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A New Metabolite with a Unique 4-Pyranone−γ-Lactam−1,4-Thiazine Moiety from a Hawaiian-Plant Associated Fungus

Chun-Shun Li,[†] Yuanqing Ding,[‡] Bao-Jun Yang,[†] Gabriella Miklossy,[†] Hong-Quan Yin,[‡] Larry A. Walker,[‡] James Turkson,[†] and Shugeng Cao*[,†,§]

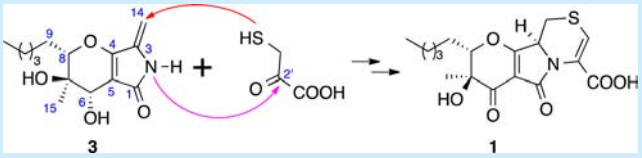
[†]Natural Products & Experimental Therapeutics, University of Hawaii Cancer Center, 701 Ilalo Street, Honolulu, Hawaii 96813, United States

[‡]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, and Division of Pharmacology, Department of BioMolecular Sciences, School of Pharmacy, The University of Mississippi, University, Mississippi 38677, United States

[§]Department of Pharmaceutical Sciences, Daniel K. Inouye College of Pharmacy, University of Hawaii at Hilo, 34 Rainbow Drive, Hilo, Hawaii 96720, United States

Supporting Information

ABSTRACT: An endophytic fungus *Paraphaeosphaeria neglecta* FT462 isolated from the Hawaiian-plant *Lycopodiella cernua* (L.) Pic. Serm produced one unusual compound (**1**, paraphaeosphaeride A) with the 4-pyranone−γ-lactam−1,4-thiazine moiety, along with two new compounds (**2** and **3**, paraphaeosphaerides B and C, respectively) and the known compound (**4**). Compounds **1**−**3** were characterized by NMR and MS spectroscopic analysis. The absolute configuration of the 3-position of compound **1** was determined as *S* by electronic circular dichroism (ECD) calculations. Compound **3** also showed STAT3 inhibition at 10 μM.



Endophytic fungi are rich producers of biologically active secondary metabolites.[1] During our ongoing search for new and bioactive compounds from about 2000 fungal strains collected in Hawaii, about 5000 fungal samples were screened against STAT3,[2] cisplatin-sensitive A2780 (A2780S, cisplatin-sensitive human ovarian cancer cell line), and cisplatin-resistant A2780 (A2780cisR, with constitutively activated STAT3). The results showed that semipure fractions produced by a fungus *Paraphaeosphaeria neglecta* FT462 were active against STAT3, A2780cisR, and A2780S at 20 μg/mL. Our previous investigation of the metabolites from FT462 enabled us to isolate a novel δ-lactone-isochromanone compound.[3] The continuous bioassay guided isolation of this strain led to the isolation of three novel metabolites with a transducer and activator of transcription 3 (STAT3) inhibition activity.

Compound **1**[4] was isolated as a yellowish oil. Its molecular formula was suggested to be $C_{17}H_{21}NO_6S$, according to its HR-ESIMS at *m*/*z* 368.1168 (calcd for $C_{17}H_{21}NO_6S$, 368.1168), with 8 degrees of unsaturation. The IR spectrum showed the existence of hydroxyl (3419 cm^{-1}) and carbonyl (1706 cm^{-1}) groups. The detailed analysis of ^1H and ^{13}C NMR spectra (Table 1) demonstrated the presence of two methyl signals, five methylenes with one (δ_H 3.34/3.56, δ_C 29.8) supposedly connected to an S atom,[5] three methines (one olefinic, one oxygenated, and one nitrogenated), and seven quarternary carbons (three olefinic, three carbonyls). $^1H-^1H$ COSY established an aliphatic spin system C-8−C-13 as shown in Figure 1, which was also verified by the corresponding HMBC correlations. Meanwhile, the observed HMBC correlations from the singlet methyl at δ_H 1.22 to an oxygenated methine (C-8, δ_C 90.1), a quaternary carbon (C-7, δ_C 74.0), and an α,β-unsaturated ketone group at δ_C 192.6 (C-6) implied that the methyl group (C-15) should be located at C-7, which was also connected to a hydroxyl group. The ketone group was then assigned at C-6.

