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Structurally Diverse Polycyclic Salicylaldehyde Derivative Enantiomers from a Marine-Derived Fungus Eurotium sp. SCSIO F452

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Abstract: To enlarge the chemical diversity of Eurotium sp. SCSIO F452, a talented marine-derived fungus, we further investigated its chemical constituents from a large-scale fermentation with modified culture. Four pairs of new salicylaldehyde derivative enantiomers, euroticins F-I (1–4), as well as a known one eurortirumin (5) were isolated and characterized. Compound 1 features an unprecedented constructed 6/6/6/5 tetracyclic structures, while 2 and 3 represent two new types of 6/6/5 scaffolds. Their structures were established by comprehensive spectroscopic analyses, X-ray diffraction, 13C NMR, and electronic circular dichroism calculations. Selected compounds showed significant inhibitory activity against α-glucosidase and moderate cytotoxic activities against SF-268, MCF-7, HepG2, and A549 cell lines.

Keywords: marine-derived fungi; Eurotium sp.; natural products; salicylaldehyde derivative enantiomers; bioactivities

1. Introduction

Natural product discovery from marine-derived fungi has attracted more and more attention in recent decades. An increasing number of secondary metabolites with intriguing scaffolds and promising bioactivities have been isolated and characterized from marine fungi [1–3]. However, an inevitable problem of duplicated isolation of some common compounds has also emerged, resulting in wasted of time and labor. To improve the discovery efficiency of novel skeletal compounds, various strategies like genome mining and bioinformatic analysis may deter natural-product chemists. Therefore, more traditional approaches, including One Strain Many Compounds (OSMAC) and large-scale culture are employed to enlarge the chemical space of microorganism [5]. The OSMAC...
approach emphasizes environmental alteration, like changing the culture medium, adding epigenetic regulators, and adjusting temperature and pH to trigger silent biosynthetic gene clusters expression and explore their chemical diversity, without the need to sequence and manipulate genes [5,6]. Another practical method is large-scale culture, which received increasing attention recently. This strategy focuses on the discovery of novel compounds in trace yield under normal scale incubation. These less explored minor compounds tend to be “dark matters” that bear novel skeletons. By scaling up, they can be better accumulated and isolable [5,7]. Therefore, it can serve as a complementary method to OSMAC, enabling natural product chemists to access more chemical entities.

Our group has long been concentrated on unveiling bioactive secondary metabolites from marine-derived fungi [8–11]. Recently, we discovered different types of enantiomeric compounds, including spirocyclic diketopiperazine alkaloids, anthraquinones, and salicylaldehyde derivatives from Eurotium sp. SCSIO F452, a fungus isolated from a South China Sea sediment sample [12–16]. In order to enlarge the chemical space of this strain, we launched a 10 folds large-scale fermentation (300 L) with a modified liquid culture media and investigated its chemical constituents. A series of unprecedented salicylaldehyde derivative enantiomers with 6/6/5/7 and 6/6/6/6 polycyclic chemical scaffolds were isolated and characterized, which warrants an in-depth investigation of this sample [17,18]. Interestingly, we further discovered four pairs of new salicylaldehyde derivative enantiomers, namely euroticins F-I (1–4), with different polycyclic architectures, as well as a known one, eurotirumin [19] (5) (Figure 1), with its chiral separation and absolute configuration confirmed for the first time. Herein we report their isolation, structural elucidation, proposed biosynthetic pathway, and bioactivity evaluation.

![Figure 1. Structures of compounds 1–5.](image)

2. Results

2.1. Structure Identification

Eurotcin F (1) was isolated as a yellow solid. Its molecular formula C_{21}H_{28}O_{4} was assigned by HRMS (ESI) m/z: [M – H]− Calcd for C_{21}H_{29}O_{4} 343.1915; Found 343.1922, corresponding to eight degrees of hydrogen deficiency. Its 1H NMR (Table 1) spectrum measured in DMSO-d_{6} exhibited four methyls at δ_{H} 0.86 (t, J = 6.7 Hz), 1.29 (s), 1.31 (s), 1.53 (s); five methylenes at δ_{H} 1.27 (overlap), 1.28 (overlap), 1.32 (overlap), 1.45 (m), 2.49 (overlap), 2.66 (d, J = 17.3 Hz); five methines at δ_{H} 3.75 (t, J = 6.7 Hz), 5.09 (s), 5.67 (d, J = 9.7 Hz), 6.27 (d, J = 9.7 Hz), 6.36 (s); one exchangeable proton at δ_{H} 8.99 (s). Its 13C NMR (Table 1) recorded 21 carbon resonances, including four methyls, five methylenes, five methines (two oxygenated, three olefinic), and seven nonprotonated carbons (five olefinic including two oxygenated, two aliphatic oxygenated). These NMR data partially resemble those of (±)-eurotiumide F [20], indicating 1 to be a salicylaldehyde analogue.
Table 1. $^1$H and $^{13}$C NMR Data for 1 in DMSO-$d_6$ and 2 in acetone-$d_6$ (700, 175, TMS, $\delta$ in ppm, $J$ in Hz).

