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Original Article

Chemical Constituents of the Marine Traditional Chinese Medicine of *Pegasus laternarius* Cuvier (Hai-E Yu)

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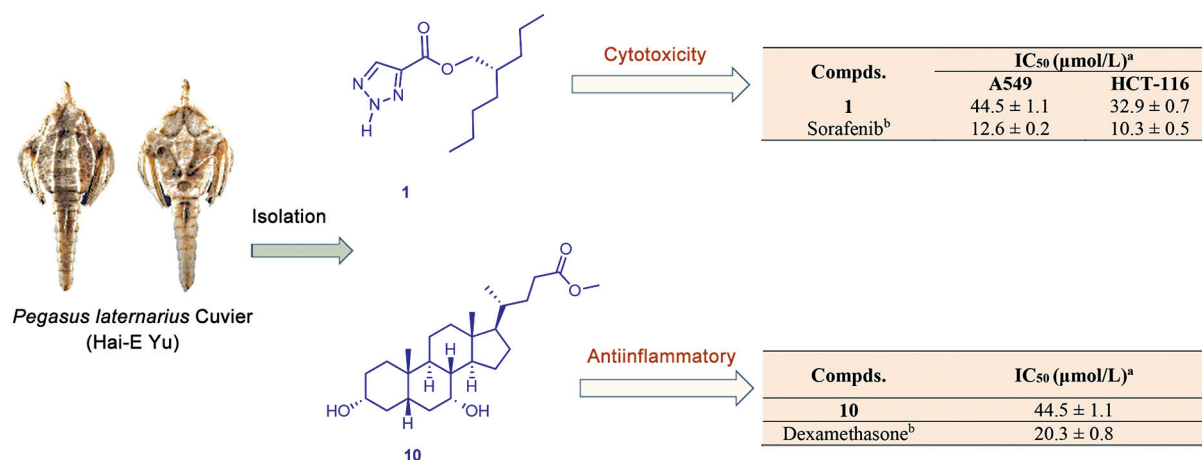
Abstract

Keywords
► marine traditional Chinese medicine
► *Pegasus laternarius* Cuvier
► cytotoxicity
► anti-inflammatory

Pegasus laternarius Cuvier (Hai-E Yu) is a marine traditional Chinese medicine that has been used to treat cancers and reduce inflammation. Previous chemical investigations have only revealed the occurrence of high levels of protein, fatty acids, and a large number of steroids, thus more active compounds in *P. laternarius* still need to be further discovered. The present study aims to search for new bioactive constituents of *P. laternarius* with cytotoxic effects and nitric oxide (NO) inhibitory activities. In this work, 16 pure compounds from the ethyl acetate fraction of *Pegasus laternarius* Cuvier were successively obtained by various chromatographic techniques, and the structure of the isolates was elucidated by spectroscopic analyses. The isolated and identified

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compounds included one 2*H*-1,2,3-triazole-4-carboxylate derivative (**1**), two oxadia-zines (**2**, **3**), two amino acids (**4**, **5**), three nucleosides (**6–8**), three steroids (**9–11**), and five others (**12–16**). All the obtained compounds were evaluated for their antitumor activity on A549 and HCT-116 cell lines along with their inhibitory effects on lipopolysaccharide-induced NO production in RAW264.7 cells. The triazole compound **1** was found to exhibit moderate cytotoxicity against two human cell lines (A549 and HCT-116) with IC_{50} values of 44.5 and 32.9 μmol/L, respectively. The steroid compound **10** inhibited NO production with IC_{50} values lower than 50 μmol/L. Consequently, this study provides insight into the cytotoxic and NO inhibitory activities of the marine traditional Chinese medicines in Hai-E-Yu.

Introduction

Marine organisms tend to produce unique secondary metabolites with specific activities due to the special marine environment characteristics of high salt, high pressure, low nutrition, lack of oxygen, and lack of sunlight, which could be considered the newest source of bioactive natural products in relation to terrestrial plants and nonmarine microorganisms.[1] Among them, a variety of species (seaweeds, shell-fishes, and minerals) have been used for thousands of years as marine traditional Chinese medicines (MTCMs) to treat diseases, and modern pharmacological studies have shown that they have antitumor, anti-inflammatory, and antiviral properties.[2] Currently, it has become an important medicinal resource for the development of new drugs for the prevention and treatment of difficult medical issues. For instance, *Concha Ostreae* polysaccharides can boost the immune system, *Sargassum* has the effects on antitumor and antiviral therapy, and *Hippocampus* exhibits a great effect on antiaging. The *in vitro* cellular studies have also suggested that the water-soluble *Margaritifera concha* protein has a strong effect on stimulating the differentiation of bone marrow stromal cells into osteoblasts and increased osteoblast proliferation.[3] Notably, a type of sulfated polysaccharide derived from seaweed has been used clinically for cardiovascular diseases, and sodium oligomannate (GV-971), an oligosaccharide compound derived from brown algae, has been used for the treatment of Alzheimer's disease by targeting the brain–gut axis.[4] Therefore, it is of great value to discover novel compounds with diverse biological activities from MTCMs.