Literature research suggested that the moiety of C-6−C-13 was very similar to that of the STAT3 inhibitor phaeosphaeride A (**4**),[6] which was totally synthesized to determine the absolute configuration.[7] The comprehensive comparison of the NMR data of compounds **1** and **4** implied that both of them should have similar rings A and B (Figure 1) except the hydroxyl group at C-6 in **4** was oxidized into a ketone in **1**. Instead of the terminal double bond in phaeosphaeride A, one nitrogenated methine at δ_C 52.2, one methylene (δ_H 3.34/3.56; δ_C 29.8) supposedly connected to a sulfur, three more carbons including one double bond (δ_C 115.1/126.8), and one carbonyl group were observed in the molecule of **1**. The HMBC (Figure 2) cross-peaks from the CH_2-14 to C-3 and C-4 and from H-3 to C-1 and C-4 implied that the terminal double bond in **4** was hydrogenated in **1**. Meanwhile, the obvious HMBC correlations from the olefinic proton H-1′ (δ_H 6.92) to a quaternary olefinic carbon C-2′ (δ_C 126.8), C-3′ (δ_C 170.6), and the sulfur connected methylene (C-14) implied that the newly present

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Table 1. NMR Spectroscopic Data for 1 in MeOH-d_4

	1	
no.	δ_H, J (Hz)[a]	δ_C[b]
1		164.8
3	5.12 (br s)	52.2
4		173.5
5		102.5
6		192.6
7		74.0
8	4.45 (dd, 10.1, 2.4)	90.1
9	1.89 (m)	28.7
10a	1.50 (m)	26.7
10b	1.72 (m)	
11	1.39 (m)	32.6
12	1.38 (m)	23.5
13	0.93 (t, 6.0)	14.3
14a	3.34 (br d, 18.0)	29.8
14b	3.56 (br d, 18.0)	
15	1.22 (s)	18.5
1′	6.92 (s)	115.1
2′		126.8
3′		170.6

[a]Spectra recorded at 500 MHz. [b]Spectra recorded at 125 MHz. Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments.

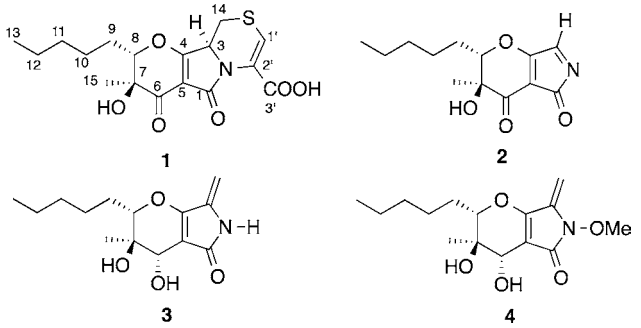

Figure 1. Structures of compounds **1**–**4**.

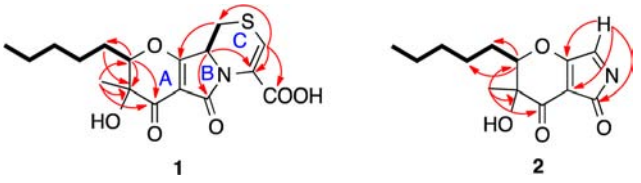

Figure 2. ^{1}H–^{1}H dqfCOSY (Bolds) and selected HMBC (Arrows) correlations of compounds **1** and **2**.

moiety with three carbons must be an α,β-disubstituted acrylic acid, which was connected to the sulfur atom at C-1′. Since the compound has 8 degrees of unsaturation, one more ring C was proposed to be present with the connection between the nitrogen atom and C-2′, which was also verified by the HMBC correlations from H-3 to C-2′. Hence, the planar structure of compound **1** containing an uncommon 2H-1,4-thiazine ring was determined as shown.