| No. | $\delta$ C a | $\delta$ H (J, Hz) b | $\delta$ C a | $\delta$ H (J, Hz) b |
|-----|--------------|---------------------|--------------|---------------------|
| 1   | 120.6        | 121.1               | 120.6        | 121.1               |
| 2   | 143.0        | 144.6               | 143.0        | 144.6               |
| 3   | 120.1        | 127.7               | 120.1        | 127.7               |
| 4   | 110.3        | 6.36, s              | 117.0        | 6.51, s              |
| 5   | 145.7        | 148.2               | 145.7        | 148.2               |
| 6   | 125.6        | 122.4               | 125.6        | 122.4               |
| 7   | 35.8         | 2.66, d (17.3)      | 95.9         | 5.58, s              |
|     |              | 2.49, overlap       |              |                     |
| 8   | 106.2        | 1.53, s              |             |                     |
| 9   | 25.8         | 6.27, d (9.7)       | 29.2         | 3.27, m              |
| 1'  | 122.6        | 5.67, d (9.7)       | 123.8        | 5.28, br t (7.4)    |
| 2'  | 75.7         | 132.6               |             |                     |
| 3'  | 28.0         | 1.31, s              | 25.9         | 1.72, s              |
| 4'  | 27.8         | 1.29, s              | 17.8         | 1.69, s              |
| 5'  | 74.1         | 5.09, s              | 41.3         | 2.30, dd (12.0, 8.1) |
| 6'  | 84.8         | 3.75, t (6.7)       | 67.9         | 4.61, ddd (8.1, 7.7, 5.0) |
| 7'  | 35.1         | 1.45, m              | 35.0         | 2.49, m              |
|     |              | 1.71, overlap       |              |                     |
| 4"  | 25.2         | 1.32, overlap        | 29.5         | 1.92, m              |
| 5"  | 31.5         | 1.27, overlap        | 49.8         | 1.33, m              |
| 6"  | 22.5         | 1.28, overlap        | 80.8         | 4.31, dq (10.4, 6.2) |
| 7"  | 14.4         | 0.86, t (6.7)       | 21.8         | 1.30, d (6.2)        |
| 5-OH| 8.99         | s                    | 55.4         | 3.45, s              |
| 7-OMe|            |                      |              |                     |

a Recorded at 175 MHz. b Recorded at 700 MHz.

Rings A and B can be elucidated as a 2,2-dimethyl-2H-chromene based on HMBC and COSY correlations (Figure 2). A 3,6-dihydro-2H-pyran ring C was deduced to fuse with ring B via C-1 and C-6 by HMBC correlations from H-2-7 ($\delta$H 2.49, overlap; 2.66, d, $J = 17.3$ Hz) to C-1 ($\delta$C 120.6), C-2 ($\delta$C 143.0), C-6 ($\delta$C 125.6), C-8 ($\delta$C 106.2); from H-1" ($\delta$H 5.09, s) to C-1, C-5 ($\delta$C 145.7), C-6, C-8. Key HMBC correlations from H-2" ($\delta$H 3.75, t, $J = 6.7$ Hz) to C-6, C-8, C-1" ($\delta$C 74.1), as well as the chemical shift of C-8 indicated that C-8 and C-2" ($\delta$C 84.8) are bridged by an oxygen atom. Therefore, a 1,3-dioxolane ring D can be deduced to fuse ring C, forming a 7,8-dioxo-bicyclo[3.2.1]octene core, which is rare in salicylaldehyde compounds. A methyl group and an $n$-pentyl group are located at C-8 and C-2", respectively, as elucidated by the HMBC correlations from H-3'-9 ($\delta$H 1.53, s) to C-7 ($\delta$C 35.8), C-8; from H-1" to C-2", C-3" ($\delta$C 35.1); from H-2" to C-4" ($\delta$C 25.2); as well as diagnostic $^1$H-$^1$H COSY cross peaks of H-2"/H-3"/H-4"/H-5"/H-6"/H-7". Hitherto, the gross structure of 1 was established to be a polycyclic salicylaldehyde derivative with an unprecedented 6/6/6/5 skeleton.

As for its relative configuration, the ROE correlation between H-1" and H-3'-9 indicated their cofacial as $\beta$-orientations. Because there is no $^1$H-$^1$H COSY correlation between H-1" and H-2", and H-1" and H-2" are displayed as sharp singlet and triplet in $^1$H NMR, respectively. The dihedral angle between H-1" and H-2" should tend to be 90 degree based on the Karplus equation, indicating that they are located on the opposite of the ring [21–23]. To further verify this conclusion, we calculated the $^{13}$C NMR spectra of (8R,1"S,2"S)-1 and (8R,1"S,2"R)-1 using gauge including atomic orbitals (GIAO) method at the mPW1PW91/6-311+G(d,p)/PCM(DMSO). The calculated $^{13}$C NMR data of (8R,1"S,2"S)-1 (DP4+ probability: 99.99%) showed a better match with the measured data of 1 than that of (8R,1"S,2"R)-1 (DP4+ probability: 0.01%) (Figure 3), which allowed the assignment of the relative configuration of 1 to be 8R*,1"S*,2"S*.
Due to the baseline circular dichroism and barely measurable optical rotation, compound 1 was deduced to be a racemate. It was separated by chiral HPLC to two optically pure enantiomers (+)-1 and (−)-1 (Figure S1). Subsequently, quantum chemical calculation of electronic circular dichroism (ECD) spectrum for (8R,1′S,2′S)-1 matched well with that of measured for (−)-1 (Figure 4). Finally, the absolute configuration of (+)-1 and (−)-1 could be determined as 8S,1′R,2′R and 8R,1′S,2′S, respectively.