Pegasus laternarius Cuvier (Hai-E Yu) is an MTCM commonly used for the treatment of tumors, cough, and antidiarrheal, and is documented in Chinese Pharmacopoeia. Previous studies have shown that the extracts of *P. laternarius* exhibited antitumor activity, anti-lipid peroxidation effect, memory-improving effect, etc. However, the constituents of *P. laternarius* have less been investigated. The previous chemical investigations have only confirmed the occurrence of a high level of protein, fatty acids, and a large number of steroids. We anticipated that more active compounds in *P. laternarius* will be further explored. In our continuing efforts to search for cytostatic and anti-inflammatory compounds from MTCMs, the chemical investigation

on *P. laternarius* was undertaken and resulted in the isolation and identification of 16 compounds (►**Fig. 1**), among them, the occurrences of compounds **1**, **6**, **12**, and **13** were first reported from *P. laternarius*. In bioassay, the 2*H*-1,2,3-triazole-4-carboxylate compound **1** showed moderate cytotoxicity with IC_{50} values of 44.5 and 32.9 μmol/L on the A549 and HCT-116 cell lines, while the steroid compound **10** inhibited NO production with IC_{50} values lower than 50 μmol/L. Herein, the isolation, structural determination, and assessment of the cytostatic and anti-inflammatory activities of the isolated compounds are further described.

