

Late-stage divergent synthesis and antifouling activity of geraniol–butenolide hybrid molecules†

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Hybrid molecules consisting of geraniol and butenolide were designed and synthesized in the late-stage divergent strategy. In the synthetic route, ring-closing metathesis was utilized for the construction of butenolide moiety. Biological evaluation of the eight synthetic hybrid compounds revealed that these molecules exhibit the antifouling activity against the cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite* with EC₅₀ values of 0.30–1.31 µg/mL. These results denote that hybridization of the geraniol and butenolide structural motifs resulted in enhancement of the antifouling activity.

Introduction

Biofouling on the surface of submerged objects has caused technical and economic problems in marine industry worldwide.¹ Settlement and growing of marine organisms on the ship's hull increase the ship's weight and surface corrosion against seawater, thereby leading to decrease of ship's maneuverability and fuel efficiency. In addition, extensive growth of marine sessile organisms on the water intake constricts the inflow of cooling seawater and diminishes the cooling efficiency at seaside power plants. In order to control biofouling, organotin compounds including tributyltin (TBT) and tributyltin oxide (TBTO) were widely used as antifoulants worldwide. Although these biocides are effective antifouling agents, they exhibit high toxicity to a wide range of marine organisms.² Due to the increasing negative influence of organotin compounds on the marine environment, the International Maritime Organization (IMO) banned the use of organotins as antifouling paints in 2008. In recent years, alternative antifouling agents such as cuprous oxide, dichlofluanid, diuron, Irgarol 1051, Sea-Nine 211, and zinc pyrithione have been used, however, it has been suggested that these antifoulants might also have a negative impact on the environment.³ Therefore, development of effective, safe, and more environmentally benign antifouling compounds is strongly demanded.

Natural products are a promising source of environmentally friendly antifouling molecules.⁴ For example, geraniol (**1**, Fig. 1) and its esters exhibit the antifouling activity.^{5,6} Furthermore, Qian's research group has reported that the butenolide is a

key structural motif exerting the antifouling activity⁷ and butenolide **2**, isolated from marine *Streptomyces* strain GWS-BW-H5, inhibits the larval settlement of barnacle *Balanus (Amphibalanus) amphitrite* with EC₅₀ value of 4.82 µg/mL and shows no toxicity (LC₅₀ = >25 µg/mL).^{7a} In order to integrate the antifoulant activity and create more potent antifouling organic compounds, we planned to combine these two active motifs. Thus, hybrid molecules **3a–3d** and **4a–4d** consisting of geraniol and butenolide were designed. Their structural differences are the substituent on the butenolide portion (**3a–3d**: R¹ = H, **4a–4d**: R¹ = C₅H₁₁) and oxidation degrees at the C5 and C12 positions. Herein, we disclose the late-stage divergent synthesis of eight artificial geraniol–butenolide hybrid molecules **3a–3d** and **4a–4d**, and their antifouling activity and toxicity against the cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite*.

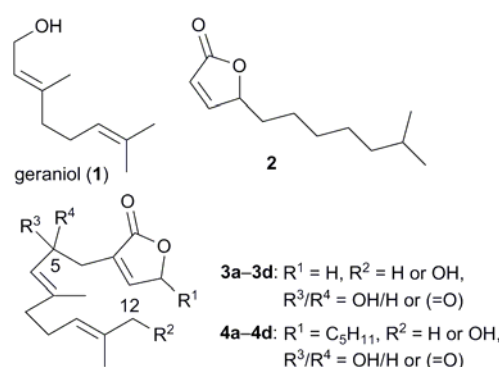


Fig. 1 Structures of geraniol (**1**), butenolide **2**, and geraniol–buteneolide hybrid molecules **3a–3d** and **4a–4d**.

Results and discussions

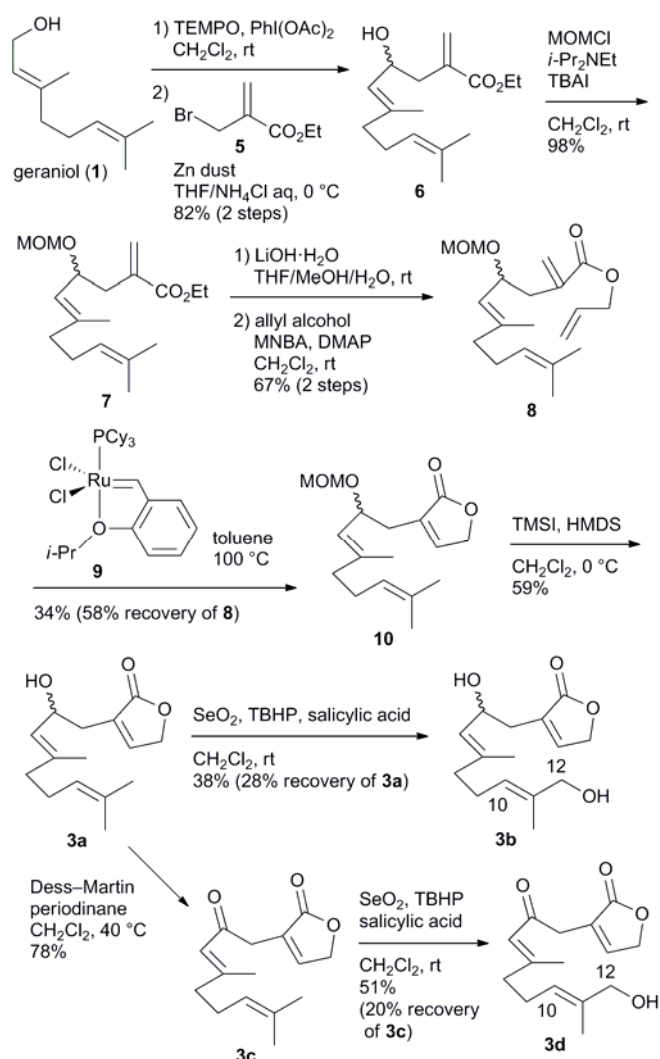
We initially embarked on synthesis of the target molecules **3a–3d** by using the synthetic route wherein these molecules could be supplied divergently at the late-stage of synthesis. Thus,

