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Chemical synthesis and antifouling activity of monoterpene–furan hybrid molecules†

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Geraniol, a monoterpene, and furan are structural motifs that exhibit antifouling activity. In this study, monoterpene–furan hybrid molecules with potentially enhanced antifouling activity were designed and synthesized. The nine synthetic hybrids showed antifouling activity against the cypris larvae of the barnacle *Balanus* (*Amphibalanus*) *amphitrite* with EC_{50} values of 1.65–4.70 μg mL^{-1}. This activity is higher than that of geraniol and the reference furan compound. This hybridization approach to increase antifouling activity is useful and can also be extended to other active structural units.

Introduction

Biofouling is the adhesion of organisms, such as barnacles, bryozoans, and mussels, on surfaces submerged in seawater.[1] The accumulation of marine organisms on submarine structures results in economic and environmental problems on a global scale. The appearance of biofouling on ship hulls causes their weight to increase and results in higher frictional resistance with seawater, which decreases the fuel efficiency and increases emission of greenhouse gases.[1a,1c] Fouling organisms block seawater pipelines in seaside power plants, which lowers the efficiency of cooling-water systems.[2] In the marine aquaculture industry, biofouling growth on fish cages restricts water exchange due to net occlusion and deforms cages due to the extra weight imposed by the settled organisms.[3] The economic costs of biofouling control in marine industries are substantial.[1,3] Several techniques to prevent biofouling have been developed, including acoustic methods,[4] electrochemical systems,[5] freshwater flushing,[6] laser cleaning,[7] mechanical cleaning,[8] photocatalytic systems,[9] ultraviolet irradiation,[10] and antifouling coatings.[1a,11] Among these methods, antifouling coatings are regarded as the most effective, economic, and convenient technique. The use of organotin compounds as biocidal antifoulant paints had been introduced in the early 1960s, and tributyltin and triphenyltin derivatives were widely employed as antifouling agents.[12] However, these organotin compounds exhibit high toxicity to a wide variety of organisms[12,13] and have negative influences on marine organisms, such as imposex, intersex, and masculinization.[12,14] These harmful effects of organotin reagents on marine ecosystems drove the International Maritime Organization (IMO) to ban their use as antifoulants on ships in 2008.[15] Copper and zinc compounds are tin-free alternative antifouling agents.[16] In addition, booster biocides, such as dichlofluanid, diuron, and Sea-Nine 211 are used as metal-free antifoulants.[16] However, these antifouling agents are toxic to several marine creatures and have negative effects on marine environments.[16,17] Therefore, the creation of effective and environmentally friendly antifouling agents has become a research topic of great significance.

Natural products have been suggested as potentially ideal sources for the development of novel and environmentally benign antifouling agents.[18] Geraniol (**1**; Figure 1), a naturally occurring monoterpene, and its derivatives are reported to show antifouling activity with low to no toxicity.[19] Additionally, furan is a structural motif found in several natural products with antifouling activity.[20] For example, dihydrofurospongin II (**2**) and euryfuran (**3**), which have been isolated from Mediterranean sponges, display inhibitory effects on the larval settlement of the barnacle *Balanus* (*Amphibalanus*) *amphitrite* without toxicity.[20c,21] Although the antifouling activity of **1** and furan-containing natural products has already been reported, little attention has been paid to the antifouling activity of hybrid molecules that possess monoterpene and furan structural scaffolds in a single compound. Therefore, we have designed monoterpene–furan hybrid molecules **4**, in which the structural variety is introduced in the monoterpene unit, as novel antifoulants in this study.[22–24] Herein, we report the concise synthesis of nine hybrids **4a**–**4i**, and their antifouling activity and toxicity toward the cypris larvae of the barnacle *Balanus* (*Amphibalanus*) *amphitrite*.

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† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