Results and Discussion

Extraction and Isolation

The air-dried whole parts of *Pegasus laternarius* Cuvier (483 g) were powdered and extracted with dichloromethane/methanol (CH_2Cl_2/MeOH, 1:1) five times at room temperature. The crude extracts were concentrated by evaporation under reduced pressure to yield 73 g of dry extract. The obtained dry extracts were suspended in H_2O and partitioned successively with petroleum ether (PE), ethyl acetate (EtOAc), and *n*-butyl alcohol (*n*-BuOH, 1 L × 5, five times). The EtOAc extract (10.8 g) was subjected to a silica gel column (Si CC, 200 – 300 mesh; PE/EtOAc, 20:1, 10:1, 5:1, 2:1, 1:1, v/v; CH_2Cl_2/MeOH, 15:1, 10:1, 5:1, 2:1, v/v) as an eluent to obtain six fractions, labeled as Fr. 1 to Fr. 6. The fraction Fr.3 (1.05 g) was submitted to silica gel column with PE/CH_2Cl_2 (95:5–50:50, v/v), leading to two new fractions, in which fraction Fr. 3–1 (104 mg) was purified by silica gel column with gradient of PE/EtOAc (90:10–50:50, v/v) to afford compound **9** (37.7 mg). The fraction Fr. 5 (0.64 g) was further submitted to silica gel column with PE/EtOAc (95:5–50:50, v/v) leading to two new fractions, Fr. 5–1 and Fr. 5–2, which were then subjected to preparative high-performance liquid chromatography (HPLC) with a solvent system of MeOH/H_2O (60:40, v/v) to provide compound **12** (t_R = 16 minutes, 3.4 mg) and **13** (t_R = 35 minutes, 2.8 mg), respectively. The fraction Fr. 6 (1.60 g) was subjected to a C18 reversed-phase column eluted with MeOH/H_2O (30:70–100:0, v/v) to yield five new fractions. The fraction Fr. 6–4 (159 mg) was chromatographed on Sephadex LH-20 (CH_2Cl_2/MeOH, 1:1, v/v) to afford three new fractions. The fraction Fr. 6–4-3 (65 mg) was purified by silica gel column and eluted with
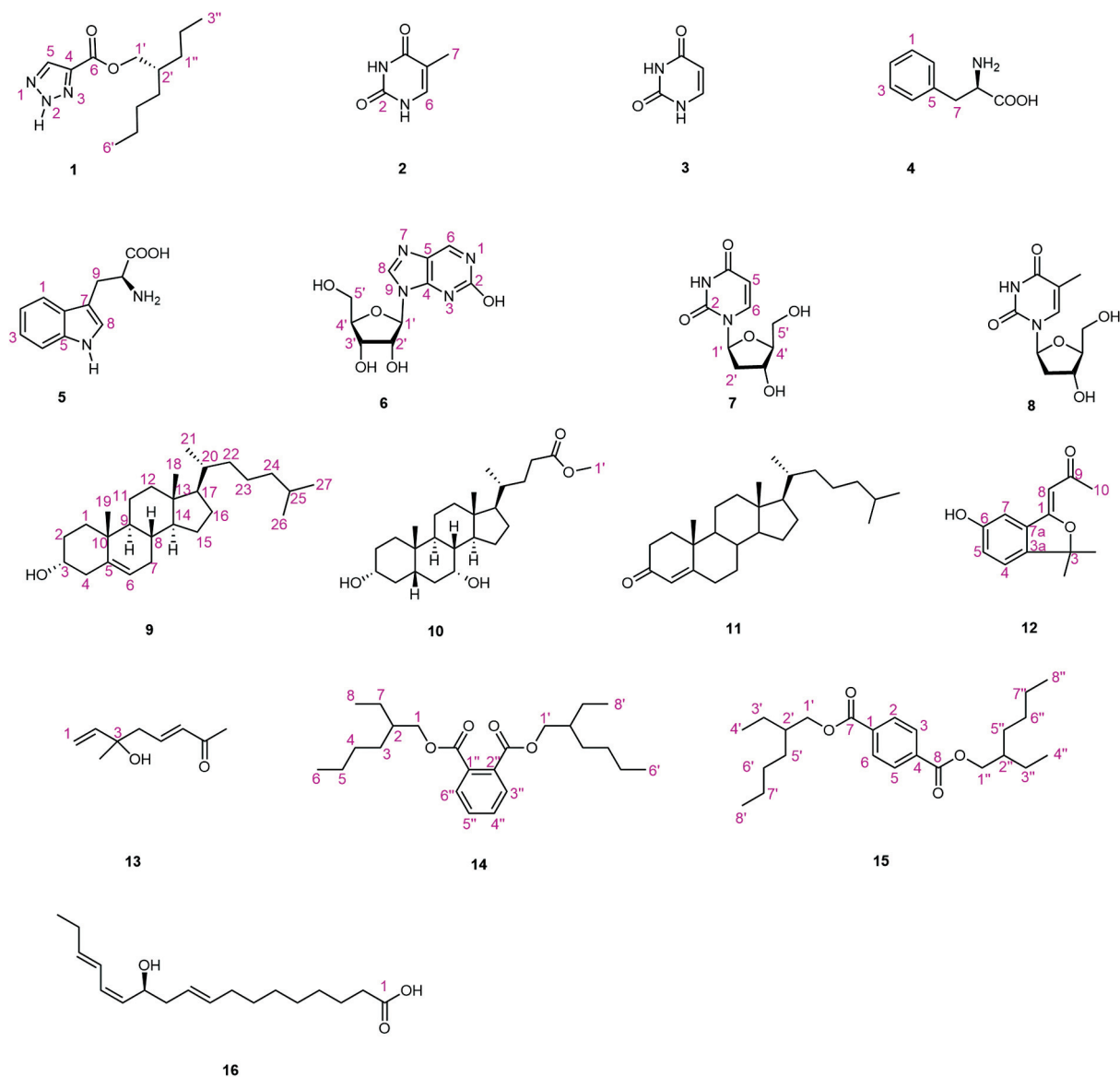
Fig. 1 Chemical structures of the isolated and identified compounds **1–16**.