Euroticin G (2) was isolated as a yellow solid, with its molecular formula determined as C_{20}H_{28}O_{5} by HRMS (ESI) m/z: [M − H]−. Calcd for C_{20}H_{27}O_{5} 347.1864; Found 347.1871.
indicating seven degrees of hydrogen deficiency. Its $^1$H NMR (Table 1) spectrum showed four methyls at $\delta_H$ 1.30 (d, $J = 6.2$ Hz), 1.69 (s), 1.72 (s), 3.45 (s), three methylenes at $\delta_H$ 1.39 (m), 1.71 (overlap), 1.92 (m), 2.49 (m), 3.27 (m), and seven methines at $\delta_H$ 1.33 (m), 2.30 (dd, $J = 12.0$, 8.1 Hz), 4.31 (dq, $J = 10.4$, 6.2 Hz), 4.61 (ddd, $J = 8.1$, 7.7, 5.0 Hz), 5.28 (br t, $J = 7.4$ Hz), 5.58 (s), 6.51 (s). The $^{13}$C NMR and DEPT (Table 1) revealed the presence of 20 carbon resonances, including four methyls (one oxygenated), three methylenes, seven methines (two olefinic, three oxygenated), six olefinic nonprotonated carbons (two oxygenated). Comparing these NMR data with eurortinum (5) [19] shows that 2 has a different 6/6/5 tricyclic pattern. Detailed analysis of 2D NMR (Figure 2) reveals it has a prenylated aromatic A ring. A 3,4-dihydro-2H-pyran ring B was fused with ring A via C-1 and C-6 by HMBC correlations from H-7 ($\delta_H$ 5.58, s) to C-2 ($\delta_C$ 144.6), C-6 ($\delta_C$ 122.4), C-2"; from H-1" ($\delta_H$ 2.30, dd, $J = 12.0$, 8.1 Hz) to C-1 (C-4 121.1), C-5 (C-4 148.2), C-6; as well as $^1$H-$^1$H COSY cross peak of H-1"/H-2". A methoxyl group is located at the acetal C-7 based on the HMBC correlation from OMe ($\delta_H$ 3.45, s) to C-7 ($\delta_C$ 95.9). Subsequently, a cyclopentane ring C was deduced to fuse with ring B by HMBC correlations from H$_2$-3" ($\delta_H$ 1.71, overlap; 2.49, m) to C-1" ($\delta_C$ 41.3), C-5" ($\delta_C$ 49.8); from H$_2$-4" ($\delta_H$ 1.39, m; 1.92, m) to C-1", C-2" ($\delta_C$ 67.9); from H-5" ($\delta_H$ 1.33, m) to C-6, C-1"; as well as the diagnostic $^1$H-$^1$H COSY cross peaks of H-2"/H$_2$-3"/H$_2$-4"/H-5"/H-1". A CH$_3$CHOH side chain attached to C-5" can be elucidated by HMBC correlations from H-6" ($\delta_H$ 4.31, dq, $J = 10.4$, 6.2 Hz) to C-1" and C-5"; from H$_3$-7" ($\delta_H$ 1.30, d, $J = 6.2$ Hz) to C-5", C-6" ($\delta_C$ 80.8); and $^1$H-$^1$H COSY cross peaks of H-5"/H-6"/H-5-7". Careful analysis of the ROESY (Figure 2) revealed correlations between H-1" and H-2", H-2" and 7-OMe, indicating their cofacial $\beta$-orientations. Therefore, the coupling constant of $J_{H-1'/H-2'}$ should be 8.1 Hz (Table 1). Then $J_{H-1'/H-5'}$ should be assigned as 12.0 Hz, indicating H-1"/H-5" to be trans relationship. From a biosynthetic point, the relative configuration of C-6" in 2 was deduced to be S by sharing the same configuration with that in compound 5, whose structure has been confirmed by X-ray experiment. Furthermore, conformation analysis revealed that there is a hydrogen bond between 6"-OH and 5-OH, which hinders the free rotation of C-5"/C-6". When the relative configuration of C-6" is assigned as S, its predominant configuration is coordinated with a diagnostic correlation between H-1" and H-6" observed in ROESY spectrum. But when C-6" is R, it is not. Thus, the structure of 2 (Figure 1) was established with its relative configuration established as 7S,1"R,2"R,5"R,6"S. This compound was proved to be a racemate by chiral HPLC separation (Figure S2), yielding (+)-2 and (−)-2. The calculated ECD spectrum of 7S,1"R,2"R,5"R,6"S-2 agreed well with that of measured for (+)-2 (Figure 5). Therefore, the absolute configuration of (+)-2 and (−)-2 can be assigned as 7S,1"R,2"R,5"R,6"S and 7R,1"S,2"S,5"S,6"R, respectively.

**Figure 5.** Comparison between PBE0/TZVP/PCM calculated and experimental ECD spectra of 2 and 4 in MeOH.

Euroticin H (3) was obtained as a yellow solid. Its molecular formula C$_{19}$H$_{24}$O$_4$ was determined by HRMS (ESI) $m/z$: [M + H]$^+$ Calcd for C$_{19}$H$_{25}$O$_4$ 317.1747; Found 317.1748,
indicating eight degrees of hydrogen deficiency. Its $^1$H NMR (Table 2) spectrum showed three methyls at $\delta_H$ 0.88 (t, $J = 7.0$ Hz), 1.42 (s), 1.43 (s); five methylenes at $\delta_H$ 1.29 (overlap), 1.30 (overlap), 1.35 (overlap), 1.53 (m), 1.83 (m), 2.11 (m), 4.81 (d, $J = 16.3$ Hz), 4.99 (d, $J = 16.3$ Hz); three methines at $\delta_H$ 6.07 (d, $J = 9.9$ Hz), 6.46 (d, $J = 9.9$ Hz), 6.60 (s); two exchangeable protons at $\delta_H$ 5.09 (br s) and 11.20 (s). The $^{13}$C NMR and DEPT (Table 2) revealed the presence of 19 carbon resonances, including three methyls, five methylenes, three olefinic methines, and eight nonprotonated carbons (one carbonyl, five olefinic including two oxygenated, two aliphatic oxygenated). Comparing the NMR data of 3 with 1 indicated it has a similar partial structure of rings A and B. An $\alpha,\beta$-unsaturated cyclopentanone was deduced to fuse with ring B via C-1 and C-6 by HMBC correlations (Figure 6) from H$_2$-7 ($\delta_H$ 4.81, dd, $J = 16.3$ Hz; 4.99, dd, $J = 16.3$ Hz) to C-1 ($\delta_C$ 128.3), C-2 ($\delta_C$ 140.1), C-6 ($\delta_C$ 112.9), C-1” ($\delta_C$ 197.5), C-2” ($\delta_C$ 92.3). A hydroxyl group and an n-pentyl group are both located at C-2”, as elucidated by the HMBC correlations from 2”-OH ($\delta_H$ 5.90, br s) to C-1”, C-2”, C-3” ($\delta_C$ 37.0); from H$_2$-3” ($\delta_H$ 1.83, m; 2.11, m) to C-1”, C-2”; from H$_2$-4” ($\delta_H$ 1.35, overlap; 1.53, m) to C-2”, C-3”; combined with diagnostic $^1$H-$^1$H COSY cross peaks of H$_2$-3”/H$_2$-4”/H$_2$-5”/H$_2$-6”/H$_2$-7”. Compound 3 was deduced to be a racemate due to its baseline ECD. We tried to separate it by chiral columns but failed. Therefore, it was established to be a racemic salicylaldehyde derivative with a new type of 6/6/5 skeleton.