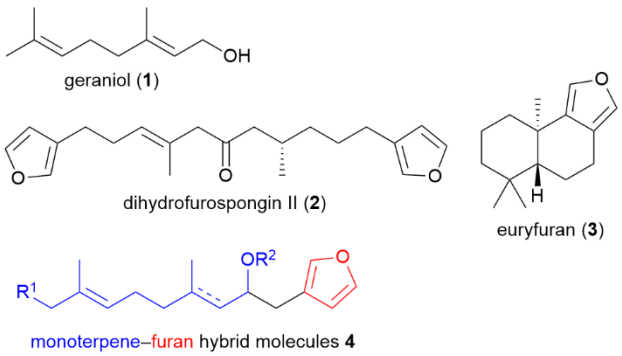
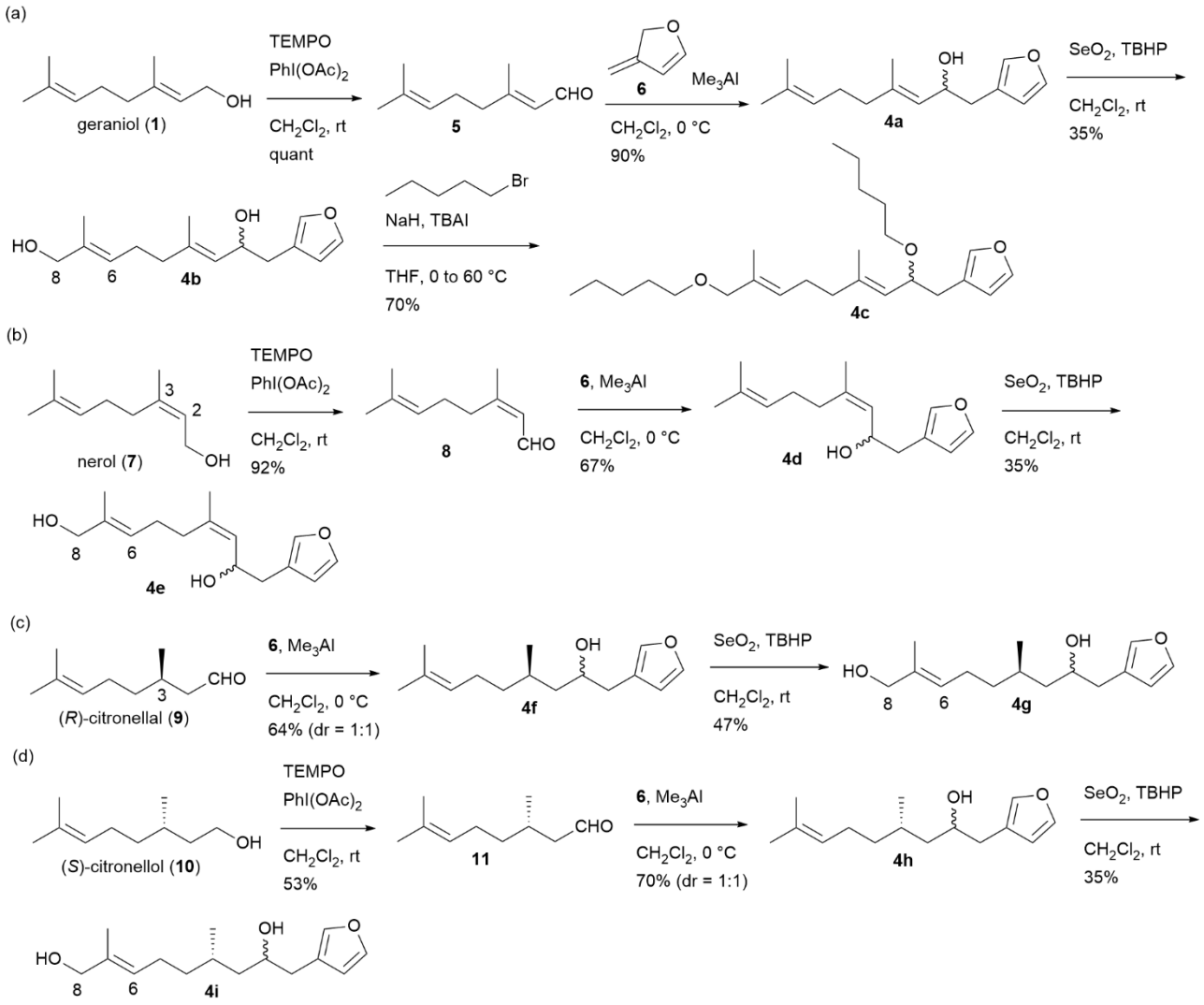


Fig. 1 Structures of antifouling active natural products **1**–**3** and monoterpene–furan hybrid molecules **4**.