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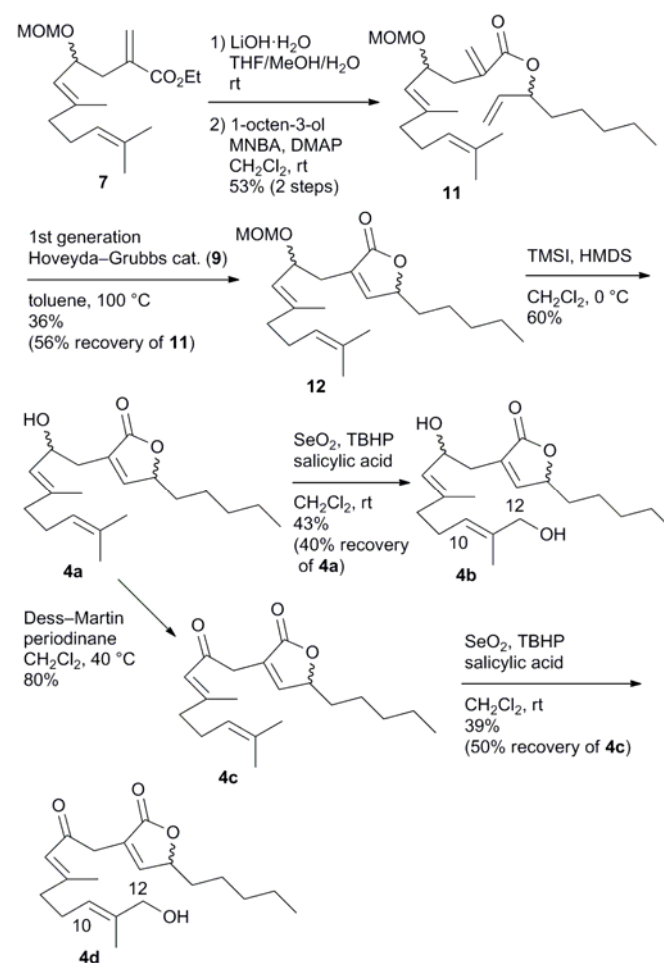
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oxidation of geraniol (**1**) with TEMPO/PhI(OAc)₂⁸ and subsequent Barbier-type allylation of the resulting α,β -unsaturated aldehyde with ethyl (2-bromomethyl)acrylate (**5**)/zinc dust⁹ in THF/aqueous NH₄Cl provided allylic alcohol **6** as a racemate in 82% yield in two steps (Scheme 1). Protection of the alcohol **6** as a MOM ether followed by hydrolysis of the resulting ethyl ester **7** with LiOH·H₂O afforded the corresponding carboxylic acid, which was connected with allyl alcohol under the Shiina esterification conditions¹⁰ to give allylic ester **8**. Ring-closing metathesis of the tetraene **8** was carried out by using first-generation Hoveyda–Grubbs catalyst (**9**)¹¹ in toluene at 100 °C to produce butenolide **10** in 34% yield along with the recovery of **8** in 58% yield. Treatment of the MOM ether **10** with TMSI/HMDS¹² furnished the alcohol **3a**. Allylic oxidation of **3a** was performed by SeO₂/TBHP/salicylic acid¹³ to afford the diol **3b**, selectively. The structure of the allylic alcohol **3b** was confirmed by the observed NOE of H-10/H₂-12. Furthermore, the allylic alcohol **3a** was oxidized by Dess–Martin periodinane¹⁴ to give the α,β -unsaturated ketone **3c**, which was derivatized to the alcohol **3d** by SeO₂-catalyzed allylic oxidation.^{13,15}

Scheme 1 Late-stage divergent synthesis of **3a–3d**.

Next, the synthesis of other target molecules **4a–4d** was investigated by the synthetic transformation similar to that used toward the synthesis of **3a–3d**. Shiina esterification¹⁰ between carboxylic acid, derived from the ethyl ester **7**, and 1-octen-3-ol took place to yield ester **11** as a mixture of four stereoisomers (Scheme 2). The tetraene **11** was subjected to ring-closing metathesis to produce butenolide **12**, which was deprotected with TMSI/HMDS¹² to afford the alcohol **4a**. Sharpless allylic oxidation¹³ of **4a** afforded the diol **4b**.¹⁵ In addition, Dess–Martin oxidation¹⁴ of the alcohol **4a** gave the ketone **4c**, which was exposed to allylic oxidation¹³ to furnish the alcohol **4d**.¹⁵

Scheme 2 Late-stage divergent synthesis of **4a–4d**.