CH$_2$Cl$_2$/MeOH (90:10–50:50, v/v) to afford the steroid compound **10** (4.5 mg). The PE extract (38.7 g) was subjected to a silica gel column using PE/CH$_2$Cl$_2$ (98:2–0:100, v/v) as an eluent to obtain seven new fractions, S.1–S.7. The fraction S.4 (1.16 g) was submitted to silica gel column with PE/CH$_2$Cl$_2$ (90:10–0:100, v/v) leading to five new fractions. The fraction S.4–1 (59 mg) was purified by a silica gel column with a gradient of PE/CH$_2$Cl$_2$ (90:10–70:30, v/v) to afford the phthalate ester compound **14** (3.6 mg) as well as the terephthalate compound **15** (20.2 mg). The 2*H*-1,2,3-triazole-4-carboxylate **1** (12.8 mg) was obtained from fraction S.5. The fraction S.7 (100 mg) was submitted to the silica gel column with PE/EtOAc (98:2–50:50, v/v) leading to three new fractions. The fraction S.7–1 (30 mg) was subjected to preparative HPLC (MeOH/H$_2$O, 80:20, v/v) leading to the octadecatrienoic acid **16** (t_R = 20 minutes, 3.1 mg). The S.7–3 was chromatographed on Sephadex LH-20 (CH$_2$Cl$_2$/MeOH, 1:1, v/v) to afford three new fractions, in which the fraction S.7–3-2 (50 mg) was purified by silica gel column with a gradient of PE/CH$_2$Cl$_2$ (95:5–60:40, v/v) to afford nucleoside **6** (4.6 mg) and cholesterone **11** (2.2 mg). The *n*-BuOH alcohol (7.09 g) was subjected to a silica gel column using CH$_2$Cl$_2$/MeOH (98:2–0:100, v/v) as an eluent to obtain five fractions, Z.1–Z.5. The fraction Z.3 (1.0 g) was subjected to a Si CC (CH$_2$Cl$_2$/MeOH, 95:5–5:5, v/v) followed by semipreparative HPLC (MeOH/H$_2$O, 10:90, v/v) to obtain the pyrimidine-2,4-dione compounds **2** (t_R = 15 minutes, 5 mg) and **3** (t_R = 8 minutes, 13 mg). The fraction Z.3 (60 mg) was chromatographed on Sephadex LH-20 (CH$_2$Cl$_2$/MeOH, 1:1, v/v) to afford three new fractions. The fraction Z.3–2 (30 mg) was subjected to preparative HPLC (MeOH/H$_2$O, 10:90, v/v) leading to *D*-phenylalanine **4** (t_R = 12 minutes, 2.1 mg) and *L*-tryptophan **5** (t_R = 8 minutes, 1.8 mg). Finally, the fraction Z.5 (42 mg) was purified by preparative HPLC (MeOH/H$_2$O, 10:90, v/v) to yield the deoxynucleosides **7** (t_R = 9 minutes, 1.2 mg) and **8** (t_R = 16 minutes, 3.1 mg).

(R)-2-Propylhexyl 2H-1,2,3-triazole-4-carboxylate (compound 1): yellow oil, $C_{12}H_{21}N_3O_2$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 8.09 (s, 1H, NH), 8.09 (s, 1H, H-5), 4.31–4.22 (m, 2H, H-1'), 1.73 (t, $J = 6.2$ Hz, 1H, H-2'), 0.95 (t, $J = 7.5$ Hz, 3H, H-3''), 0.90 (t, $J = 7.0$ Hz, 3H, H-6'). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 134.40 (C-4), 129.64 (C-5), 166.12 (C-6), 67.92 (C-1'), 39.06 (C-2'), 29.84 (C-3'), 29.13 (C-4'), 23.11 (C-5'), 14.17 (C-6'), 30.72 (C-1'), 24.13 (C-2''), 11.23 (C-3''). The data were consistent with a reported study.[5]

Thymine (compound 2): white powder, $C_5H_6N_2O_2$, ^1H NMR (600 MHz, DMSO-d_6) δ$_H$ 10.98 (s, 1H, NH), 10.58 (s, 1H, NH), 7.24 (s, 1H, H-6), 1.72 (s, 3H, H-7). ^{13}C NMR (150 MHz, DMSO-d_6) δ$_C$ 164.95 (C-4), 151.52 (C-2), 137.74 (C-6), 107.69 (C-5), 11.82 (C-7). The data were consistent with a reported study.[6]

Uracil (compound 3): white powder, $C_4H_4N_2O_2$, ^1H NMR (600 MHz, DMSO-d_6) δ$_H$ 11.00 (s, 1H, NH), 10.81 (s, 1H, NH), 7.38 (d, $J = 7.6$ Hz, 1H, H-6), 5.44 (d, $J = 7.6$ Hz, 1H, H-5). ^{13}C NMR (150 MHz, DMSO-d_6) δ$_C$ 164.37 (C-4), 151.55 (C-2), 142.22 (C-6), 100.25 (C-5). The data were consistent with a reported study.[6]

D-Phenylalanine (compound 4): white powder, $C_9H_{11}NO_2$, ^1H NMR (400 MHz, D$_2$O) δ$_H$ 7.22 (m, 1H, H-2), 7.21 (m, 1H, H-1, 3, 4 and 6), 4.05 (m, 1H, H-8), 3.18 (m, 2H, H-7a), 3.02 (m, 2H, H-7b). ^{13}C NMR (100 MHz, D$_2$O) δ$_C$ 174.81 (C-9), 135.47 (C-5), 129.42 (C-1 and C-3), 129.11 (C-4 and C-6), 127.64 (C-2), 56.22 (C-8), 36.82 (C-7). The data were consistent with a reported study.[7]