Results and discussion

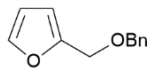
To install structural variation in the monoterpene moiety of the hybrid molecules, geraniol (**1**), nerol (**7**), (*R*)-citronellal (**9**), and (*S*)-citronellol (**10**) were chosen as scaffolds. We first examined the synthesis of geraniol–furan hybrid molecules. Treatment of **1** (Scheme 1a) with TEMPO/PhI(OAc)$_2$[25] quantitatively furnished geranial (**5**).[26] The furan domain was next introduced in accordance with the protocol reported by Miles *et al.*[27] The carbonyl-ene reaction between **5** and 3-methylene-2,3-dihydrofuran (**6**) with Me_3Al proceeded smoothly at 0 °C in CH_2Cl_2, affording the first hybrid molecule **4a** in 90% yield.[28] Treatment of **4a** with SeO_2/TBHP[29] provided the second hybrid molecule **4b** in 35% yield.[30] Diol **4b** was treated with 1-bromopentane/NaH/TBAI to give the third hybrid molecule **4c** in 70% yield. To clarify the influence of the C2/C3 alkene geometry on the biological activity, nerol (**7**; Scheme 1b) was used as the starting material instead of geraniol (**1**). Neral (**8**),[26] which was obtained by TEMPO oxidation[25] of **7**, was treated with **6** in the presence of Me_3Al,[27] producing allylic alcohol **4d** in 67% yield. Allylic oxidation of **4d** with SeO_2/TBHP[29] afforded diol **4e** in 35% yield.[30] Next, (*R*)-citronellal (**9**; Scheme 1c), which has a C3 chiral center, was used as the monoterpene unit. The carbonyl-ene reaction[27] of **9** and **6** gave alcohol **4f** in 64% yield as a 1:1 diastereomeric mixture, which was converted into diol **4g** with SeO_2/TBHP[29] in 47% yield.[30] Moreover, similar transformations were applied to (*S*)-citronellol (**10**; Scheme 1d), which afforded alcohol **4h** and diol **4i**.[30]



Scheme 1 Synthesis of monoterpene–furan hybrid molecules **4a**–**4i**.

With the designed monoterpene–furan hybrid molecules **4a**–**4i** in hand, we then evaluated the biological activity of these nine hybrids against the cypris larvae of the barnacle *Balanus* (*Amphibalanus*) *amphitrite*. The antifouling activity and toxicity of each compound were evaluated as the 50% effective concentration (EC_{50}) and the 50% lethal concentration (LC_{50}). First, the activity of $CuSO_4$ was assessed as a positive control, and the obtained values (EC_{50} = 0.29 μg mL^{-1}; LC_{50} = 2.10 μg mL^{-1}) were comparable to the reported ones (Table 1).[31] Geraniol (**1**) and (*S*)-citronellol (**10**) showed antifouling activity with EC_{50} values of 19.3 and 12.7 μg mL^{-1}, respectively, without toxicity (LC_{50} > 50 μg mL^{-1}). The reference furan compound **12**[32] displayed an EC_{50} value of 21.8 μg mL^{-1} and no toxicity. Hybrid molecule **4a** exhibited an inhibitory effect (EC_{50} = 4.28 μg mL^{-1}) commensurate with a higher antifouling activity than that of **1** and **12**. The antifouling effect of diol **4b** (EC_{50} = 2.73 μg mL^{-1}) was slightly increased in comparison with that of **4a**. The alkylated lipophilic compound **4c** showed an EC_{50} value of 4.57 μg mL^{-1}. Hybrids **4d** and **4e**, which were derived from nerol (**7**), retained settlement-inhibitory activity (**4d**: EC_{50} = 4.68 μg mL^{-1}; **4e**: 4.70 μg mL^{-1}), revealing that the difference in the C2/C3 alkene geometry between **4a**/**4b** and **4d**/**4e** has little influence on the antifouling activity. Hybrid molecules **4f**–**4i** exhibited antifouling activity (EC_{50} = 1.65–4.48 μg mL^{-1}) similar to that of **4a**–**4e**, which clarified that the C2/C3 alkene moieties in **4a**–**4e** are not essential for their antifouling activity. In addition, the toxicity was also evaluated, which revealed that hybrids **4d**, **4f**, **4g**, and **4h** showed weak toxicity (LC_{50} = 24.0–47.9 μg mL^{-1}), while LC_{50} values of the other hybrids were over 50 μg mL^{-1}. These results indicate that all nine hybrid molecules **4a**–**4i** have greater antifouling activity than the structural scaffolds **1**, **10**, and **12** and exhibit low toxicity.

Table 1 Antifouling activity (EC_{50}) and toxicity (LC_{50}) of $CuSO_4$, geraniol (**1**), (*S*)-citronellol (**10**), furan **12**, and hybrid molecules **4a**–**4i**[a]



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Compound	EC_{50} (μg mL^{-1})	LC_{50} (μg mL^{-1})
$CuSO_4$	0.29	2.10
1	19.3	>50
10	12.7	>50
12	21.8	>50
4a	4.28	>50
4b	2.73	>50
4c	4.57	>50
4d	4.68	47.9
4e	4.70	>50
4f	4.41	35.2
4g	4.20	31.7
4h	1.65	24.0
4i	4.48	>50

[a] Against the cypris larvae of the barnacle *Balanus* (*Amphibalanus*) *amphitrite*.