Having completed the synthesis of all eight designed geraniol–butenolide hybrid molecules **3a–3d** and **4a–4d**, we next evaluated the antifouling activity of these synthetic products against the cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite*. The larvae were treated in 24-well polystyrene plates with various concentrations of the compounds in the dark for 96 h. Our results are summarized in Table 1. The hybrid molecule **3a** inhibited the larval settlement with EC₅₀ value of 1.18 μ g/mL, elucidating that the antifouling activity of **3a** is increased in comparison with that of geraniol (**1**, EC₅₀ = 2.71 μ g/mL)⁶ and the butenolide **2** (EC₅₀ = 4.82 μ g/mL).^{7a} The diol **3b**, hydroxylated product of **3a**, exhibited

about three times higher antifouling activity than that of **3a** ($EC_{50} = 0.44 \mu\text{g/mL}$). Both of the lipophilic compound **3c** and the alcohol **3d** retained the antifoulant activity without regard to the presence of hydroxy moiety at the C12 positions ($EC_{50} = 0.30$ and $0.74 \mu\text{g/mL}$). The antifouling activity of the synthetic products **4a–4d** bearing the pentyl substituent on the butenolide portion proved to be similar to that observed in **3a–3d** ($EC_{50} = 0.58–1.31 \mu\text{g/mL}$). Furthermore, the toxicity of **3a–3d** and **4a–4d** were also evaluated, and it was found that the alcohol **4a** was moderately toxic ($LC_{50} = 8.83 \mu\text{g/mL}$) and other molecules showed low or no toxicity. These findings denote that the geraniol–butenolide hybrid molecules have the antifoulant activity higher than that of geraniol (**1**) and the butenolide **2**, and are promising antifouling agents toward the field testing in the sea.

Table 1 Antifouling activity (EC_{50}) and toxicity (LC_{50}) of geraniol (**1**), butenolide **2**, and geraniol–butenolide hybrid molecules^a

Compound	EC_{50}	LC_{50}
1	2.71	>50
2^b	4.82	>25
3a	1.18	46.7
3b	0.44	>50
3c	0.30	37.0
3d	0.74	25.1
4a	0.81	8.83
4b	0.58	>50
4c	1.31	>50
4d	0.80	>50

^a Against the cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite*. EC_{50} and LC_{50} in $\mu\text{g/mL}$. ^b Reported by Qian's research group, see reference 7a.

Conclusions

In conclusion, we have tried to fulfill the urgent need for creation of the effective and environmentally friendly antifouling agents. In order to integrate the antifouling activity, we designed the geraniol–butenolide hybrid molecules **3a–3d** and **4a–4d**, wherein the structural differences are the substituent on the butenolide moiety and the C5- and C12-oxidation degrees. All eight designed compounds were synthesized by utilizing the ring-closing metathesis as a key transformation and the late-stage divergent strategy. Biological evaluation of the synthetic products against the cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite* established that all eight hybrid molecules are more antifouling active than each of geraniol (**1**) and the butenolide **2**, and most of them show low or no toxicity. These results mean that hybridization of the geraniol and butenolide structures led to enhancement of the antifouling activity. The results on the field tests of these synthetic hybrid molecules in the sea will be reported in due course.

Experimental

General methods

Reagents were used as received from commercial suppliers unless otherwise indicated. All reactions were carried out under an atmosphere of argon. Reaction solvents were purchased as dehydrated solvents and stored with active molecular sieves 4\AA under argon prior to use for reactions. All solvents for work-up procedure were used as received. Chemical shifts in the NMR spectra are reported in ppm with reference to the internal residual solvent (^1H NMR, CDCl_3 7.26 ppm, C_6D_6 7.16 ppm; ^{13}C NMR, CDCl_3 77.0 ppm). The following abbreviations are used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (J) are in hertz.

Allylic alcohol **6**

To a solution of geraniol (**1**) (0.50 mL, 2.85 mmol) in CH_2Cl_2 (29 mL) were added $\text{PhI}(\text{OAc})_2$ (1.10 g, 3.42 mmol) and TEMPO (44.5 mg, 0.285 mmol) at room temperature. After the mixture was stirred at the same temperature for 4 h, the reaction was quenched with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ at 0°C . The mixture was diluted with EtOAc, washed with saturated aqueous NaHCO_3 , H_2O , and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 20:1, 10:1) gave the corresponding aldehyde (902 mg), which was used for the next step without further purification.

To a solution of the aldehyde obtained above (902 mg) in THF (24 mL) and saturated aqueous NH_4Cl (4.8 mL) were added ethyl (2-bromomethyl)acrylate (**5**) (0.58 mL, 4.28 mmol) and zinc dust (559 mg, 8.55 mmol) at 0°C . After the mixture was stirred at the same temperature for 20 min, the mixture was filtered through a Celite pad and washed with EtOAc. The mixture was washed with H_2O and brine and dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 5:1) gave allylic alcohol **6** (622 mg, 82% in two steps): colorless oil; $R_f = 0.54$ (hexane/EtOAc = 2:1); IR (neat) 3414, 2924, 2850, 1715, 1630 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 6.24 (d, $J = 1.4$ Hz, 1 H), 5.64 (s, 1 H), 5.19 (dd, $J = 8.5, 1.2$ Hz, 1 H), 5.10–5.06 (m, 1 H), 4.60–4.54 (m, 1 H), 4.27–4.20 (m, 2 H), 2.56–2.46 (m, 2 H), 2.10–1.99 (m, 4 H), 1.68 (s, 3 H), 1.68 (d, $J = 1.2$ Hz, 3 H), 1.61 (s, 3 H), 1.32 (t, $J = 7.2$ Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 167.4, 138.5, 137.2, 131.6, 127.5, 126.9, 123.9, 67.6, 60.9, 40.7, 39.5, 26.5, 25.7, 17.7, 16.7, 14.3; HRMS (ESI–TOF) calcd for $\text{C}_{16}\text{H}_{26}\text{O}_3\text{Na}$ [$M + \text{Na}$]⁺ 289.1780, found 289.1779.