**Table 2.** $^1$H and $^{13}$C NMR data for 3 in acetone-$d_6$ and 4 in DMSO-$d_6$ (700, 175, TMS, $\delta$ in ppm, $J$ in Hz).

| No. | $\delta_C$ a | $\delta_H$ (J, Hz) b | $\delta_C$ c | $\delta_H$ (J, Hz) d |
|-----|--------------|----------------|---------------|----------------|
| 1   | 128.3        | 41.1           | 2.80, d (17.8)| 2.51, overlap   |
| 2   | 140.1        | 169.4          |               |                |
| 3   | 130.8        | 126.7          | 6.01, s       |                |
| 4   | 112.9        | 6.60, s        | 209.5         |                |
| 5   | 158.1        | 76.4           |               |                |
| 6   | 112.9        |                |               |                |
| 7   | 57.3         | 4.99, d (16.3) | 4.81, d (16.3)|                |
| 1’  | 122.4        | 6.46, d (9.9)  | 121.7         | 6.68, d (15.8) |
| 2’  | 138.0        | 6.07, d (9.9)  | 149.5         | 6.49, d (15.8) |
| 3’  | 77.1         | 69.5           |               |                |
| 4’  | 28.0         | 1.43, s        | 29.5          | 1.24, s        |
| 5’  | 27.7         | 1.42, s        | 29.5          | 1.24, s        |
| 1”  | 197.5        | 37.4           | 1.50, dt (12.6, 3.9)| 1.39, dt (12.6, 4.8)|
| 2”  | 92.3         | 23.2           | 1.25 overlap  | 1.10 m         |
| 3”  | 37.0         | 2.11, m        | 29.5          | 1.21, overlap  |
| 4”  | 23.6         | 1.83, m        | 28.7          | 1.22, overlap  |
| 5”  | 32.8         | 1.53, m        | 31.2          | 1.20, overlap  |
| 6”  | 23.2         | 1.29, overlap  | 22.1          | 1.24, overlap  |
| 7”  | 14.3         | 0.88, t (7.0)  | 14.0          | 0.84, t (7.2)  |
| 5-OH| 11.2         | s              |               |                |
| 2”-OH| 5.90          | br s           |               |                |

a Recorded at 125 MHz. b Recorded at 500 MHz. c Recorded at 175 MHz. d Recorded at 700 MHz.
Eurotirumin H (4) was obtained as a yellow oil. Its molecular formula C_{17}H_{28}O_3 was determined by HRMS (ESI) m/z: [M + Na]^+ Calcd for C_{17}H_{28}O_3Na 303.1931; Found 303.1938, indicating four degrees of hydrogen deficiency. The $^1$H and $^{13}$C NMR data (Table 2) of 4 were partially similar with those of flavoglaucin [16]. The conservative aromatic nuclei in flavoglaucin was changed to an α,β-unsaturated cyclopentanone in 2, which can be evidenced by the HMBC correlations (Figure 6) from H-1" (δ_H 2.80, d, J = 17.8 Hz; 2.51, overlap) to C-2 (δ_C 169.4), C-3 (δ_C 126.7), C-4 (δ_C 209.5), C-5 (δ_C 76.4). A hydroxyl group located at C-5 can be elucidated by the chemical shift of C-5 (δ_C 76.4) and HMBC correlations from H-2-1" (δ_H 1.50, dt, J = 12.6, 3.9 Hz; 1.39, dt, J = 12.6, 4.8 Hz) to C-1 (δ_C 41.1), C-4, C-5. The prenyl group in flavoglaucin was transformed to a 3-hydroxyl-3-methylbutene group attached to C-2 in 4, as elucidated by HMBC correlations from H-1' (δ_H 6.68, d, J = 15.8 Hz) to C-1, C-2, C-3, C-2' (δ_C 149.5), C-3' (δ_C 69.5); from H-2' (δ_H 6.49, d, J = 15.8 Hz) to C-2, C-3', C-4' (δ_C 29.5), C-5' (δ_C 29.5). The double bond between C-1' and C-2' was assigned as E geometry by the large coupling constant (J_H-1'/H-2' = 15.8 Hz) [24]. It was confirmed to be a racemate and further separated to yield (+)-4 and (−)-4 (Figure S3). The calculated ECD of S-4 fitted well that of (−)-4 (Figure 5). Accordingly, (+)-4 can be assigned as R. To be noted, this compound represents the first de aromatized prenylated salicylaldehyde derivative from fungus.

Compound 5 was isolated as a yellow solid. It was identified as eurotirumin [19] by comparison of HRMS and NMR data (Table S3). It was not demonstrated as a racemate or not. In our separation, we proposed it to be a racemate because of its low optical rotation. Then we successfully separated it by chiral HPLC (Figures S4 and S5) and fortunately obtained suitable crystals of (−)-5 from MeOH for single-crystal X-ray diffraction experiment, which confirmed its planar and absolute configuration as 1"R,2"R,5"S,6"S with the Flack parameters of −0.06(15) (CCDC 2087463) (Figure 7). Thus, (−)-5 can be assigned as 1"S,2"S,5"S,6"R.

![Figure 6](image_url)  
Figure 6. Key $^1$H-$^1$H COSY and HMBC correlations of compounds 3 and 4.

![Figure 7](image_url)  
Figure 7. X-ray ORTEP drawing of compound (−)-5.
2.2. Proposed Biosynthesis Pathway

The putative biosynthetic pathway of 1–5 is proposed in Scheme 1. For 1, intermediate Ia was formed by polyketide pathway from the starting precursor (one acetyl-CoA, seven malonyl-CoA) and cyclization. Then Ia underwent prenylation to produce IIa. A series of selective reduction, dehydration, hydrogenation, and oxidation of IIa could produce IIIa, which went through nucleophilic addition and oxidation to become Va. Another nucleophilic addition of Va would produce compound 1. For 2–5, a similar PKS pathway with one acetyl-CoA and six malonyl-CoA and prenylation gave IIb, which could generate three different precursors (IIIb1, IIIb2, IIIb3) for three different routes. In route 1, an aldol condensation of IIIb1 furnished IVb1, which can be oxidized to Vb1. Subsequent nucleophilic addition occurred between 2"-OH and C-7 or 5-OH and C-6" would produce compounds 2 and 5, respectively. In route 2, IIIb2 can transform to Vb2 by oxidation and aldol concentration. Dehydration and oxidation of Vb2 could yield compound 3. In route 3, decarbonylation of IIIb3 gave IVb3. It can transform to Vb3 by tautomerism with loss of one H2. Another decarbonylation of Vb3 yielded VIb3, which went through oxidation and reduction to furnish compound 4.