Conclusions

Monoterpene and furan are key structural units that show antifouling activity without toxicity. In this study, we designed nine hybrid molecules **4a**–**4i** in which monoterpene and furan moieties were combined to achieve enhanced antifouling activity. These nine monoterpene–furan hybrids, which possess structural variety in the monoterpene domain, were concisely synthesized via carbonyl-ene reactions between the aldehydes and 3-methylene-2,3-dihydrofuran (**6**). Then, the biological activity of the synthetic hybrids **4a**–**4i** toward the cypris larvae of the barnacle *Balanus* (*Amphibalanus*) *amphitrite* was evaluated. All nine hybrids showed higher antifouling activity than geraniol (**1**), (*S*)-citronellol (**10**), or furan **12**, combined with low toxicity. These findings indicate that combining two antifouling-active scaffolds, i.e., monoterpene and furan, increases the antifouling activity. This hybridization approach to achieve enhanced antifouling activity is useful and can be employed using other active structural motifs for the creation of novel antifouling agents.

Dedication

We dedicate this work to the memory of Prof. Daisuke Uemura, who sadly passed away on 13th April 2021.

Experimental

General methods

Unless otherwise indicated, all reagents were purchased from common commercial suppliers and used as received. All reactions were carried out under an argon atmosphere. Heated reactions were conducted using an oil bath. Reaction solvents were purchased as dehydrated solvents and stored over activated molecular sieves 4Å under argon prior to use. All solvents for the work-up procedures were used as received. Analytical thin layer chromatography (TLC) was performed using aluminum TLC plates (Merck TLC silica gel $60F_{254}$). Column chromatography was performed on Fuji Silysia silica gel BW-300 or Kanto Chemical silica gel 60N. IR spectra were recorded on a JASCO FT/IR-460 plus. ^1H and ^{13}C NMR spectra were recorded on JEOL JNM-AL400 or Varian 400-MR spectrometer. Chemical shifts in the NMR spectra are reported in ppm with reference to the internal residual solvent (for ^1H NMR, $CDCl_3$: 7.26 ppm; for ^{13}C NMR, $CDCl_3$: 77.0 ppm). The following abbreviations are used to designate the multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Coupling constants (*J*) are given in Hertz. High-resolution mass spectra were recorded on a Bruker micrOTOF II (ESI–TOF–MS) spectrometer.

Geranial (5). $PhI(OAc)_2$ (17.4 g, 54.0 mmol) and TEMPO (703 mg, 4.50 mmol) were added to a solution of geraniol (**1**; 7.8 mL, 45.0 mmol) in CH_2Cl_2 (450 mL) at room temperature. The mixture was stirred at the same temperature for 14 h. The reaction was then quenched with saturated aqueous $Na_2S_2O_3$ at 0 °C. The mixture was diluted with Et_2O, washed with saturated aqueous $NaHCO_3$, H_2O, and brine, and then dried over Na_2SO_4.

After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 20:1) to afford geranial (**5**;[26] 8.50 g, quant): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.1 (d, J = 8.0 Hz, 1 H), 5.89 (dd, J = 8.0, 0.8 Hz, 1 H), 5.07 (t, J = 6.0 Hz, 1 H), 2.27–2.16 (m, 4 H), 2.17 (s, 3 H), 1.69 (s, 3 H), 1.62 (s, 3 H).