Methoxymethyl ether **7**

To a solution of alcohol **6** (2.86 g, 10.7 mmol) in CH_2Cl_2 (54 mL) were added *i*-Pr₂NEt (11.0 mL, 64.2 mmol), MOMCl (4.1 mL, 53.5 mmol), and TBAI (2.03 g, 5.50 mmol) at room temperature. After the mixture was stirred at the same temperature for 1 h, the reaction was quenched with saturated aqueous NH_4Cl . The mixture was diluted with EtOAc, washed with saturated aqueous NH_4Cl , saturated aqueous NaHCO_3 , H_2O , and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 20:1) gave methoxymethyl ether **7** (3.26 g, 98%): colorless oil; $R_f = 0.58$ (hexane/EtOAc = 4:1); IR (neat) 2927, 2883, 1717 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 6.20 (d, $J = 1.4$ Hz, 1 H), 5.60 (d, $J = 1.4$ Hz, 1 H), 5.06–4.99 (m, 2 H), 4.63 (d, $J = 6.6$ Hz, 1 H),

4.58–4.53 (m, 1 H), 4.45 (d, $J = 6.6$ Hz, 1 H), 4.22 (q, $J = 7.2$ Hz, 2 H), 3.31 (s, 3 H), 2.60 (dd, $J = 13.8, 7.7$ Hz, 1 H), 2.46 (dd, $J = 13.8, 6.0$ Hz, 1 H), 2.09–2.01 (m, 4 H), 1.68 (s, 3 H), 1.65 (d, $J = 1.2$ Hz, 3 H), 1.60 (s, 3 H), 1.31 (t, $J = 7.2$ Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 167.0, 140.3, 137.2, 131.5, 127.0, 124.6, 123.9, 93.2, 70.5, 60.6, 55.2, 39.6, 38.6, 26.4, 25.7, 17.7, 16.6, 14.3; HRMS (ESI–TOF) calcd for $\text{C}_{18}\text{H}_{30}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 333.2042, found 333.2044.

Ester 8

To a solution of ethyl ester **7** (52.8 mg, 0.170 mmol) in THF (1.0 mL), MeOH (0.3 mL), and H_2O (0.3 mL) was added $\text{LiOH}\cdot\text{H}_2\text{O}$ (14.3 mg, 0.340 mmol) at room temperature. After the mixture was stirred at the same temperature for 13 h, the mixture was neutralized with aqueous HCl at 0 °C. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted with EtOAc three times and the combined organic phase was dried over Na_2SO_4 . Concentration gave the corresponding carboxylic acid (46.5 mg), which was used for the next step without further purification.

To a solution of the carboxylic acid obtained above (46.5 mg) in CH_2Cl_2 (1.6 mL) were added allyl alcohol (13 μL , 0.191 mmol), DMAP (48.2 mg, 0.394 mmol), and MNBA (67.9 mg, 0.197 mmol) at room temperature. After the mixture was stirred at the same temperature for 1 h, the mixture was filtered through short column chromatography (hexane/EtOAc = 1:1). Concentration and column chromatography (hexane/EtOAc = 15:1) gave ester **8** (37.3 mg, 67% in two steps): colorless oil; $R_f = 0.49$ (hexane/EtOAc = 7:1); IR (neat) 2927, 2878, 1721 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 6.23 (d, $J = 1.2$ Hz, 1 H), 6.00–5.90 (m, 1 H), 5.63 (d, $J = 1.2$ Hz, 1 H), 5.34 (dq, $J = 17.1, 1.2$ Hz, 1 H), 5.23 (dq, $J = 10.4, 1.2$ Hz, 1 H), 5.07–5.03 (m, 1 H), 5.00 (dd, $J = 9.3, 1.2$ Hz, 1 H), 4.66 (dd, $J = 5.6, 1.2$ Hz, 2 H), 4.62 (d, $J = 6.8$ Hz, 1 H), 4.58–4.52 (m, 1 H), 4.44 (d, $J = 6.8$ Hz, 1 H), 3.30 (s, 3 H), 2.61 (dd, $J = 13.8, 7.6$ Hz, 1 H), 2.47 (dd, $J = 13.8, 6.0$ Hz, 1 H), 2.09–2.00 (m, 4 H), 1.67 (s, 3 H), 1.64 (d, $J = 1.2$ Hz, 3 H), 1.60 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.6, 140.4, 137.0, 132.2, 131.5, 127.4, 124.5, 123.9, 118.0, 93.2, 70.5, 65.3, 55.2, 39.6, 38.5, 26.4, 25.7, 17.7, 16.6; HRMS (ESI–TOF) calcd for $\text{C}_{19}\text{H}_{30}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 345.2042, found 345.2047.