L-Tryptophan (compound 5): white powder, $C_{11}H_{12}N_2O_2$, ^1H NMR (400 MHz, CD$_3$OD) δ$_H$ 7.70 (d, $J = 7.9$ Hz, 1H, H-1), 7.36 (d, $J = 8.1$ Hz, 1H, H-4), 7.19 (s, 1H, H-8), 7.12 (t, $J = 7.5$ Hz, 1H, H-3), 7.05 (t, $J = 7.5$ Hz, 1H, H-2), 3.86 (dd, $J = 9.3, 4.0$ Hz, 1H, H-10), 3.52 (dd, $J = 15.2, 4.1$ Hz, 1H, H-9a), 3.15 (dd, $J = 15.2, 9.4$ Hz, 1H, H-9b). ^{13}C NMR (100 MHz, CD$_3$OD) δ$_C$ 138.43 (C-5), 128.51 (C-6), 125.13 (C-8), 120.13 (C-3), 119.35 (C-2), 112.44 (C-4), 28.54 (C-9). The data were consistent with a reported study.[8]

2-Hydroxypurine nucleoside (compound 6): white powder, $C_{10}H_{12}N_4O_5$, ^1H NMR (400 MHz, D$_2$O) δ$_H$ 8.26 (s, 1H, H-8), 8.16 (s, 1H, H-6), 6.05 (d, $J = 5.8$ Hz, 1H, H-1'), 4.43 (dd, $J = 6.4, 2.6$ Hz, 1H, H-3'), 4.30–4.25 (m, 1H, H-4'), 3.91 (dd, $J = 12.8, 1.5$ Hz, 1H, H-5'a), 3.83 (dd, $J = 12.8, 3.9$ Hz, 1H, H-5'b). ^{13}C NMR (100 MHz, D$_2$O) δ$_C$ 160.86 (C-2), 148.62 (C-4), 148.00 (C-6), 139.85 (C-8), 124.75 (C-5), 88.42 (C-1'), 85.67 (C-4'), 73.92 (C-3'), 70.53 (C-2'), 61.43 (C-5'). The data were consistent with a reported study.[9]

2'-Deoxyuridine (compound 7): white solid, $C_9H_{12}N_2O_5$, ^1H NMR (600 MHz, DMSO-d_6) δ$_H$ 7.85 (d, $J = 8.1$ Hz, 1H, H-6), 6.15 (dd, $J = 7.5, 6.2$ Hz, 1H, H-1'), 5.63 (d, $J = 8.1$ Hz, 1H, H-5), 4.23 (dt, $J = 6.2, 3.2$ Hz, 1H, H-3'), 3.79–3.76 (m, 1H, H-4'), 3.57 (dd, $J = 11.9, 3.8$ Hz, 1H, H-5'a), 3.53 (dd, $J = 11.9, 3.8$ Hz, 1H, H-5'b), 2.12–2.03 (m, 2H, H-2'). ^{13}C NMR (150 MHz, DMSO-d_6) δ$_C$ 163.17 (C-4), 150.48 (C-2), 140.55 (C-6), 100.78 (C-5), 87.44 (C-4'), 84.15 (C-1'), 70.44 (C-3'), 61.31 (C-5'), 48.63 (C-2'). The data were consistent with a reported study.[10]

Thymidine (compound 8): white solid, $C_{10}H_{14}N_2O_5$, ^1H NMR (600 MHz, DMSO-d_6) δ$_H$ 7.69 (d, $J = 1.3$ Hz, 1H, H-6), 6.16 (dd, $J = 7.6, 6.2$ Hz, 1H, H-1'), 4.23 (dt, $J = 6.2, 3.1$ Hz, 1H, H-3'), 3.75 (dd, $J = 3.6$ Hz, 1H, H-4'), 3.61–3.52 (m, 2H, H-5'), 2.11–2.03 (m, 2H, H-2'), 1.76 (d, $J = 1.3$ Hz, 3H, 5-CH$_3$). ^{13}C NMR (150 MHz, DMSO-d_6) δ$_C$ 163.83 (C-4), 150.54 (C-2), 136.20 (C-6), 109.45 (C-5), 87.32 (C-4'), 83.82 (C-1'), 70.51 (C-3'), 61.41 (C-5'), 39.49 (C-2') 12.33 (5-CH$_3$). The data were consistent with a reported study.[10]