Alcohol 4a. Me_3Al (1.07 M in hexane, 8.8 mL, 9.42 mmol) was added to a mixture of geranial (**5**; 1.20 g, 7.85 mmol) and 3-methylene-2,3-dihydrofuran (**6**; 0.94 mL, 13.2 mmol) in CH_2Cl_2 (16 mL) at 0 °C. The mixture was stirred at the same temperature for 40 min. The reaction was then quenched with saturated aqueous sodium potassium tartrate. The mixture was diluted with Et_2O, washed with H_2O and brine, and then dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 20:1) to afford alcohol **4a**[28] (1.66 g, 90%): colorless oil; R_f = 0.20 (hexane/EtOAc = 7:1); IR (neat) 3367, 2968, 2918, 2853, 1666 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (t, J = 1.6 Hz, 1 H), 7.29 (brs, 1 H), 6.33 (brs, 1 H), 5.23 (dd, J = 8.6, 1.2 Hz, 1 H), 5.09–5.06 (m, 1 H), 4.55–4.49 (m, 1 H), 2.66 (dd, J = 14.4, 6.8 Hz, 1 H), 2.61 (dd, J = 14.4, 5.6 Hz, 1 H), 2.11–2.00 (m, 2 H), 2.04–2.00 (m, 2 H), 1.69 (s, 3 H), 1.66 (d, J = 1.2 Hz, 3 H), 1.61 (s, 3 H), 1.51 (d, J = 3.4 Hz, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.7, 140.1, 139.1, 131.6, 126.9, 123.8, 120.8, 111.5, 68.4, 39.6, 33.3, 26.4, 25.7, 17.8, 16.7; HRMS (ESI–TOF) calcd for $C_{15}H_{22}O_2Na$ [M + Na]$^+$ 257.1517, found 257.1512.

Diol 4b. A mixture of SeO_2 (384 mg, 3.46 mmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 13.8 mL, 69.0 mmol) in CH_2Cl_2 (50 mL) was stirred at room temperature for 10 min. Then, alcohol **4a** (5.06 g, 21.6 mmol) in CH_2Cl_2 (10 mL + 5.0 mL + 5.0 mL) was added to the mixture at room temperature, where the mixture was stirred for 4 h. The reaction was then quenched with saturated aqueous $Na_2S_2O_3$. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted twice with EtOAc and the combined organic phase was dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 2:1) to afford diol **4b** (1.91 g, 35%): colorless oil; R_f = 0.34 (hexane/EtOAc = 1:1); IR (neat) 3336, 2980, 2919, 2852, 1669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (t, J = 1.4 Hz, 1 H), 7.29 (brs, 1 H), 6.32 (brs, 1 H), 5.36 (td, J = 6.8, 1.2 Hz, 1 H), 5.22 (dd, J = 8.5, 1.2 Hz, 1 H), 4.54–4.48 (m, 1 H), 3.99 (s, 2 H), 2.66 (dd, J = 14.4, 7.5 Hz, 1 H), 2.60 (dd, J = 14.4, 5.9 Hz, 1 H), 2.19–2.13 (m, 2 H), 2.08–2.04 (m, 2 H), 1.66 (s, 3 H), 1.65 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.7, 140.1, 138.4, 135.1, 127.2, 125.4, 120.7, 111.5, 68.8, 68.4, 39.1, 33.2, 25.7, 16.6, 13.8; HRMS (ESI–TOF) calcd for $C_{15}H_{22}O_3Na$ [M + Na]$^+$ 273.1467, found 273.1468.

Alkylated compound 4c. Diol **4b** (41.8 mg, 0.167 mmol) in THF (0.5 mL + 0.3 mL + 0.2 mL), 1-bromopentane (0.17 mL, 1.34 mmol), and tetrabutylammonium iodide (12.3 mg, 33.4 µmol) were added to a suspension of NaH (60%, 20.1 mg, 0.835 mmol) in THF (0.7 mL) at 0 °C. The mixture was then stirred at 60 °C for 17 h, before NaH (60%, 20.1 mg, 0.835 mmol) and 1-bromopentane (0.17 mL, 1.34 mmol) were added at room temperature. The mixture was then stirred at 60 °C for 3 h, before the reaction was quenched with saturated aqueous NH_4Cl. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 40:1) to afford alkylated compound **4c** (45.8 mg, 70%): pale yellow oil; R_f = 0.44 (hexane/EtOAc = 20:1); IR (neat) 2956, 2930, 2871, 2858, 1669 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (t, J = 1.6 Hz, 1 H), 7.24 (brs, 1 H), 6.30 (brs, 1 H), 5.35 (td, J = 6.8, 1.2 Hz, 1 H), 5.08 (dd, J = 9.0, 1.4 Hz, 1 H), 4.08 (dt, J = 8.8, 6.6 Hz, 1 H), 3.81 (s, 2 H), 3.44 (dt, J = 9.2, 6.6 Hz, 1 H), 3.34 (t, J = 6.6 Hz, 2 H), 3.24 (dt, J = 9.2, 6.6 Hz, 1 H), 2.70 (dd, J = 14.4, 6.4 Hz, 1 H), 2.51 (dd, J = 14.4, 6.4 Hz, 1 H), 2.15–2.12 (m, 2 H), 2.07–2.04 (m, 2 H), 1.64 (s, 3 H), 1.59–1.53 (m, 7 H), 1.35–1.28 (m, 8 H), 0.91–0.87 (m, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2, 139.9, 138.9, 132.7, 127.1, 126.2, 121.4, 111.9, 76.8, 75.9, 69.8, 68.1, 39.3, 31.3, 29.7, 29.5, 28.5, 28.4, 26.0, 22.6, 22.5, 16.6, 14.0, 13.9; HRMS (ESI–TOF) calcd for $C_{25}H_{42}O_3Na$ [M + Na]$^+$ 413.3032, found 413.3008.