Butenolide 10

To a solution of tetraene **8** (33.0 mg, 0.102 mmol) in toluene (10 mL) was added first-generation Hoveyda–Grubbs catalyst (**9**) (2.5 mg, 4.16 μmol) at room temperature. After the mixture was stirred at 100 °C for 2 days, the mixture was filtered through short column chromatography (hexane/EtOAc = 2:1). Concentration and column chromatography (hexane/EtOAc = 15:1, 8:1, 4:1) gave butenolide **10** (10.1 mg, 34%) and tetraene **8** (19.2 mg, 58% recovery). Butenolide **10**: colorless oil; $R_f = 0.31$ (hexane/EtOAc = 2:1); IR (neat) 2925, 2852, 1757 cm^{-1} ; ^1H NMR (400 MHz, C_6D_6) δ 6.32 (t, $J = 1.3$ Hz, 1 H), 5.13–5.09 (m, 2 H), 4.83–4.77 (m, 1 H), 4.73 (d, $J = 6.6$ Hz, 1 H), 4.42 (d, $J = 6.6$ Hz, 1 H), 3.85 (d, $J = 1.3$ Hz, 2 H), 3.15 (s, 3 H), 2.60 (dd, $J = 7.6, 1.2$ Hz, 1 H), 2.49 (dd, $J = 5.6, 1.2$ Hz, 1 H), 2.11–1.96 (m, 4 H), 1.66 (s, 3 H), 1.62 (d, $J = 1.2$ Hz, 3 H), 1.53 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.1, 146.2, 141.2, 131.7,

130.6, 123.9, 123.7, 93.3, 70.1, 69.7, 55.4, 39.6, 31.6, 26.4, 25.7, 17.7, 16.6; HRMS (ESI–TOF) calcd for $\text{C}_{17}\text{H}_{26}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 317.1729, found 317.1733.

Alcohol 3a

To a solution of methoxymethyl ether **10** (38.5 mg, 0.131 mmol) in CH_2Cl_2 (1.3 mL) were added HMDS (0.27 mL, 1.29 mmol) and TMSI (93 μL , 0.653 mmol) at 0 °C. After the mixture was stirred at the same temperature for 10 min, the reaction was quenched with saturated aqueous NaHCO_3 . The mixture was diluted with EtOAc, washed with saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$, H_2O , and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane, hexane/EtOAc = 7:1, 1:1) gave alcohol **3a** (19.5 mg, 59%): colorless oil; $R_f = 0.49$ (hexane/EtOAc = 1:1); IR (neat) 3483, 2968, 2917, 2847, 1754, 1663 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.28 (t, $J = 1.5$ Hz, 1 H), 5.22 (dd, $J = 8.3, 1.2$ Hz, 1 H), 5.08–5.05 (m, 1 H), 4.80 (d, $J = 1.5$ Hz, 2 H), 4.68–4.63 (m, 1 H), 2.60–2.48 (m, 2 H), 2.10–2.00 (m, 4 H), 1.70 (d, $J = 1.2$ Hz, 3 H), 1.69 (s, 3 H), 1.61 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.6, 146.6, 139.6, 131.8, 131.0, 126.6, 123.7, 70.4, 66.7, 39.5, 33.7, 26.4, 25.7, 17.8, 16.8; HRMS (ESI–TOF) calcd for $\text{C}_{15}\text{H}_{22}\text{O}_3\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 273.1467, found 273.1470.

Diol 3b

To a solution of salicylic acid (0.3 mg, 2.17 μmol) in CH_2Cl_2 (0.5 mL) were added SeO_2 (0.1 mg, 0.901 μmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 6.8 μL , 34.0 μmol) at room temperature. After the mixture was stirred at the same temperature for 10 min, to the mixture was added alcohol **3a** (5.4 mg, 21.6 μmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.1 mL) at room temperature. After the mixture was stirred at the same temperature for 4 h, the reaction was quenched with saturated aqueous Na_2SO_3 . The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 2:1, 1:2) gave diol **3b** (2.2 mg, 38%) and alcohol **3a** (1.5 mg, 28% recovery). Diol **3b**: colorless oil; $R_f = 0.34$ (EtOAc); IR (neat) 3398, 2919, 2855, 1739, 1651 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.29 (t, $J = 1.2$ Hz, 1 H), 5.35 (td, $J = 6.8, 1.2$ Hz, 1 H), 5.20 (dd, $J = 8.5, 1.2$ Hz, 1 H), 4.81 (d, $J = 1.2$ Hz, 2 H), 4.67–4.62 (m, 1 H), 4.00 (s, 2 H), 2.60–2.49 (m, 2 H), 2.20–2.14 (m, 2 H), 2.07 (d, $J = 7.6$ Hz, 2 H), 1.69 (d, $J = 1.2$ Hz, 3 H), 1.66 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.7, 146.9, 138.9, 135.2, 130.8, 126.9, 125.1, 70.5, 68.8, 66.7, 39.0, 33.6, 25.7, 16.7, 13.8; HRMS (ESI–TOF) calcd for $\text{C}_{15}\text{H}_{22}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 289.1416, found 289.1421.