(3β)-Cholest-5-en-3-ol (compound 9): white amorphous powder, $C_{27}H_{46}O$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 5.34 (d, $J = 5.4$ Hz, 1H, H-6), 3.57 (m, 1H, H-3), 1.00 (s, 3H, H-19), 0.91 (d, $J = 6.5$ Hz, 3H, H-21), 0.87 (d, $J = 1.8$ Hz, 3H, H-26), 0.85 (d, $J = 1.8$ Hz, 3H, H-27), 0.67 (s, 3H, H-18). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 140.84 (C-5), 121.78 (C-6), 71.87 (C-3), 56.85 (C-14), 56.25 (C-17), 50.22 (C-9), 39.87 (C-16), 39.60 (C-24), 37.34 (C-1), 36.58 (C-10), 36.28 (C-22), 35.87 (C-8 and C-20), 31.99 (C-7), 31.73 (C-2), 28.31 (C-12), 28.09 (C-25), 24.37 (C-15), 23.92 (C-24), 22.90 (C-27), 22.64 (C-26), 21.17 (C-11), 19.48 (C-19), 18.80 (C-21), 11.94 (C-18). The data were consistent with a reported study.[11]

Methyl-3α,7α-dihydroxy-5β-cholan-24-oate (compound 10): colorless gelatinous solid, $C_{25}H_{42}O_4$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 3.84 (m, 1H, H-7), 3.68 (s, 3H, H-1'), 3.46 (m, 1H, H-3), 0.93 (d, $J = 6.5$ Hz, 3H, H-21), 0.89 (s, 3H, H-19), 0.65 (s, 3H, H-18). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 177.84 (C-24), 72.20 (C-3), 68.73 (C-7), 35.20 (C-17), 50.60 (C-14), 42.87 (C-13), 41.61 (C-5), 40.01 (C-12), 39.77 (C-4), 39.57 (C-8), 35.50 (C-1 and C-20), 35.19 (C-6 and C-10), 32.98 (C-9), 30.94 (C-2), 30.79 (C-22), 30.74 (C-23), 28.29 (C-16), 23.85 (C-15), 22.91 (C-19), 20.72 (C-11), 18.38 (C-21), 11.92 (C-18), 55.9 (C-1'). The data were consistent with a reported study.[12]

Cholest-4-en-3-one (compound 11): white solid, $C_{27}H_{44}O$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 5.72 (s, 1H, H-4), 1.25 (s, 2H, H-24), 1.17 (s, 3H, H-19), 0.90 (d, $J = 6.5$ Hz, 3H, H-21), 0.87 (d, $J = 1.8$ Hz, 3H, H-27), 0.85 (d, $J = 1.8$ Hz, 3H, H-26), 0.70 (s, 3H, H-18). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 199.79 (C-3), 171.85 (C-5), 123.82 (C-4), 56.19 (C-17), 55.96 (C-14), 53.90 (C-9), 42.47 (C-13), 39.71 (C-12), 39.57 (C-24), 38.69 (C-10), 36.19 (C-22), 35.83 (C-20), 35.76 (C-1), 35.70 (C-8), 34.06 (C-2), 33.03 (C-6), 32.13 (C-7), 28.25 (C-25), 28.08 (C-16), 24.25 (C-15), 23.89 (C-23), 22.88 (C-27), 22.62 (C-26), 21.10 (C-11), 18.71 (C-21), 17.46 (C-19), 12.02 (C-18). The data were consistent with a reported study.[13]

Matriisobenzofuran (compound 12): yellow powder, $C_{13}H_{14}O_3$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 8.18 (d, $J = 1.6$ Hz, 1H, H-7), 7.94 (dd, $J = 8.6, 1.8$ Hz, 1H, H-5), 7.49 (d, $J = 8.6$ Hz, 1H, H-4), 6.66 (s, 1H, H-8), 2.66 (s, 3H, H-10), 1.70 (s, 6H, 3-CH$_3$). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 197.91 (C=O, C-9), 164.94 (C-1), 157.54 (C-6), 132.93 (C-3a), 128.66 (C-7a), 125.08 (C-5), 122.54 (C-7), 111.40 (C-4), 101.17 (C-8), 69.55 (C-3), 28.94 (3-CH$_3$), 27.02 (C-10). The data were consistent with a reported study.[14]

6-Hydroxy-6-methylocta-3,7-dien-2-one (compound 13): colorless oil, $C_9H_{14}O_2$, ^1H NMR (400 MHz, CDCl$_3$) δ$_H$ 6.81 (dt, $J = 15.3, 7.5$ Hz, 1H, H-5), 6.11 (d, $J = 16.0$ Hz, 1H, H-6), 5.95 (dd, $J = 17.3, 10.7$ Hz, 1H, H-2), 5.26 (d, $J = 17.3$ Hz, 1H, H-1a), 5.12 (d, $J = 10.7$ Hz, 1H, H-1b), 2.47 (d, $J = 7.2$ Hz, 2H, H-4), 2.26 (s, 3H, H-8), 1.34 (s, 3H, H-9). ^{13}C NMR