Neral (8). $PhI(OAc)_2$ (548 mg, 1.70 mmol) and TEMPO (22.2 mg, 0.142 mmol) were added to a solution of nerol (**7**; 0.25 mL, 1.42 mmol) in CH_2Cl_2 (14 mL) at room temperature. Then, the mixture was stirred at the same temperature for 4 h, before the reaction was quenched with saturated aqueous $Na_2S_2O_3$ 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. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 30:1) to afford neral (**8**;[26] 198 mg, 92%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (d, J = 8.4 Hz, 1 H), 5.88 (d, J = 8.4 Hz, 1 H), 5.13–5.08 (m, 1 H), 2.59 (t, J = 7.6 Hz, 2 H), 2.24 (q, J = 7.6 Hz, 2 H), 1.99 (s, 3 H), 1.69 (s, 3 H), 1.60 (s, 3 H).

Alcohol 4d. Me_3Al (1.07 M in hexane, 0.90 mL, 0.963 mmol) was added to a mixture of neral (**8**; 120 mg, 0.788 mmol) and 3-methylene-2,3-dihydrofuran (**6**; 0.11 mL, 1.59 mmol) in CH_2Cl_2 (1.6 mL) at 0 °C. Then, the mixture was stirred at the same temperature for 1 h, before the reaction was quenched with saturated aqueous sodium potassium tartrate. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 10:1) to furnish alcohol **4d** (124 mg, 67%): colorless oil; R_f = 0.20 (hexane/EtOAc = 7:1); IR (neat) 3376, 2966, 2917, 2854, 1667 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (brs, 1 H), 7.29 (brs, 1 H), 6.32 (brs, 1 H), 5.25 (d, J = 8.3 Hz, 1 H), 5.12–5.08 (m, 1 H), 4.51–4.45 (m, 1 H), 2.64 (dd, J = 14.4, 7.1 Hz, 1 H), 2.58 (dd, J = 14.4, 5.6 Hz, 1 H), 2.10–2.01 (m, 4 H), 1.74 (d, J = 1.1 Hz, 3 H), 1.69 (s, 3 H), 1.60 (s, 3 H), 1.55 (brs, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.7, 140.1, 139.3, 132.3, 127.9, 123.8, 120.9, 111.5, 68.0, 33.2, 32.4, 26.5, 25.7, 23.4, 17.7; HRMS (ESI–TOF) calcd for $C_{15}H_{22}O_2Na$ [M + Na]$^+$ 257.1517, found 257.1513.

Diol 4e. A mixture of SeO_2 (7.6 mg, 68.3 µmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 0.11 mL, 0.550 mmol) in CH_2Cl_2 (1.1 mL) was stirred at room temperature for 10 min. Then, alcohol **4d** (40.0 mg, 0.171 mmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.2 mL) was added to the mixture at room temperature. Then, the mixture was stirred at the same temperature for 1 h, before

the reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted twice with EtOAc and the combined organic phase was dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 2:1) to afford diol **4e** (14.9 mg, 35%): colorless oil; R_f = 0.32 (hexane/EtOAc = 1:1); IR (neat) 3349, 2919, 2854, 1668 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, J = 1.2 Hz, 1 H), 7.28 (s, 1 H), 6.30 (brs, 1 H), 5.42–5.38 (m, 1 H), 5.23 (dd, J = 9.0, 1.0 Hz, 1 H), 4.46–4.41 (m, 1 H), 3.97 (s, 2 H), 2.63 (dd, J = 14.4, 6.8 Hz, 1 H), 2.57 (dd, J = 14.4, 5.6 Hz, 1 H), 2.22–2.00 (m, 4 H), 1.94 (brs, 2 H), 1.74 (d, J = 1.4 Hz, 3 H), 1.65 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.8, 140.1, 138.0, 135.6, 128.2, 125.0, 120.7, 111.5, 68.6, 68.0, 33.3, 31.9, 25.4, 23.3, 13.8; HRMS (ESI–TOF) calcd for $C_{15}H_{22}O_3Na$ $[M + Na]^+$ 273.1467, found 273.1470.