The NMR chemical shifts of positions 7–15 of **1** were very similar to those of **4**, and H_2-9 of **1** showed a strong ROESY correlation to H_3-15, indicating that **1** should have the same stereochemistry as compound **4** at the 7- and 8-positions. A comparison of experimental CD and calculated ECD spectra has been employed to assign the absolute configuration of C-3 in compound **1**.[8−11] Both 3*S* and 3*R* configurations of compound **1** were submitted for the conformational search by using the OPLS_2005 force field in MacroModel[12] with an energy window of 130 kJ/mol, yielding 139 and 149 conformers, respectively. Fifty-three conformers of 3*S* configuration within an energy cutoff of 20 kJ/mol were included for geometric optimization, followed by harmonic vibrational frequency analysis at the B3LYP/6-311++G** level in the gas phase. Thirteen conformers (specifically 3*S*1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 18, and 38, Figure S1) were found to respectively occupy 41.1%, 9.2%, 7.8%, 6.7%, 7.1%, 7.5%, 1.7%, 1.6%, 1.8%, 1.5%, 1.9%, 1.5%, and 6.7%, counting 96.1% for total (Table S1). Hydrogen bonds (C7)O–H···O(C6) and (C3′)O–H···O(C1) were found in all of the above predominant conformers, among which the structural difference is the free rotation of the side chain about bonds C-8–C-9, C-9–C-10, C-10–C-11, and C-11–C-12, respectively (Figure S1). The calculated ECDs of individual conformers of compound **1** at the B3LYP-SCRF-(COSMO)/6-311++G**//B3LYP/6-311++G** level in methanol were found very similar (Figure 3), indicating that the free

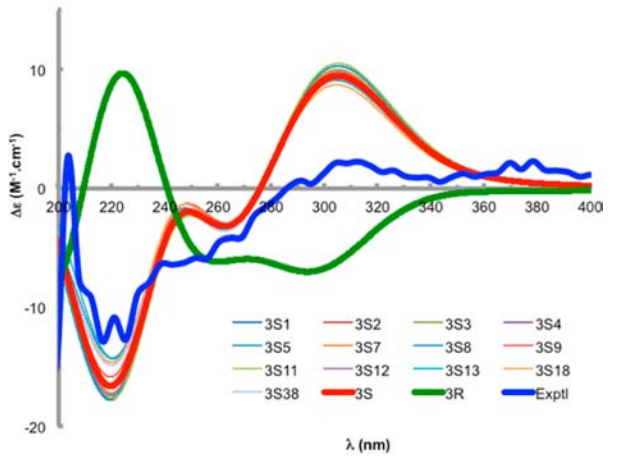

Figure 3. Experimental CD and calculated ECDs of 3*S* and 3*R* configurations of compound **1** (blue, experimental CD in methanol; green, calculated ECD of 3*R* configuration; red and others, weighted ECD and calculated ECD of individual conformers of 3*S* configuration).

rotation of the side chain does not significantly change the ECD spectrum of the 3*S* configuration of compound **1**. Thus, for the 3*R* configuration, only the first conformer found in the conformational search was submitted for geometric optimization, harmonic vibrational frequency computation, and ECD calculation at the above levels. The weighted ECD of the 3*S* configuration of compound **1** coincides with those of its individual conformers very well and well matches the experimentally recorded CD spectrum, whereas that of the 3*R* configuration is opposite in most wavelength regions, specifically at about 210–241 and 286–400 nm (Figure 3). Unambiguously, the absolute configuration at C-3 position was assigned as *S*. Computations at the quantum mechanics levels were performed using the Gaussian 09.[13]

Compound **2**[14] was isolated as a yellowish oil that gave an $[M + H]^+$ ion in the HRESIMS at m/z 252.1239, appropriate for the molecular formula $C_{13}H_{18}NO_4$ (calcd 252.1236). The comprehensive analysis of the NMR data (Table 2) implied that the structure **2** was similar to that of **4**. However, the terminal olefin, the methoxy group, and the oxygenated

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Table 2. NMR Spectroscopic Data for Compounds 2 and 3 in MeOH-d_4