(100 MHz, CDCl$_3$) δ$_C$ 198.54 (C = O, C-7), 144.05 (C-2), 143.36 (C-5), 134.19 (C-6), 112.77 (C-1), 72.77 (C-3), 45.16 (C-4), 27.91 (C-8), 26.91 (C-9). The data were consistent with a reported study.[15]

Bis(2-ethylhexyl) phthalate (compound 14): colorless gelatinous solid, C$_{24}$H$_{38}$O$_4$, ^{1}H NMR (400 MHz, CDCl$_3$) δ$_H$ 7.71 (dd, J = 5.7, 3.3 Hz, 2H, H-3″ and H-6″), 7.53 (m, 2H, H-4″ and H-5″), 4.31–4.16 (m, 2H, H-1 and H-1'), 1.74–1.64 (m, 2H, H-2 and H-2'), 0.92 (m, 6H, H-6 and H-6'), 0.90 (m, 6H, H-8 and H-8'). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 167.92 (2C = O), 132.62 (C-1″ and C-2″), 131.03 (C-4″ and C-5″), 128.96 (C-3″ and C-6″), 68.32 (C-1 and C-1'), 38.89 (C-2 and C-2'), 30.52 (C-3 and C-3'), 29.08 (C-4 and C-4'), 23.91 (C-7 and C-7'), 23.14 (C-5 and C-5'), 14.20 (C-8 and C-8'), 11.11 (C-6 and C-6'). The data were consistent with a reported study.[16]

Bis(2-ethylhexyl) terephthalate (compound 15): yellow oil, C$_{24}$H$_{38}$O$_4$, ^{1}H NMR (400 MHz, CDCl$_3$) δ$_H$ 8.10 (s, 4H, H-2, 3, 5 and 6), 4.32–4.21 (m, 4H, H-1' and H-1″), 1.80–1.67 (m, 2H, H-2' and H-2″), 1.47–1.27 (m, 16H, H-3', 5', 6', 7' and H-3″, 5″, 6″, 7″), 0.97–0.92 (m, 6H, H-8' and H-8″), 0.92–0.88 (m, 6H, H-4' and H-4″). ^{13}C NMR (100 MHz, CDCl$_3$) δ$_C$ 166.08 (C-7 and C-8), 134.38 (C-1 and C-4), 129.62 (C-2, 3, 5 and 6), 67.89 (C-1'), 39.04 (C-2'), 30.70 (C-3'), 29.11 (C-4'), 24.11 (C-7'), 23.09 (C-5'), 14.15 (C-6'), 11.21 (C-8'). The data were consistent with a reported study.[17]

(*S,9E,13Z,15E*)-12-Hydroxyoctadeca-9,13,15-octadeca-trienoic acid (compound 16): yellow oil, C$_{18}$H$_{30}$O$_3$, ^{1}H NMR (400 MHz, CD$_3$CD) δ$_H$ 6.00 (dd, J = 15.2, 11.0 Hz, 1H, H-14), 5.46 (t, J = 11.0 Hz, 1H, H-15), 5.14 (dd, J = 15.2, 6.6 Hz, 1H, H-10), 0.46 (t, J = 7.5 Hz, 3H, H-18). ^{13}C NMR (100 MHz, CD$_3$CD) δ$_C$ 175.50 (C-1), 136.68 (C-13), 134.58 (C-16), 133.05 (C-9), 129.34 (C-15), 126.65 (C-14), 125.55 (C-10), 73.27 (C-12), 36.29 (C-11), 34.94 (C-2), 30.70 (C-7), 30.25 (C-6), 30.17 (C-4), 30.11 (C-5), 28.61 (C-3), 25.99 (C-8), 21.70 (C-17), 14.56 (C-18). The data were consistent with a reported study.[18]

All the isolates were evaluated for their antitumor activity on A549 and HCT-116 cell lines and their inhibitory effects on lipopolysaccharide (LPS)-induced NO production in RAW264.7 cells. Inhibiting NO production in LPS-stimulated RAW 264.7 cells represents a possible way to screen agents with anti-inflammatory activity.[19] The bioassay screening results indicated that the 2*H*-1,2,3-triazole-4-carboxylate derivative **1** displayed cytotoxicity with IC$_{50}$ values at 44.5 and 32.9 μmol/L on the A549 and HCT-116 cell lines (►**Table 1**), respectively. In addition, the steroid compound **10** inhibited NO production with an IC$_{50}$ value at 44.5 μmol/L (►**Table 2**), suggesting the anti-inflammatory activity of the compound.

Table 1 Cytotoxicity of compound 1 from *P. laternarius*

Compds.	IC$_{50}$ (μmol/L)^a	
	A549	HCT-116
1	44.5 ± 1.1	32.9 ± 0.7
Sorafenib^b	12.6 ± 0.2	10.3 ± 0.5

^{a}Data were expressed as means ± standard deviation (*n* = 3).
^{b}Sorafenib was used as a positive control.