Alcohol 4f. Me_3Al (1.07 M in hexane, 0.90 mL, 0.963 mmol) was added to a mixture of (*R*)-citronellal (**9**; 0.15 mL, 0.828 mmol) and 3-methylene-2,3-dihydrofuran (**6**; 0.12 mL, 1.68 mmol) in CH_2Cl_2 (1.6 mL) at 0 °C. Then, the mixture was stirred at the same temperature for 1 h, before the reaction was quenched with saturated aqueous sodium potassium tartrate. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 10:1) to furnish alcohol **4f** (124 mg, 64%): colorless oil; R_f = 0.28 (hexane/EtOAc = 7:1); IR (neat) 3399, 2963, 2917, 2849 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (t, J = 1.7 Hz, 1 H), 7.31 (s, 1 H), 6.31 (brs, 1 H), 5.10 (t, J = 6.8 Hz, 1 H), 3.87–3.81 (m, 1 H), 2.66–2.58 (m, 1 H), 2.52–2.43 (m, 1 H), 2.03–1.93 (m, 2 H), 1.68 (s, 3 H), 1.60 (s, 3 H), 1.55–1.09 (m, 5 H), 0.94 (d, J = 6.8 Hz, 1.5 H), 0.92 (d, J = 6.6 Hz, 1.5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.0, 140.1, 140.1, 131.2, 131.1, 124.7, 121.1, 121.0, 111.4, 69.5, 69.1, 44.3, 44.3, 37.9, 36.8, 33.8, 33.2, 29.5, 29.1, 25.8, 25.5, 25.4, 20.3, 19.2, 17.7; HRMS (ESI–TOF) calcd for $C_{15}H_{24}O_2Na$ $[M + Na]^+$ 259.1674, found 259.1673.

Diol 4g. A mixture of SeO_2 (7.5 mg, 67.6 µmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 0.11 mL, 0.550 mmol) in CH_2Cl_2 (1.1 mL) was stirred at room temperature for 10 min. Then, alcohol **4f** (40.0 mg, 0.171 mmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.2 mL) was added to the mixture at room temperature, before the mixture was stirred at the same temperature for 1 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted twice with EtOAc and the combined organic phase was dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 10:1, 4:1, 2:1) to afford diol **4g** (19.9 mg, 47%): colorless oil; R_f = 0.31 (hexane/EtOAc = 1:1); IR (neat) 3349, 2923, 2856 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (brs, 1 H), 7.30 (brs, 1 H), 6.31 (brs, 1 H), 5.40 (td, J = 6.8, 1.2 Hz, 1 H), 3.99 (s, 2 H), 3.87–3.81 (m, 1 H), 2.65–2.58 (m, 1 H), 2.53–2.44 (m, 1 H), 2.13–1.96 (m, 2 H), 1.67 (s, 3 H), 1.55–1.13 (m, 5 H), 0.96 (d, J = 6.6 Hz, 1.5 H), 0.93 (d, J = 6.6 Hz, 1.5 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.1, 140.1, 140.1, 134.6, 134.5, 126.4, 126.3, 121.1, 121.0, 111.3, 69.4, 69.1, 69.0, 68.9, 44.3, 44.1, 37.5, 36.3, 33.9, 33.3, 29.3, 29.0, 25.1, 25.0, 20.3, 19.2, 13.7; HRMS (ESI–TOF) calcd for $C_{15}H_{24}O_3Na$ $[M + Na]^+$ 275.1623, found 275.1623.

(*S*)-Citronellal (11). $PhI(OAc)_2$ (535 mg, 1.66 mmol) and TEMPO (21.6 mg, 0.138 mmol) were added to a solution of (*S*)-citronellol (**10**; 0.25 mL, 1.38 mmol) in CH_2Cl_2 (14 mL) at room temperature, where the mixture was stirred for 3 h. The reaction was quenched with saturated aqueous $Na_2S_2O_3$ 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. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 30:1) to afford (*S*)-citronellal (**11**;[33] 113 mg, 53%): ^1H NMR (400 MHz, $CDCl_3$) δ 9.75 (t, J = 2.4 Hz, 1 H), 5.10–5.06 (m, 1 H), 2.41 (ddd, J = 15.6, 5.6, 2.4 Hz, 1 H), 2.23 (ddd, J = 15.6, 8.0, 2.4 Hz, 1 H), 2.11–1.96 (m, 3 H), 1.68 (d, J = 0.8 Hz, 3 H), 1.60 (s, 3 H), 1.41–1.22 (m, 2 H), 0.97 (d, J = 6.8 Hz, 3 H).