Scheme 1. Proposed biosynthetic pathway of compounds 1–5.
2.3. Bioactivity Evaluation

Compounds (+)-1, (−)-1, (+)-2, (−)-2, (±)-3, (+)-4, (−)-4, (+)-5, and (−)-5 were evaluated for their in vitro cytotoxicity against four human cancer lines SF-268 (human glioblastoma carcinoma), MCF-7 (breast cancer), HepG-2 (liver cancer), and A549 (lung cancer), as well as antioxidative activity, α-glucosidase inhibitory, and antimicrobial activity against the bacteria Staphylococcus aureus and Bacillus subtilis [17,26]. All the compounds except (+)-2, (−)-2, and (±)-3 exhibited moderate cytotoxic activities with IC50 values ranging from 12.74 to 55.5 µM (Table 3). Compounds (+)-1, (−)-1, (+)-2, (−)-2 showed weak DPPH radical scavenging activity with EC50 values ranging from 41.40 to 77.07 µM (Table 4). Compounds (±)-3 and (+)-2 exhibited significant inhibitory against α-glucosidase with IC50 values of 16.31 ± 1.68 µM and 38.04 ± 2.73 µM, which are even better or comparative to that of the positive control Acarbose (IC50 of 32.92 ± 1.03 µM), while compounds (−)-2 and (+)-5 showed weak activity (IC50 of 89.41 ± 7.86 µM) (Table 4). None of these compounds exhibited antimicrobial activity at a concentration of 100 µM.

Table 3. Cytotoxic activities of compounds (+)-1, (−)-1, (+)-2, (−)-2, (±)-3, (+)-4, (−)-4, (+)-5, and (−)-5 against tumor cells a.

| Compounds | SF-268 (µM) | MCF-7 (µM) | HepG2 (µM) | A549 (µM) |
|-----------|-------------|-------------|-------------|-----------|
| (+)-1     | 21.88 ± 0.96| 27.17 ± 2.03| 28.00 ± 1.68| 33.78 ± 0.34|
| (−)-1     | 37.31 ± 2.46| 28.00 ± 1.57| 30.72 ± 3.55| 33.43 ± 1.50|
| (+)-2     | >100        | >100        | >100        | >100      |
| (−)-2     | >100        | >100        | >100        | >100      |
| (±)-3     | >100        | >100        | >100        | >100      |
| (+)-4     | 12.74 ± 0.46| 20.51 ± 1.54| 19.88 ± 5.09| 16.90 ± 0.92|
| (−)-4     | 23.73 ± 3.88| 23.56 ± 2.99| 19.53 ± 2.70| 22.15 ± 1.54|
| (+)-5     | 21.98 ± 0.88| 55.59 ± 4.85| 41.18 ± 2.63| 47.34 ± 0.71|
| (−)-5     | 35.65 ± 1.79| 50.67 ± 2.48| 40.69 ± 2.76| 42.61 ± 1.43|
| Adriamycin| 1.19 ± 0.03 | 2.02 ± 0.04 | 1.99 ± 0.07  | 1.73 ± 0.04 |

a The results were mean ± SD (SD = standard deviation). Positive control: Adriamycin.

Table 4. Antioxidative and α-glucosidase inhibitory activities of compounds (+)-1, (−)-1, (+)-2, (−)-2, (±)-3, (+)-4, (−)-4, (+)-5, and (−)-5.

| Compounds | Antioxidative Activity EC50 (µM) | α-Glucosidase Inhibitory IC50 (µM) |
|-----------|---------------------------------|-----------------------------------|
| (+)-1     | 42.34 ± 3.85                    | >100                              |
| (−)-1     | 41.40 ± 2.68                    | >100                              |
| (+)-2     | 76.90 ± 0.99                    | 38.04 ± 2.73                      |
| (−)-2     | 77.07 ± 1.88                    | 79.71 ± 1.74                      |
| (±)-3     | >100                            | 16.31 ± 1.68                      |
| (+)-4     | >100                            | >100                              |
| (−)-4     | >100                            | >100                              |
| (+)-5     | >100                            | 89.41 ± 7.86                      |
| (−)-5     | >100                            | >100                              |
| Ascorbic acid a | 11.35 ± 0.56                     |                                   |
| Acarbose b | 32.92 ± 1.03                     |                                   |

a Positive control for antioxidative activity; b Positive control for α-glucosidase inhibitory activity.

3. Materials and Methods

3.1. General Experimental Procedures

Optical rotations were measured with an MCP 500 automatic polarimeter (Anton Paar, Graz, Austria) with MeOH as solvent. UV spectra were recorded on a UV-2600 spectrometer (Shimadzu, Kyoto, Japan). Circular dichroism spectra were measured with a Chirascan circular dichroism spectrometer (Applied Photophysics, Ltd., Surrey, UK). Crystallographic data were collected on an XtaLAB AFC12 (Rigaku, Kyoto, Japan): Kappa single diffractometer using Cu Kα radiation. 1H, 13C NMR, and 2D NMR spectra were
recorded on the AVANCE 500 MHz and 700 MHz NMR with TMS as an internal standardthe (Bruker Biospin GmbH, Rheinstetten, Germany). HRESIMS spectra data were recorded on a MaXis quadrupole-time-of-flight mass spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany). Thin layer chromatography (TLC) was performed on plates precoated withsilica gel GF254 (10–40 µm, Qingdao Marine Chemical Factory, Qingdao, China). Columnchromatography (CC) was performed over silica gel (200–300 mesh and 300–400 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and ODS (50 µM, YMC). High performanceliquid chromatography was performed on an Agilent 1260 HPLC equipped with a DAD detector (Agilent Technologies Co., Ltd., Palo Alto, CA, USA), using YMC-pack ODS-A column (250 × 10 mm, 5 µm, YMC CO., LTD., Kyoto, Japan) and COSMOSIL Cholesterol packed column (250 × 10 mm, 5 µm, Nacalai tesque, Kyoto, Japan). All solventsused in CC and HPLC were of analytical grade (Tianjin Damao Chemical Plant, Tianjin, China) and chromatographic grade (Oceanpak Axelative Chemical, Gothenburg, Sweden),respectively. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H2SO4 in EtOH.