Table 2 Nitric oxide inhibition of compound 10 in RAW264.7 cells

Compds.	IC$_{50}$ (μmol/L)^a
10	44.5 ± 1.1
Dexamethasone^b	20.3 ± 0.8

^{a}Data were expressed as means ± standard deviation (*n* = 3).
^{b}Dexamethasone was used as a positive control.

Conclusion

Chemical investigations on *Pegasus laternarius* (Hai-E Yu) were undertaken, and 16 compounds were isolated and identified. Among them, compounds **1**, **6**, **12**, and **13** were first reported from *P. laternarius*. The bioassay results showed that the triazole compound **1** exhibited moderate cytotoxicity with IC$_{50}$ values of 44.5 and 32.9 μmol/L on the A549 and HCT-116 cell lines, respectively, while the steroid compound **10** showed NO production inhibition activity with IC$_{50}$ value at concentration lower than 50 μmol/L. This study provides valuable information for understanding the MTCM of Hai-E Yu and searching for anticancer and anti-inflammatory candidates from MTCMs.

Experimental Section

General Experimental Procedures

^{1}H and ^{13}C NMR spectra were acquired on a Bruker AVANCE III 400 and 600 spectrometer. HRESIMS spectra were recorded on an Agilent G6250 Q-TOF (Agilent, Santa Clara, California, United States). All solvents used for column chromatography and HPLC were of analytical grade (purchased from Shanghai Chemical Reagents Co., Ltd., Shanghai, China) and chromatographic grade (purchased from Dikma Technologies Inc., Beijing, China), respectively. Sephadex LH-20 (Pharmacia, Peapack, New Jersey, United States) was also used for column chromatography. Commercial silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China, 100–200 and 300–400 mesh) was used for column chromatography, and precoated silica gel GF254 plates (Sinopharm Chemical Reagent Co., Shanghai, China) were used for analytical thin-layer chromatography. Reversed-phase HPLC was performed on an Agilent 1260 series liquid chromatograph equipped with a DAD G1315D detector at 210 nm (Agilent, Santa Clara, California, United States). An Agilent semipreparative XDB-C18 column (5 μm, 250 mm × 9.4 mm) was employed for the purification.

Animal Materials

The animal *Pegasus laternarius* Cuvier was collected from Yangjiang City, Guangdong Province, China in July 2022, and was authenticated by Dr. Lin Gong (Institute of Oceanology, Chinese Academy of Sciences). For reference and future studies, a voucher specimen of the animal was cataloged (No. 202207–16) in Laboratory 1808 of Shandong Laboratory of Yantai Drug Discovery.

Cytotoxicity Assay

This part was conducted by referring to our previous paper.[20] The materials used in the study were CCK8 kit (Shanghai Lfe-iLab Biotechnology Co., Ltd., Shanghai, China), human lung carcinoma cell line A549 and colon cancer cell line HCT-116 (Shanghai Beyotime Biotechnology, Shanghai, China), and sorafenib (Promega, https://www.promega.com.cn/). OD at 450 nm was measured to assess cell viability with the inhibitory ratios calculated as $[A_{(control)} - A_{(sample)}]/A_{(control)} \times 100\%$. GraphPad Prism 7 (GraphPad Software, San Diego, CA, United States) was used to calculate IC_{50} values. All data were expressed as the mean $\pm$ standard deviation of three independent experiments.

Determination of NO Production and the Cell Viability Assay

The experiment was conducted according to a reported study.[19] Dexamethasone was used as a control drug (Promega, https://www.promega.com.cn/). The NO production level was identified by measuring the nitrite concentration in the cell culture supernatants. In brief, the RAW264.7 cells (10^5 cells/well) were stabilized with or without 1 µg/mL of LPS for 24 hours in the presence or absence of the test compounds. Then the cell culture supernatant (100 µL) was reacted with 100 µL of Griess reagent. The viability of the remaining cells after the Griess assay was detected by colorimetric assay using CCK8. The compounds were tested for NO assay at a concentration of 50 µmol/L. If the NO inhibition of compounds in RAW264.7 cells was more than 50% at 50 µmol/L, the IC_{50} values were tested, and the concentrations were set as 100, 50, 20, 10, 5, 1, 0.1 µmol/L, respectively.

Supporting Information

Spectroscopic characterization processes (^{1}H NMR and ^{13}C NMR) for compounds **1–16** are included in the Supporting Information (►**Figs. S1–S32** [available in the online version]).

Conflict of Interest
None declared.

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