Alcohol 4h. Me_3Al (1.07 M in hexane, 0.80 mL, 0.856 mmol) was added to a mixture of (*S*)-citronellal (**11**; 113 mg, 0.733 mmol) and 3-methylene-2,3-dihydrofuran (**6**; 0.11 mL, 1.48 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C, where the mixture was stirred for 1 h. Then, the reaction was quenched with saturated aqueous sodium potassium tartrate. The mixture was diluted with EtOAc, washed with H_2O and brine, and then dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 15:1) to afford alcohol **4h** (121 mg, 70%): colorless oil; R_f = 0.28 (hexane/EtOAc = 7:1); IR (neat) 3399, 2963, 2923, 2849 cm^{-1}; HRMS (ESI–TOF) calcd for $C_{15}H_{24}O_2Na$ $[M + Na]^+$ 259.1674, found 259.1669; ^1H and ^{13}C NMR data were identical to those of alcohol **4f**.

Diol 4i. A mixture of SeO_2 (7.5 mg, 67.6 µmol) and TBHP (5.0 M in 2,4,6-trimethylpentane, 0.11 mL, 0.550 mmol) in CH_2Cl_2 (1.1 mL) was stirred at room temperature for 10 min. Then, alcohol **4h** (40.0 mg, 0.171 mmol) in CH_2Cl_2 (0.2 mL + 0.2 mL + 0.2 mL) was added to the mixture at room temperature, where the mixture was stirred for 1 h, before the reaction was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was diluted with EtOAc and washed with H_2O and brine. The aqueous phase was extracted twice with EtOAc and the combined organic phase was dried over Na_2SO_4. After filtration and concentration of the filtrate, the obtained residue was subjected to column chromatography (hexane/EtOAc = 10:1, 4:1, 2:1) to afford diol **4i** (15.1 mg, 35%): colorless oil; R_f = 0.31 (hexane/EtOAc = 1:1); IR (neat) 3349, 2923, 2850 cm^{-1}; HRMS (ESI–TOF) calcd for $C_{15}H_{24}O_3Na$ $[M + Na]^+$ 275.1623, found 275.1623; ^1H and ^{13}C NMR data were identical to those of alcohol **4g**.

Antifouling activity and toxicity

Adult *Balanus* (*Amphibalanus*) *amphitrite* were collected in Kobe, Japan during 2021–2022 and maintained in aquaria by feeding them brine shrimp. Nauplii released from adults were cultured in filtered sea water (FSW) containing penicillin/streptomycin (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) and fed *Chaetoceros calcitrans* at 22 °C under a 12h:12h light:dark cycle. The nauplii metamorphosed into cyprids after 5–6 days. Only swimming cyprids were collected.

These were rinsed with FSW and aged for 2–3 days prior to use at 5 °C in the dark. The effects of the compounds on the barnacle cyprids were tested using 24-well polystyrene plates (Corning Inc., NY, USA). Each compound was dissolved in MeOH. Aliquots of the solution were applied to the wells of the 24-well polystyrene plates (0.1, 0.3, 1.0, 3.0, 10, and 50 μg) and air-dried. Approximately 10 cypris larvae were added to each well, and the wells were filled with FSW to a final volume of 1.0 mL. The plates were kept at 25 °C in the dark for 96 h. The number of larvae that settled (including metamorphosed larvae), died, and did not settle was counted under a microscope. In the experiments, each concentration level was tested in three wells and the assay was repeated three times. The assay was conducted using $CuSO_4$ (0.01, 0.03, 0.1, 0.3, 1.0, 3.0, and 10 μg) as a positive control. An assay without any compound was performed as a negative control. The settlement inhibition was calculated on the basis of the negative control settlement.[34] The antifouling activity and toxicity were expressed as EC_{50} and LC_{50} values, respectively. Probit analysis was used to calculate the EC_{50} and LC_{50} values.[31b,31c]

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We are grateful to Dr. Noriyuki Endo (Himeji EcoTech Co., Ltd.) for valuable discussions and Mr. Kosuke Hattori (Okayama University), Mr. Yuji Sugita (Okayama University), and Ms. Shiori Kitade (University of Hyogo) for experimental support. We appreciate the Division of Instrumental Analysis at Okayama University for assistance with the NMR and HRMS measurements. This work was supported by the Iketani Science and Technology Foundation, the Fukuoka Naohiko Memorial Foundation, and the JSPS via KAKENHI grant JP21H01938.

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