backbone flexibility. Additionally, diols with a double bond between C-1 and C-2 and two hydroxy groups at each side of the polyyne chain appear to enhance polyacetylene cytotoxicity. The present results may help to guide a polyacetylene scaffold-based design for future PPARγ partial agonists with reduced toxicity.

**EXPERIMENTAL SECTION**

**General Experimental Procedures.** Optical rotations were measured on a JASCO P-2000 multi-option polarimeter. 1H, 13C, and 2D NMR spectra (COSY, HSQC, and HMBC) were recorded at
25 °C for compounds 1–21 in CD$_3$OD and for compounds 22 and 23 in CDCl$_3$ on UNITYINOVA 400/600 (Varian) and Avance 300/700 spectrometers (Bruker). Chemical shifts are expressed in δ (ppm) with the CD$_3$OD or CDCl$_3$ peak used as reference. LC-HRESIMS was carried out using the same column on a Dionex Ultimate 3000 UHPLC coupled with a Thermo QExactive Hybrid Quadrupole Orbitrap mass spectrometer equipped with an H-ESI II probe in the positive and negative mode, with most compounds only showing ionization in the positive mode. All solvents were obtained from VWR Chemicals and Carl Roth. Open column chromatography (CC) was carried out with silica gel (15–40 μm, Merck), Sephadex LH-20 (GE Healthcare), and RP-18 silica gel (25–40 μm, Fuji silica) as stationary phases. Semipreparative HPLC experiments were performed on a Merck-Hitachi semipreparative system (flow rate: 3 mL/min) equipped with a LiChroCART 10 × 250 mm column packed with LiChrospher 100 RP-18 (particle size: 10 μm). Analytical HPLC was conducted on an Agilent 1260 system (flow rate: 0.3 mL/min), using a Zorbax SB-C18 narrow bore (3.5 μm) 2.1 × 150 mm column (Agilent).

**Plant Material.** The dried root bark of *O. horridus* (1.75 kg) was ground manually and extracted successively with dichloromethane and methanol. The dichloromethane extract (145 g) was subjected to silica gel chromatography and eluted with a hexane–ethyl acetate gradient to afford 117 fractions (OD 1–117, each 1.5 L). Fractions with a characteristic polyacetylene UV pattern were further subjected to chromatography using reversed-phase C$_{18}$ material and Sephadex LH-20, with aqueous methanol as mobile phase. All compounds were finally purified with semipreparative HPLC. The petroleum ether extract of *P. ginseng* was obtained from Phoenix, Arizona, with protection from direct sunlight. A voucher specimen (No. IPW_Opl-horr_012013) was deposited at the University of Graz.

**Extraction and Isolation.** The dried root bark of *O. horridus* was subjected to chromatography using reversed-phase C$_{18}$ material. The petroleum ether extract of *P. ginseng* was purchased from Molekula (Munich, Germany). All compounds were stored as solutions dissolved in 100% DMSO.

**Biological Evaluation.** HEK293T cells were purchased from the ATCC (Manassas, VA, USA), Dulbecco’s modified Eagle medium (DMEM; 4.5 g/L glucose) was obtained from Lonza (Basel, Switzerland), and fetal bovine serum (FBS) was from ThermoFisher Scientific (Waltham, MA, USA). Plasmids pSG5-hPPARγ and tk-PPRE-luc were gifts from Prof. Walter Wahli and Prof. Beatrice Desvergne (Center for Integrative Genomics, University of Lausanne, Switzerland). pEGFP-N1 was purchased from Takara Bio USA (Mountain View, CA, USA). Pioglitazone was purchased from Molekula (Munich, Germany). All compounds were reseeded in a 96-well plate and treated with 0.1% DMSO as vehicle control or 10 μM test compound and incubated for 18 h. Cells were lysed using the Promega luciferase cell culture lysis reagent and the medium was replaced with DMEM containing 5% DMSO to measure luciferase activity.

**Material for Biological Evaluation.** HEK293T cells were purchased from the ATCC (Manassas, VA, USA), Dulbecco’s modified Eagle medium (DMEM; 4.5 g/L glucose) was obtained from Lonza (Basel, Switzerland), and fetal bovine serum (FBS) was from ThermoFisher Scientific (Waltham, MA, USA). Plasmids pSG5-hPPARγ and tk-PPRE-luc were gifts from Prof. Walter Wahli and Prof. Beatrice Desvergne (Center for Integrative Genomics, University of Lausanne, Switzerland). pEGFP-N1 was purchased from Takara Bio USA (Mountain View, CA, USA). Pioglitazone was purchased from Molekula (Munich, Germany). All compounds were stored as solutions dissolved in 100% DMSO.

**Biological Evaluation.** For PPARγ activation experiments, luciferase reporter assays were performed in HEK 293 cells. Cells were cultured under standard conditions (37 °C, 5% CO$_2$; passage every 3 days) in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/mL benzylpenicillin, and 100 μg/mL streptomycin (DMEM complete). A total of 6 × 10$^5$ cells were seeded in 15 cm Petri dishes and incubated overnight. Cells were transfected using the calcium phosphate method with the following plasmids. All compounds were measured on a Tecan Spectra fluorometer (Mannedorf, Switzerland). Luminescence values were normalized to the EGFP fluorescence and expressed as fold changes relative to the vehicle control. Relative viability was calculated from fluorescence values relative to those of the vehicle controls.