3.2. Fungal Material, Fermentation, and Extraction

As described previously [12].

3.3. Purification

The EtOAc extract (356 g) was subjected to a silica gel column using step gradient elution with CH2Cl2/Acetone (1:0 to 0:1) and yielded five fractions Frs.1-5 monitored by TLC. Fr.1 was separated by silica gel CC (PE/EtOAc 1:0 to 0:1) to obtain four subfractions (Frs.1.1–1.4). Fr.1.2 was separated by silica gel CC (PE/EtOAc 1:0 to 0:1) to obtain four subfractions (Frs.1.1.1–1.1.4). Fr.1.2.1 was purified by silica gel CC (PE/EtOAc 1:0 to 0:1) to produce six parts (Frs.1.2.1–1.2.6). Fr.1.2 was separated by ODS CC with a gradient elution of MeOH/H2O (3 mL/min, 70% CH3CN/H2O, ODS-A column) to yield 1 (0.8 mg, tR = 19.0 min). Fr.1.2.1.2 was purified by HPLC (3 mL/min, 69% CH3CN/H2O, ODS-A column) to yield 5 (4.5 mg, tR = 18.2 min). Fr.1.2.1.3 was purified by HPLC (3 mL/min, 73% CH3CN/H2O, ODS-A column) to yield 2 (2.1 mg, tR = 12.4 min). Fr.1.2.1.4 was purified by HPLC (3 mL/min, 67% CH3CN/H2O, ODS-A column) to yield 3 (2.0 mg, tR = 28.5 min). Fr.1.2.2 was purified by silica gel CC (PE/EtOAc 1:0 to 0:1) to obtain five subfractions (Frs.1.2.2.1–1.2.2.5). Fr.1.2.2.2 was purified by HPLC (3 mL/min, 43% CH3CN/H2O, Cholesterol column) to yield 4 (4.0 mg, tR = 20.0 min).

Compounds 1–5 were isolated as racemates. Compound 1 was subjected to chiral HPLC (Daicel chiralpak IA, 250 × 46 mm, 5 µM) using n-hexane/isopropanol (v/v: 91:9; flow rate: 1 mL/min) as mobile phase to yield (+)-1 (0.8 mg, tR = 14.5 min) and (−)-1 (0.8 mg, tR = 9.1 min). Compound 2 was subjected to chiral HPLC (Daicel chiralpak IA, 250 × 46 mm, 5 µM) using n-hexane/isopropanol (v/v: 87:13; flow rate: 1 mL/min) to yield (+)-2 (0.8 mg, tR = 19.0 min) and (−)-2 (0.8 mg, tR = 12.7 min). Compound 4 was subjected to chiral HPLC (Daicel chiralpak IA, 250 × 46 mm, 5 µM) using n-hexane/isopropanol (v/v: 90:10; flow rate: 1 mL/min) to yield (+)-4 (0.6 mg, tR = 9.5 min) and (−)-4 (0.6 mg, tR = 11.2 min). Compound 5 was subjected to chiral HPLC (Daicel chiralpak IA, 250 × 46 mm, 5 µM) using n-hexane/isopropanol (v/v: 92:8; flow rate: 1 mL/min) to yield (+)-5 (0.8 mg, tR = 8.8 min) and (−)-5 (0.8 mg, tR = 10.3 min). Compound 3 was not separated successfully.

(+)- Euroticin F [(±)-1]. yellow solid; [a]25 D= 0 (c 0.1, MeOH); HRMS (ESI) m/z: [M − H]− Calcd for C21H22O4 343.1915; Found 343.1922. 1H and 13C NMR see Table 1.

(+)-1. yellow solid; [a]25 D= +91 (c 0.075, MeOH); ECD (MeOH) λmax (Δε) 336 (+1.1), 275 (+3.8), 267 (+3.8), 211 (+5.4) nm.

(−)-1. yellow solid; [a]25 D= −90 (c 0.075, MeOH); ECD (MeOH) λmax (Δε) 331 (−1.5), 275 (−4.6), 267 (−4.6), 213 (−4.5) nm.
(±)-Euroticin G [(±)-2]. yellow solid; [α]25 D = 0 (c 0.1, MeOH); UV (MeOH) λ_max (log ε) 296 (3.4), 206 (4.3) nm; HRMS (ESI) m/z: [M − H]− Calcd for C_{20}H_{27}O_{5} 347.1864; Found 347.1871. 1H and 13C NMR see Table 1.

(+)-2. yellow solid; [α]25 D = +37 (c 0.1, MeOH); ECD (MeOH) λ_max (Δε) 296 (−2.0), 226 (+2.7), 216 (+3.1) nm.

(−)-2. yellow solid; [α]25 D = −35 (c 0.1, MeOH); ECD (MeOH) λ_max (Δε) 296 (+3.2), 236 (−2.8), 216 (−3.2) nm.

(±)-Euroticin H [(±)-3]. yellow solid; [α]25 D = 0 (c 0.02, MeOH); UV (MeOH) λ_max (log ε) 304 (3.8), 241 (3.8), 209 (3.8) nm; HRMS (ESI) m/z: [M + H]⁺ Calcd for C_{19}H_{25}O_{4} 317.1747; Found 317.1748. 1H and 13C NMR see Table 2.

(±)-Euroticin I [(±)-4]. yellow oil; [α]25 D = 0 (c 0.03, MeOH); UV (MeOH) λ_max (log ε) 273 (4.8) nm; HRMS (ESI) m/z: [M + Na]⁺ Calcd for C_{17}H_{28}O_{3}Na 303.1931; Found 303.1938. 1H and 13C NMR see Table 2.

(+)-4. yellow oil; [α]25 D = +22 (c 0.06, MeOH); ECD (MeOH) λ_max (Δε) 362 (+1.4), 275 (−11.4), 213 (+16.9) nm.