Ketone 3c

To a solution of alcohol **3a** (5.3 mg, 21.2 μmol) in CH_2Cl_2 (0.3 mL) was added Dess–Martin periodinane (27.0 mg, 63.7 μmol) at room temperature. After the mixture was stirred at 40 °C for 22 h, to the mixture was added Dess–Martin periodinane (27.0 mg, 63.7 μmol) at room temperature. After the mixture was stirred at 40 °C for 3 h, the mixture was filtered through short column chromatography (EtOAc). Concentration and column chromatography (hexane/EtOAc = 8:1) gave ketone **3c**

(4.1 mg, 78%): colorless oil; R_f = 0.65 (hexane/EtOAc = 1:1); IR (neat) 2966, 2921, 2844, 1757, 1689, 1618 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.48 (t, J = 1.5 Hz, 1 H), 6.11 (s, 1 H), 5.07 (brs, 1 H), 4.86 (d, J = 1.5 Hz, 2 H), 3.47 (d, J = 1.5 Hz, 2 H), 2.18–2.17 (m, 4 H), 2.16 (d, J = 1.0 Hz, 3 H), 1.70 (s, 3 H), 1.62 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 194.6, 173.9, 161.2, 147.8, 132.7, 127.4, 122.7, 122.4, 70.6, 41.4, 39.5, 26.2, 25.7, 19.8, 17.8; HRMS (ESI–TOF) calcd for $\text{C}_{15}\text{H}_{20}\text{O}_3\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 271.1310, found 271.1310.

Alcohol 3d

To a solution of salicylic acid (1.7 mg, 12.3 μmol) in CH_2Cl_2 (0.5 mL) were added SeO_2 (0.7 mg, 6.31 μmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 38 μL , 0.190 mmol) at room temperature. After the mixture was stirred at the same temperature for 10 min, to the mixture was added ketone **3c** (6.1 mg, 24.6 μmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.1 mL) at room temperature. After the mixture was stirred at the same temperature for 4 h, the reaction was quenched with saturated aqueous Na_2SO_3 . The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 2:1, 1:1) gave alcohol **3d** (3.3 mg, 51%) and ketone **3c** (1.2 mg, 20% recovery). Alcohol **3d**: colorless oil; R_f = 0.43 (EtOAc); IR (neat) 3435, 2954, 2920, 2851, 1750, 1685, 1617 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.47 (t, J = 2.0 Hz, 1 H), 6.13 (s, 1 H), 5.38–5.35 (m, 1 H), 4.86 (d, J = 2.0 Hz, 2 H), 4.01 (s, 2 H), 3.47 (s, 2 H), 2.27–2.23 (m, 4 H), 2.17 (s, 3 H), 1.67 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 194.5, 174.0, 160.6, 147.9, 136.0, 127.4, 123.9, 122.6, 70.7, 68.6, 40.9, 39.7, 25.5, 19.7, 13.8; HRMS (ESI–TOF) calcd for $\text{C}_{15}\text{H}_{20}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 287.1259, found 287.1258.

Ester 11

To a solution of ethyl ester **7** (535 mg, 1.72 mmol) in THF (10 mL), MeOH (3.4 mL), and H_2O (3.4 mL) was added LiOH· H_2O (143 mg, 3.42 mmol) at room temperature. After the mixture was stirred at the same temperature for 2 days, the mixture was neutralized with aqueous HCl at 0 $^\circ\text{C}$. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted with EtOAc three times and the combined organic phase was dried over Na_2SO_4 . Concentration and short column chromatography (hexane/EtOAc = 4:1) gave the corresponding carboxylic acid (395 mg), which was used for the next step without further purification.

To a solution of the carboxylic acid obtained above (395 mg) in CH_2Cl_2 (14 mL) were added 1-octen-3-ol (0.26 mL, 1.70 mmol), DMAP (410 mg, 3.36 mmol), and MNBA (578 mg, 1.68 mmol) at room temperature. After the mixture was stirred at the same temperature for 1 h, the mixture was filtered through short column chromatography (hexane/EtOAc = 3:1). Concentration and column chromatography (hexane/EtOAc = 20:1) gave ester **11** (361 mg, 53% in two steps): colorless oil; R_f = 0.56 (hexane/EtOAc = 2:1); IR (neat) 2929, 2852, 1717, 1634 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 6.23 (d, J = 1.5 Hz, 1 H), 5.80 (ddd, J = 17.2, 10.5, 6.4 Hz, 1 H), 5.61 (s, 1 H), 5.34–5.31 (m, 1 H), 5.25 (d, J = 17.2 Hz, 1 H), 5.16 (d, J = 10.5 Hz, 1 H), 5.06–

5.04 (m, 1 H), 4.99 (d, J = 9.3 Hz, 1 H), 4.63 (d, J = 6.6 Hz, 1 H), 4.55 (q, J = 7.7 Hz, 1 H), 4.44 (d, J = 6.6 Hz, 1 H), 3.30 (s, 3 H), 2.65–2.58 (m, 1 H), 2.45 (dd, J = 13.6, 5.0 Hz, 1 H), 2.09–2.01 (m, 4 H), 1.67 (s, 3 H), 1.64 (s, 3 H), 1.63–1.57 (m, 2 H), 1.60 (s, 3 H), 1.37–1.30 (m, 6 H), 0.88 (t, J = 6.7 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.2, 140.4, 137.3, 136.6, 131.5, 127.2, 124.6, 123.9, 116.4, 93.2, 75.1, 70.4, 55.2, 39.6, 38.6, 34.3, 31.6, 26.4, 25.7, 24.8, 22.6, 17.7, 16.6, 14.0; HRMS (ESI–TOF) calcd for $\text{C}_{24}\text{H}_{40}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 415.2824, found 415.2828.