For the resazurin assay, HEK293 cells (5 × 10$^4$ cells per well) were seeded in a 96-well plate and incubated overnight. On the following day, the medium was replaced with 0.4% DMSO as vehicle control or 10 μM test compound and incubated for 18 h. Cells were lysed using the Promega luciferase cell culture lysis 5× reagent (Manheim, Germany). Luminescence and fluorescence values were measured on a Tecan Spectra fluorometer (Mannedorf, Switzerland). Luminescence values were normalized to the EGFP fluorescence and expressed as fold changes relative to the vehicle control.

**Statistical Analysis.** All experiments were performed at least three times, each in four technical replicates. Data were analyzed using the GraphPad Prism 6 software (La Jolla, CA, USA) and R version 3.4.4.

**Docking.** For the docking, the X-ray crystal structure 3R5N35 with a resolution of 2.0 Å and a good electron density distribution was employed. In this structure, PPARγ was crystallized with two molecules of magonol, a partial agonist on the PPARγ. After calculation of the starting conformations in Omega 2.5.1.4, 36,37 the docking was performed using the software GOLD 5.2.38,39

**Supporting Information.** An animation illustrating this process can be found in the Supporting Information. For C$_{6}$H$_{12}$O$_{6}$, 291.1960 m/z 309.2063 [M + H]$^+$ (calcd for C$_{6}$H$_{12}$O$_{6}$, 309.2066).

11-Hydroxyoplopantriol (7): [α]$_D^{25}$ +234.5 (c 0.29, MeOH), +176.9 (c 0.29, CHCl$_3$); UV (CH$_3$CN–H$_2$O) $\lambda_{max}$ 231, 244, and 258 nm; 1H NMR and 13C NMR, see Table 1; HRESIMS m/z 503.5325 [2M + H – 3H$_2$O]$^+$ (calcd for C$_{31}$H$_{40}$O$_{12}$, 503.5325) m/z 279.1958 [M + H]$^+$ (calcd for C$_{19}$H$_{28}$O$_{10}$, 279.1960).

Oplontalin C 18-acetate (14): [α]$_D^{25}$ +234.5 (c 0.06, MeOH); UV (CH$_3$CN–H$_2$O) $\lambda_{max}$ 215, 242, 254, 268, and 283 nm; 1H NMR and 13C NMR, see Table 1; HRESIMS m/z 629.3839 [2M + H – 2H$_2$O]$^+$ (calcd for C$_{35}$H$_{47}$O$_{12}$, 629.3842) m/z 333.2060 [M + H]$^+$ (calcd for C$_{20}$H$_{25}$O$_{9}$, 333.2060).

Oplontalin C 15: [α]$_D^{25}$ +234.5 (c 0.07, MeOH); UV (CH$_3$CN–H$_2$O) $\lambda_{max}$ 215, 242, 254, 268, and 283 nm; 1H NMR and 13C NMR, see Table 1; HRESIMS m/z 527.3534 [2 + H – 3H$_2$O]$^+$ (calcd for C$_{27}$H$_{35}$O$_{12}$, 527.3525) m/z 291.1962 [M + H]$^+$ (calcd for C$_{15}$H$_{23}$O$_{8}$, 291.1960).
protonation state of His32 was set to NE2H, and a water molecule (HOH35) was extracted and set to “toggle and spin” in the advanced options. Both of the crystallized ligands were extracted, and the binding site was defined by one or more ligands. The two ligands were chosen, and a cavity file was generated. Per compound, up to 15 binding poses per ligand (GA run) were reported, and the setting “allow early termination” was disabled. The slow and most accurate GA search option was employed, and ChemPLP was used as scoring function. Finally, the docking poses were analyzed in LigandScout 3.12, and the most probable poses were selected with regard to structure–activity relationship (see Table 1). In order to achieve a distinct structure–activity analysis, only the comparatively highly active and the inactive compounds were considered. With regard to the flexibility of the receptor–ligand system, the interaction pattern of the docking poses was investigated before and after minimization of MMFF94 energy in LigandScout. Due to this minimization process, some of the molecules were able to form additional interactions with essential amino acids.

**ASSOCIATED CONTENT**

* Supporting Information The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b00691.

Biological evaluation, molecular docking, detailed isolation procedure, spectroscopic data, UV patterns, HRESIMS and NMR spectra (PDF)

Animation of Sephadex LH-20 fractionation of polyacetylene isomers (MP4)

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**Author Contributions**
Mirta Resater and Xin Liu contributed equally to this work.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We gratefully acknowledge the funding provided by the Austrian Science Fund (FWF): S10704, S10705, and S10711 (NFN: Drugs from Nature Targeting Inflammation (DNTI)). NAWI Graz is thanked for supporting the Graz Central Lab Plant, Environmental & Microbial Metabolomics.

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