(−)-4. yellow oil; [α]25 D = −22 (c 0.06, MeOH); ECD (MeOH) λ_max (Δε) 365 (−1.9), 276 (+10.5), 2714 (−13.7) nm.

(±)-Eurotirumin [(±)-5]. yellow solid; [α]25 D = 0 (c 0.02, MeOH); UV (MeOH) λ_max (log ε) 274 (3.5), 202 (4.2) nm; HRMS (ESI) m/z: [M − H]⁻ Calcd for C_{19}H_{23}O_{4} 315.1602; Found 315.1607. 1H and 13C NMR see Table S1.

(+)-5. yellow solid; [α]25 D = +70.8 (c 0.05, MeOH); ECD (MeOH) λ_max (Δε) 318 (+0.9), 239 (−2.8) nm.

(−)-5. yellow solid; [α]25 D = −71.4 (c 0.05, MeOH); ECD (MeOH) λ_max (Δε) 312 (−1.1), 237 (+3.1) nm.

3.4. X-ray Crystallographic Analysis

Crystallographic data were collected on an XtaLAB AFC12 (Rigaku, Kyoto, Japan): Kappa single diffractometer using Cu Ka radiation. The structure was solved with the ShelXT (Germany) structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimization. The crystallographic data for (−)-5 was deposited in the Cambridge Crystallographic Data Centre (CCDC deposition number: 2087463). The data can be obtained freely from the Cambridge Crystallographic Data Centre by visiting sites of www.ccdc.cam.ac.uk/conts/retrieving.html (accessed on 1 July 2021).

Crystal Data for Compound (−)-5: C_{18.9875}H_{23.9625}O_{4} (M = 316.19 g/mol): orthorhombic, space group P2_{1}2_{1}2_{1}, a = 4.5232(10) Å, b = 10.1857(3) Å, c = 35.1507(9) Å, V = 1619.46(7) Å^3, Z = 4, T = 100.0(10) K, μ(CuKα) = 0.726 mm⁻¹, D_calcd = 1.297 g/cm³, 6838 reflections measured (9.04° ≤ 2θ ≤ 148.45°), 3174 unique (R_int = 0.0331, R_{sigma} = 0.0418) which were used in all calculations. The final R₁ was 0.0508 (l > 2σ(l)) and wR(F²) was 0.1386 (all data). The goodness of fit on F² was 1.049. Flack parameter = −0.06(15).

3.5. ECD and 13C NMR Calculation Methods

The theoretical calculations of 13C NMR of 1 and ECD of 1, 2, and 4 were carried out using the Gaussian 09 [27] and ORCA 4.2.1 [28,29] software packages. Conformational analysis was initially performed using Spartan’14 (Wavefunction, Irvine, CA, USA). More details about the experimental procedures and the optimized conformation geometries, thermodynamic parameters, and populations of all conformations are provided in Supporting Information.

3.6. Cytotoxicity, Antioxidative, and α-Glucosidase Inhibitory Activity, and Antimicrobial Activity Assays

3.6.1. Cytotoxicity Assay

The cells of SF-268 (human glioblastoma carcinoma), MCF-7 (breast cancer), HepG-2 (liver cancer), and A549 (lung cancer) were purchased from Stem Cell Bank, Chinese
Academy of Sciences. The cells were cultured in DMEM medium (Gibco) containing 10% fetal bovine serum (Gibco) at 37 °C in a humidified atmosphere with 5% (v/v) CO₂. The cells were incubated in cultural flasks until sub-confluent (~80%). Then, cells (180 µL) with a density of 3 × 10⁴ cells/mL of media were seeded onto 96-well plates and incubated for 24 h at 37 °C, 5% CO₂. Subsequently, 20 µL of different concentrations of compounds ranging from 1 to 128 µM in DMSO were added to each plate well. Equal volume of DMSO was used as a negative control. The plates were further incubated for 72 h. After incubation, cell monolayers were fixed with 50% (wt/v) trichloroacetic acid (50 µL) and stained for 30 min with 0.4% (wt/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by washing repeatedly with 1% acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution (200 µL) for OD determination at 570 nm using a microplate reader. Adriamycin was used as positive control possessing potent cytotoxic activity. All data were obtained in triplicate and presented as means ± SD. IC₅₀ values were calculated with the Sigma Plot 10.0 software (Systat Software Inc., CA, USA) using a non-linear curve-fitting method [17].

3.6.2. Antioxidative Assay
Sample stock solutions (10 mM) were diluted to final concentrations of 2, 4, 8, 16, 32, 64, and 128 µM in ethanol. Then, 100 µL 0.2 mmol/L DPPH ethanol solution was added to 100 µL sample solutions of different concentrations on 96-well plates, and allowed to react at room temperature. Ascorbic acid was used as a positive control possessing potent antioxidant activity. After 12 h, the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity (AA) using the following formula: AA(%) = [1 – (Asample – Ablank)/Acontrol] × 100%. All data were obtained in triplicate and are presented as means ± S.D. EC₅₀ values were calculated with the SigmaPlot 10.0 software (Systat Software Inc., CA, USA) using a non-linear curve-fitting method [17].

3.6.3. α-Glucosidase Inhibitory Activity Assay
Inhibitory α-glucosidase activities were determined spectrophotometrically in a 96-well microtiter plate based on p-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate. In brief, 20 µL 0.2 U/mL α-glucosidase enzyme solution, 50 µL 0.1 mol/L PBS (pH 6.8), and 10 µL of the test compounds in DMSO were mixed and preincubated at 37 °C prior to initiation of the reaction by adding the substrate. After 10 min of preincubation, 20 µL 5 mmol/L PNPG solution was added and then incubated together at 37 °C. After 15 min of incubation, 20 µL 0.2 mol/L Na₂CO₃ was added to the test tubes to stop the reaction. The absorbance values were measured at 405 nm and converted into percentage inhibitory activity using the following formula: AA(%) = [1 – Asample/(Anegative control – Ablank)] × 100%. Acarbose was used as positive control. All data were obtained in triplicate and presented as means ± SD. IC₅₀ values were calculated with the Sigma Plot 10.0 software using a non-linear curve-fitting method [30].