	2		3	
no.	δ_H, J (Hz)[a]	δ_C[b]	δ_H, J (Hz)[a]	δ_C[b]
1		171.7[c]		169.2
3	7.04 (s)	131.2		140.7
4		171.7[c]		157.2
5		104.5		105.4
6		193.1	4.10 (br s)	66.9
7		73.7		72.8
8	4.22 (dd, 10.2. 2.1)	89.0	4.12 (br d, 12.0)	87.9
9a	1.86 (m)	29.1	1.65 (m)	29.2
9b	1.78 (m)		1.90 (m)	
10a	1.68 (m)	26.8	1.42 (m)	27.8
10b	1.46 (m)		1.56 (m)	
11	1.38 (m)	32.6	1.34 (m)	32.6
12	1.37 (m)	23.8	1.34 (m)	23.6
13	0.93 (t, 6.0)	14.4	0.91 (t, 6.0)	14.3
14a			5.04 (br s)	92.5
14b			4.89 (br s)	
15	1.19 (s)	18.9	1.29 (s)	19.7

[a]Spectra recorded at 500 MHz. [b]Spectra recorded at 125 MHz. Data based on ^{1}H, ^{13}C, HSQC, and HMBC experiments. [c]Overlapped.

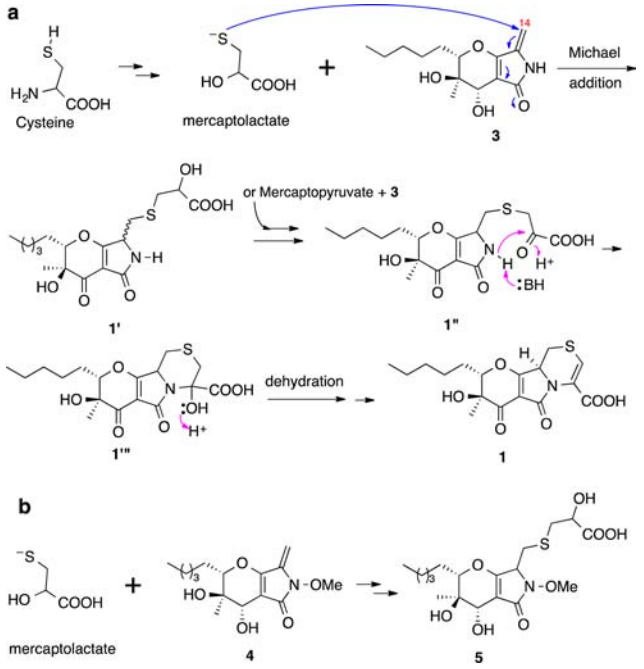
methine at δ_C 64.1 in **4** were absent in compound **2**. The observation of one ketone group at δ_C 193.1 and the HMBC correlations from the protons of the singlet methyl (C-15) to the ketone implied that the hydroxyl at C-6 in **4** was oxidized into a ketone in **2**. HMBC correlations from the new aromatic proton at δ_H 7.04 to C-1 and C-5 proved the presence of a novel γ-lactam ring. Compound **2** was determined tentatively to have the same configuration as **1** based on the similarity of their NMR data and the same sign of their optical rotations.

Compound **3**[15] was also isolated as a yellowish oil. The HRESIMS showed the quasi-molecular ion peak at m/z 290.1367 (calcd for $C_{14}H_{21}NO_4Na$, 290.1368) implying the molecular formula $C_{14}H_{21}NO_4$, one CH_2O less than that of **4**. The ^{1}H and ^{13}C NMR data (Table 2) of **3** and **4** were very similar except that the methoxy group at N-2 in **4** was absent in **3**, which is also consistent with the observation from the HR-ESIMS. The absolute configuration of compound **3** was determined to be the same as that for **4**.

A biosynthetic origin of compound **1** was proposed as shown in Scheme 1. The precursor of the side chain at the 14-position in compound **1** could be cysteine, which is converted to mercaptolactate readily in microorganisms.[16] Nucleophilic addition of the mercaptolactate thiol to C-14 of compound **3** generates the intermediate **1′**, and **1′** is oxidized to the another intermediate **1″**. It is also plausible that **1″** is generated from mercaptopyruvate and compound **3**. The nitrogen atom of **1″** undergoes intramolecular nucleophilic addition to the ketone of the mercaptopyruvate moiety, leading to the formation of yet another intermediate **1‴**. Dehydration of **1‴** yields the final product **1** (Scheme 1a). We tried to identify intermediates **1′**−**1‴**, but were unsuccessful. However, the intermediate (**5**, Scheme 1b) generated from mercaptolactate and compound **4** was reported in SciFinder, although no reference was cited.