Butenolide 12

To a solution of tetraene **11** (60.3 mg, 0.154 mmol) in toluene (17 mL) was added first-generation Hoveyda–Grubbs catalyst (**9**) (3.8 mg, 6.64 μmol) at room temperature. After the mixture was stirred at 100 $^\circ\text{C}$ for 3 days, the mixture was filtered through short column chromatography (hexane/EtOAc = 1:2). Concentration and column chromatography (hexane/EtOAc = 20:1, 7:1) gave butenolide **12** (20.4 mg, 36%) and tetraene **11** (34.0 mg, 56% recovery). Butenolide **12**: colorless oil; R_f = 0.40 (hexane/EtOAc = 2:1); IR (neat) 2954, 2928, 2856, 1756 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.14 (s, 1 H), 5.05–5.01 (m, 2 H), 4.88 (t, J = 6.5 Hz, 1 H), 4.65 (d, J = 6.6 Hz, 1 H), 4.63–4.57 (m, 1 H), 4.47 (d, J = 6.6 Hz, 1 H), 3.32 (s, 1.5 H), 3.31 (s, 1.5 H), 2.59 (dd, J = 14.8, 7.6 Hz, 1 H), 2.44 (dd, J = 14.8, 6.1 Hz, 1 H), 2.11–2.01 (m, 4 H), 1.71–1.57 (m, 2 H), 1.68 (s, 3 H), 1.68 (s, 3 H), 1.60 (s, 3 H), 1.45–1.29 (m, 6 H), 0.89 (t, J = 7.0 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 173.6, 150.2, 150.1, 141.2, 131.7, 130.5, 124.0, 123.7, 93.3, 81.3, 69.8, 69.7, 55.4, 39.6, 33.6, 31.6, 26.4, 25.7, 24.8, 24.8, 22.5, 17.8, 16.7, 14.0; HRMS (ESI–TOF) calcd for $\text{C}_{22}\text{H}_{36}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 387.2511, found 387.2514.

Alcohol 4a

To a solution of methoxymethyl ether **12** (19.0 mg, 52.1 μmol) in CH_2Cl_2 (1.0 mL) were added HMDS (38 μL , 0.182 mmol) and TMSI (13 μL , 91.3 μmol) at 0 $^\circ\text{C}$. After the mixture was stirred at the same temperature for 1 h, to the mixture were added HMDS (38 μL , 0.182 mmol) and TMSI (13 μL , 91.3 μmol) at 0 $^\circ\text{C}$. After the mixture was stirred at the same temperature for 1 h, the reaction was quenched with saturated aqueous NaHCO_3 . The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane, hexane/EtOAc = 20:1, 4:1) gave alcohol **4a** (10.1 mg, 60%): colorless oil; R_f = 0.44 (hexane/EtOAc = 2:1); IR (neat) 3426, 2924, 2856, 1749 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.16 (d, J = 1.2 Hz, 0.5 H), 7.16 (d, J = 1.2 Hz, 0.5 H), 5.22–5.19 (m, 1 H), 5.08–5.05 (m, 1 H), 4.94–4.90 (m, 1 H), 4.67–4.61 (m, 1 H), 2.57–2.45 (m, 2 H), 2.08–1.99 (m, 4 H), 1.73–1.62 (m, 2 H), 1.69 (s, 3 H), 1.68 (s, 3 H), 1.60 (s, 3 H), 1.44–1.30 (m, 6 H), 0.89 (t, J = 7.1 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.1, 150.6, 150.5, 139.5, 139.4, 131.7, 130.8, 130.8, 126.5, 123.7, 81.7, 81.6, 66.8, 66.7, 39.5, 33.7, 33.5, 31.5, 26.5, 25.7, 24.7, 22.5, 17.8, 16.8, 16.8, 14.0; HRMS (ESI–TOF) calcd for $\text{C}_{20}\text{H}_{32}\text{O}_3\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 343.2249, found 343.2247.

Diol 4b

To a mixture of salicylic acid (0.4 mg, 2.90 μmol) and SeO_2 (0.2 mg, 1.80 μmol) in CH_2Cl_2 (0.5 mL) was added and TBHP (5.0 M in 2,4,6-trimethylpentane, 4.9 μL , 24.5 μmol) at room temperature. After the mixture was stirred at the same temperature for 10 min, to the mixture was added alcohol **4a** (9.7 mg, 30.3 μmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.1 mL) at room temperature. After the mixture was stirred at the same temperature for 2 days, the reaction was quenched with saturated aqueous Na_2SO_3 . The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 3:1, 1:1) gave diol **4b** (4.4 mg, 43%) and alcohol **4a** (3.9 mg, 40% recovery). Diol **4b**: colorless oil; R_f = 0.29 (hexane/EtOAc = 1:1); IR (neat) 3398, 2924, 2856, 1741 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.17 (d, J = 1.4 Hz, 1 H), 5.36–5.33 (m, 1 H), 5.21–5.18 (m, 1 H), 4.94–4.91 (m, 1 H), 4.66–4.60 (m, 1 H), 4.00 (s, 2 H), 2.54–2.50 (m, 2 H), 2.17–2.04 (m, 4 H), 1.70–1.62 (m, 2 H), 1.69 (s, 3 H), 1.69 (s, 3 H), 1.66 (s, 3 H), 1.44–1.26 (m, 6 H), 0.91–0.88 (m, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 174.3, 150.8, 138.8, 135.2, 130.7, 126.9, 125.1, 81.7, 68.8, 66.9, 66.8, 39.0, 33.6, 33.5, 31.6, 29.7, 25.7, 24.7, 22.5, 16.7, 14.0, 13.8; HRMS (ESI–TOF) calcd for $\text{C}_{20}\text{H}_{32}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 359.2198, found 359.2200.