3.6.4. Antimicrobial Activity Assay
All the compounds were tested for antibacterial activity against Staphylococcus aureus and Bacillus subtilis by the Mueller–Hinton broth microdilution method. Tested strains were cultured for 16 h on a rotary shaker at 37 °C. Cultures were diluted with sterilized medium to achieve an optical absorbance of 0.04-0.06 at 600 nm, then further diluted 10-fold before adding into 96-well microtiter plates. Compounds were dissolved in acetone, serially diluted to 7 concentrations (1.56–100 µg/mL), and tested in the 96-well plate in triplicate. The minimum inhibitory concentration (MIC) that completely inhibited visible growth of the tested strains were recorded after 18 h cultivation from three independent experiments, with vancomycin as the positive control and acetone as a blank control [26].
4. Conclusions

In conclusion, euroticins F-I (1–4), four pairs of new salicylaldehyde derivative enantiomers, as well as a known one, eurotirumin (5), were isolated from a South China Sea fungus Eurotium sp. SCSIO F452. Compound 1 features an unprecedented constructed 6/6/6/5 tetracyclic structures, while 2 and 3 represent two new types of 6/6/5 scaffolds. Compound 4 is the first de-aromatized prenylated salicylaldehyde derivative. Compounds 1–5 are all occurred as racemates. Their optical pure enantiomers, except for 3 were well separated with absolute configuration unambiguously resolved by single crystal X-ray diffraction and ECD calculation for the first time. Selected compounds showed significant inhibitory activity against α-glucosidase and moderate cytotoxic activities. Our work would further enlarge the chemical diversity and pharmacological prosperity of salicylaldehyde derivatives.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/md19100543/s1, Figure S1: The chiral HPLC chromatogram of 1; Figure S2: The chiral HPLC chromatogram of 2; Figure S3: The chiral HPLC chromatogram of 4; Figure S4: The chiral HPLC chromatogram of 5; Figure S5: Comparison between experimental ECD spectra of (±)-5 and (−)-5; Figure S6: Structures of compounds applied for theoretical calculations; Figure S7: The 1H NMR (700 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S8: The 13C NMR (175 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S9: The HSQC (700 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S10: The HMBC (700 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S11: The 1H-1H COSY (700 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S12: The ROESY (700 MHz) spectrum of euroticin F (1) in DMSO-d$_6$; Figure S13: The HRESIMS spectrum of euroticin F (1); Figure S14: The 1H NMR (700 MHz) spectrum of euroticin G (2) in acetone-d$_6$; Figure S15: The 13C NMR (175 MHz) spectrum of euroticin G (2) in acetone-d$_6$; Figure S16: The HSQC (700 MHz) spectrum of euroticin G (2) in acetone-d$_6$; Figure S17: The HMBC (700 MHz) spectrum of euroticin G (2) in acetone-d$_6$; Figure S18: The 1H-1H COSY (700 MHz) spectrum of euroticin G (2) in acetone-d$_6$; Figure S19: The ROESY (700 MHz) spectrum of euroticin D (2) in acetone-d$_6$; Figure S20: The HRESIMS spectrum of euroticin G (2); Figure S21: The UV spectrum of euroticin G (2); Figure S22: The 1H NMR (500 MHz) spectrum of euroticin H (3) in acetone-d$_6$; Figure S23: The 13C NMR (125 MHz) spectrum of euroticin H (3) in acetone-d$_6$; Figure S24: The HSQC (500 MHz) spectrum of euroticin H (3) in acetone-d$_6$; Figure S25: The HMBC (500 MHz) spectrum of euroticin H (3) in acetone-d$_6$; Figure S26: The 1H-1H COSY (500 MHz) spectrum of euroticin H (3) in acetone-d$_6$; Figure S27: The HSQC (700 MHz) spectrum of euroticin H (3); Figure S28: The HRESIMS spectrum of euroticin H (3); Figure S29: The 1H NMR (700 MHz) spectrum of euroticin I (4) in DMSO-d$_6$; Figure S30: The 13C NMR (175 MHz) spectrum of euroticin I (4) in DMSO-d$_6$; Figure S31: The HSQC (700 MHz) spectrum of euroticin I (4) in DMSO-d$_6$; Figure S32: The HMBC (700 MHz) spectrum of euroticin I (4) in DMSO-d$_6$; Figure S33: The 1H-1H COSY (700 MHz) spectrum of euroticin I (4) in DMSO-d$_6$; Figure S34: The HRESIMS spectrum of euroticin I (4); Figure S35: The UV spectrum of euroticin I (4); Figure S36: The 1H NMR (700 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S37: The 13C NMR (175 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S38: The HSQC (700 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S39: The HMBC (700 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S40: The 1H-1H COSY (700 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S41: The ROESY (700 MHz) spectrum of eurotirumin (5) in acetone-d$_6$; Figure S42: The HRESIMS spectrum of eurotirumin (5); Figure S43: The UV spectrum of eurotirumin (5); Table S1: Calculated relative thermal energies (ΔE), relative free energies (ΔG)$^0$, and equilibrium populations (P)$^0$ of low-energy conformers (8R,1'S,2''S)-1, (8R,1'S,2''R)-1, (7S,1'R,2''R,5''R,6''S)-2, (7S,1'R,2''R,5''R,6''R)-2, and (5)-4 in MeOH solution; Table S2: Calculated 13C NMR data for (8R,1'S,2''R)-1 and (8R,1'S,2''S)-1 and their goodness of fit with the measured shifts of 1; Table S3: 1H and 13C NMR Data for 5 in acetone-d$_6$ (700, 175, TMS, δ in ppm, J in Hz).
Author Contributions: W.-M.Z. (Wei-Mao Zhong) performed the isolation, purification, and structural characterization of all the compounds and prepared the manuscript; Y.-C.C. and W.-M.Z. (Wei-Min Zhang) contributed to the bioactivity evaluation; X.-Y.W. performed $^{13}$C NMR and ECD calculations; J.-F.W., X.-F.S. and Q.Z. contributed to the isolation of compounds and revised the manuscript; X.-P.T. contributed to the isolation and identification of the fungal strain; F.-Z.W. and S.Z. designed and supervised the research and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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