The treatment of MDA-MB-231 breast cancer cells with the new compounds **1**−**3** were conducted at 10 μM. The results showed that **3** inhibited intracellular phospho-Tyrosine STAT3 (pY705STAT3) (Figure 4), suggesting the potential inhibition of aberrant STAT3 activity in the tumor cells. Compound **3**

Scheme 1. Proposed Biosynthetic Pathways for Compounds 1 and 5



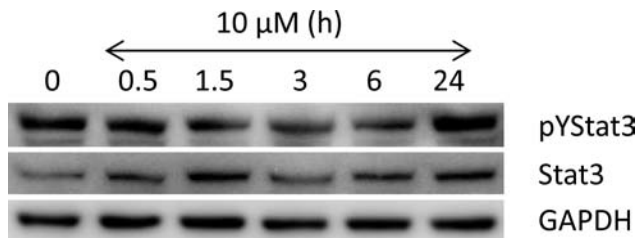


Figure 4. MDA-MB-231 treated with 10 μM of compound **3**.

was also tested as active against A2780 and A2780cisR (cisplatin-resistant A2780) with an IC_{50} of 15.0 and 52.4 μM, respectively, indicating that **3** may have another target in the cancer cells.

There are a few publications about 4-pyranone−γ-lactams or related compounds such as cordylactam from *Cordyceps* sp.,[17] pyranonigrins from *Aspergillus niger*,[18] curvupallides from *Curvularia pallescens*,[19] and phaeosphaerides from *Phaeosphaeria avenaria*,[6] but the tricyclic 4-pyranone−γ-lactam−1,4-thiazine moiety as shown in paraphaeosphaeride A (**1**) has not been reported in natural products before. The discovery of paraphaeosphaeride A (**1**) might provide a new target for synthetic chemists, and its uncommon structure skeleton is also worthy of further biosynthetic investigation.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectroscopic data for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01650.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: scao@cc.hawaii.edu.

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Notes

The authors declare no competing financial interest.

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(4) Paraphaeosphaeride A (**1**): yellowish oil; $[\alpha]_{25}^{D}$ −171.4 (*c* = 0.53, MeOH). UV (MeOH) λ_{max} 235, 365 nm; IR (film) ν_{max} 3419, 2954, 2929, 2859, 1706, 1594, 1484, 1385, 1352, 1278, 1245, 1192 cm^{-1}; ^{1}H (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) data, see Table 1; HR-ESIMS *m*/*z* 368.1168 $[M + H]^+$ (calcd for $C_{17}H_{22}NO_6S$, 368.1168).

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(14) Paraphaeosphaeride B (**2**): yellowish oil; $[\alpha]_{25}^{D}$ −53.8 (*c* = 0.36, MeOH). UV (MeOH) λ_{max} 217, 243, 300 (sh) nm; IR (film) ν_{max} 3397, 3198, 1709, 1674, 1634, 1599, 1531, 1512, 1436, 1311, 1184, 1115, 1064 cm^{-1}; ^{1}H (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) data, see Table 2; HR-ESIMS *m*/*z* 252.1239 $[M + H]^+$ (calcd for $C_{13}H_{18}NO_4$, 252.1236).

(15) Paraphaeosphaeride C (**3**): yellowish oil; $[\alpha]_{25}^{D}$ −128.6 (*c* = 0.14, MeOH). UV (MeOH) λ_{max} 200, 262 nm; IR ν_{max} 3396, 2959, 2927, 2856, 1591, 1532, 1261, 1086, 1022 cm^{-1}; ^{1}H (methanol-d_4, 500 MHz) and ^{13}C NMR (methanol-d_4, 125 MHz) data, see Table 2; HR-ESIMS *m*/*z* 290.1367 $[M + Na]^+$ (calcd for $C_{14}H_{21}NO_4Na$, 290.1368).

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