Ketone **4c**

To a solution of alcohol **4a** (61.3 mg, 0.191 mmol) in CH_2Cl_2 (1.9 mL) was added Dess–Martin periodinane (243 mg, 0.574 mmol) at room temperature. After the mixture was stirred at 40 $^\circ\text{C}$ for 3 h, to the mixture was added Dess–Martin periodinane (243 mg, 0.574 mmol) at room temperature. After the mixture was stirred at 40 $^\circ\text{C}$ for 1 day, the mixture was filtered through short column chromatography (hexane/EtOAc = 2:1). Concentration and column chromatography (hexane/EtOAc = 9:1) gave ketone **4c** (48.4 mg, 80%): colorless oil; R_f = 0.56 (hexane/EtOAc = 3:1); IR (neat) 2954, 2928, 2861, 1756, 1690, 1619 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.38 (d, J = 1.2 Hz, 1 H), 6.10 (s, 1 H), 5.06 (brs, 1 H), 4.99–4.95 (m, 1 H), 3.44 (s, 2 H), 2.17–2.15 (m, 4 H), 2.15 (s, 3 H), 1.79–1.65 (m, 2 H), 1.69 (s, 3 H), 1.61 (s, 3 H), 1.46–1.25 (m, 6 H), 0.89 (t, J = 6.8 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 194.7, 173.5, 161.0, 151.6, 132.6, 127.2, 122.7, 122.4, 81.9, 41.4, 39.5, 33.5, 31.5, 26.2, 25.7, 24.7, 22.5, 19.8, 17.8, 14.0; HRMS (ESI–TOF) calcd for $\text{C}_{20}\text{H}_{30}\text{O}_3\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 341.2093, found 341.2095.

Alcohol **4d**

To a solution of salicylic acid (2.3 mg, 16.7 μmol) in CH_2Cl_2 (0.5 mL) were added SeO_2 (0.8 mg, 7.21 μmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 53 μL , 0.265 mmol) at room temperature. After the mixture was stirred at the same temperature for 10 min, to the mixture was added ketone **4c** (10.6 mg, 33.3 μmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.1 mL) at room temperature. After the mixture was stirred at the same temperature for 2 h, the reaction was quenched with saturated aqueous Na_2SO_3 . The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4 . Concentration and column chromatography (hexane/EtOAc = 9:1, 2:1, 1:1) gave alcohol **4d** (4.3 mg, 39%)

and ketone **4c** (5.3 mg, 50% recovery). Alcohol **4d**: colorless oil; R_f = 0.39 (hexane/EtOAc = 1:1); IR (neat) 3460, 2954, 2925, 2861, 1754, 1689, 1616 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 7.37 (d, J = 1.2 Hz, 1 H), 6.12 (d, J = 0.7 Hz, 1 H), 5.37–5.35 (m, 1 H), 4.98 (ddd, J = 7.6, 5.6, 1.6 Hz, 1 H), 4.01 (s, 2 H), 3.43 (d, J = 1.2 Hz, 2 H), 2.27–2.20 (m, 4 H), 2.16 (d, J = 1.2 Hz, 3 H), 1.80–1.62 (m, 2 H), 1.67 (s, 3 H), 1.48–1.26 (m, 6 H), 0.90 (t, J = 7.2 Hz, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 194.7, 173.5, 160.4, 151.7, 136.1, 127.3, 123.9, 122.6, 82.0, 68.6, 40.9, 39.6, 33.5, 31.5, 25.5, 24.7, 22.5, 19.7, 14.0, 13.8; HRMS (ESI–TOF) calcd for $\text{C}_{20}\text{H}_{30}\text{O}_4\text{Na}$ [$\text{M} + \text{Na}$] $^+$ 357.2042, found 357.2045.

Antifouling activity and toxicity

Adult barnacles of *Balanus (Amphibalanus) amphitrite* were collected at Mega fishing port (Himeji, Hyogo, Japan) and maintained in aquaria at 20 ± 1 $^\circ\text{C}$ by feeding with brine shrimp (*Artemia salina*) nauplii for one week. Cypris larvae of barnacle *Balanus (Amphibalanus) amphitrite* were obtained by larval culture in the laboratory according to the method reported by Nogata and co-workers.¹⁶ Obtained cypris larvae were aged for 2–3 days prior to use at 5 $^\circ\text{C}$ in the dark. The effects of test compounds on the barnacle cyprids settlement were tested using 24-well polystyrene plates (Corning, NY, USA) according to our previous report.¹⁷ Each test compound was dissolved in 2 μL of DMSO and aliquots of the solution were applied to wells of 24-well polystyrene plates (0.1, 0.3, 1.0, 3.0, 10, and 50 μg). DMSO alone showed no effects on larval settlement at the concentration used in this assay (0.2%). Approximately 10 cypris larvae were added to each well filled with filtered natural seawater (28 psu) at final volume of 1.0 mL. After the incubation at 25 $^\circ\text{C}$ in the dark for 96 h, the number of larvae, which settled (including metamorphosed larvae), died, or did not settle, was counted under a microscope. Each level of the experiments was carried out with three wells and the assay was repeated three times. The assay was performed with CuSO_4 (0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 10 μg) as a positive control. The assay without compound was performed as a control. The antifouling activity and toxicity were expressed as EC_{50} and LC_{50} values, respectively. The EC_{50} and LC_{50} values were calculated by probit analysis according to Nogata's report.¹⁸ When probit analysis could not be applied to calculate the values, these were estimated by straight-line graphical interpolation.

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