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Chrysamides A–C, Three Dimeric Nitrophenyl *trans*-Epoxyamides Produced by the Deep-Sea-Derived Fungus *Penicillium chrysogenum* SCSIO41001

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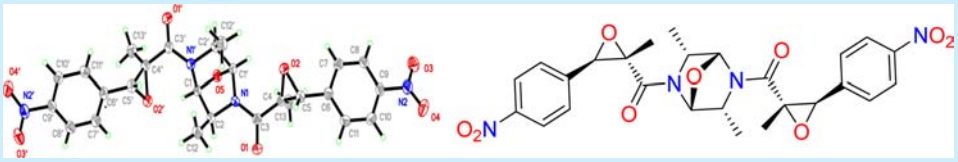
Supporting Information



ABSTRACT: Three dimeric nitrophenyl *trans*-epoxyamides, chrysamides A–C (**1**–**3**), were obtained from the deep-sea-derived fungus *Penicillium chrysogenum* SCSIO41001. Their structures were characterized by spectroscopic analysis, electronic circular dichroism computations, and X-ray single-crystal diffraction analysis. Notably, compound **1** possesses a novel centrosymmetric dimer skeleton featuring an unprecedented 7-oxa-2,5-diazabicyclo[2.2.1]heptane ring system, which represents the first example of dimeric nitrophenyl *trans*-epoxyamide in nature. Compound **3** suppresses the production of proinflammatory cytokine interleukin-17. A possible biosynthetic pathway of **1**–**3** was proposed.

Naturally occurring nitro compounds are relatively rare in nature, only about 200 nitro compounds have been isolated from plants, fungi, bacteria, and mammals.[1] These compounds display great structural diversity, and they exhibit a wide range of biological activities, including antibacterial,[2] antifouling,[3] cytotoxic,[4] nematicidal,[5] antimicrobial,[6] and kinase inhibitory activities.[7] So far, only five nitrobenzoyl sesquiterpenoids have been isolated from marine fungi *Aspergillus* species,[4,8−10] and two nitro alkaloids were isolated from marine fungus *Penicillium citrinum*.[11] Bioactive metabolites with a dimeric skeleton, an unusual class of natural products, possess an intriguing pharmacological potential.[12−16] The pharmacological importance of these metabolites grabbed the attention of scientists to isolate and synthesize these types of compounds. The dimeric chemical structures make better understanding for the biosynthetic mechanism and to synthesize these compounds for the future prospect.[17]

Marine extremophilic microorganisms tend to produce fascinating novel types of bioactive secondary metabolites.[18,19] In order to discover the biologically active and structurally unique natural products from the marine-derived fungi, *Penicillium chrysogenum* SCSIO41001 was isolated from the deep sea sediment of the Indian Ocean. Further chemical investigations of its organic extract led to the isolation of three new dimeric nitrophenyl *trans*-epoxyamides, chrysamides A–C (**1**–**3**) (Figure 1). To the best of our knowledge, compounds **1** and **2** possess an unprecedented centrosymmetric dimer skeleton, while compound **1** features a piperazine ring coupled via an ether bridge forming a novel 7-oxa-2,5-diazabicyclo[2.2.1]heptane ring system, which was an unusual incidence of dimeric nitrophenyl *trans*-epoxyamide in nature. Compound **3** showed inhibitory

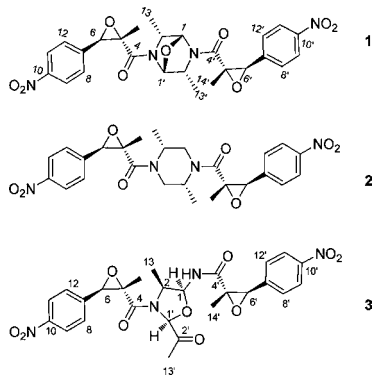


Figure 1. Structures of compounds **1**–**3**.

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−3 (500/125 MHz, $CDCl_3$, TMS, δ, ppm)

	1		2		3	
position	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	90.0, CH	6.31, d (2.5)	44.0, CH_2	a 4.28, overlap, b 3.23, overlap	80.7, CH	6.07,dd (7.0,10.0)
2	57.8, CH	4.15, m	50.4, CH	4.45,brs	54.6, CH	4.55, m
4	170.1, C		169.5, C		169.0, C	
5	62.9, C		64.5, C		64.3, C	
6	60.5, CH	4.12, s	62.8, CH	4.28, s	61.1, CH	4.11, s
7	140.1, C		141.6, C		140.5, C	
8,12	127.2, CH	7.54, d (8.5)	128.2, CH	7.53, d (8.0)	128.1, CH	7.43, d (8.5)
9,11	123.2, CH	8.28, d (8.5)	124.2, CH	8.27, d (8.0)	124.1, CH	8.22, d (8.5)
10	147.6, C		148.5, C		148.6, C	
13	12.9, CH_3	1.36, d (6.5)	16.6, CH_3	1.24, overlap	14.0, CH_3	1.38, d (6.5)
14	13.7, CH_3	1.37, s	15.5, CH_3	1.31, s	13.9, CH_3	1.31, s
1′	90.0, CH	6.31, d (2.5)	44.0, CH_2	a 4.2, overlap, b 3.23, overlap	88.0, CH	5.80, s
2′	57.8, CH	4.15, m	50.4, CH	4.45,brs	204.0, C	
3′					NH	7.13, d (10.0)
4′	170.1, C		169.5, C		170.6, C	
5′	62.9, C		64.5, C		63.0, C	
6′	60.5, CH	4.12, s	62.8, CH	4.28, s	62.6, CH	3.99, s
7′	140.1, C		141.6, C		140.7, C	
8′12′	127.2, CH	7.54, d (8.5)	128.2, CH	7.53, d (8.0)	128.1, CH	7.48, d (8.5)
9′11′	123.2, CH	8.28, d (8.5)	124.2, CH	8.27, d (8.0)	124.2, CH	8.25, d (8.5)
10′	147.6, C		148.5, C		148.5, C	
13′	12.9, CH_3	1.36, d (6.5)	16.6, CH_3	1.24, overlap	27.3, CH_3	2.41, s
14′	13.7, CH_3	1.37, s	15.5, CH_3	1.31, s	12.5, CH_3	1.29, s

effect on the production of proinflammatory cytokine IL-17. Herein, the isolation, structural elucidation, and bioactivity of compounds **1**−**3** are reported.

Compound **1** was isolated as colorless crystals ($CHCl_3$). The molecular formula was established to be $C_{26}H_{26}N_4O_9$ on the basis of the HRESIMS peak at m/z 573.1400 $[M + Cl]^-$, with 16 unsaturation equivalents. The IR spectrum showed absorptions at 1662, 1602, 1516, and 1458 cm^{-1}, which supported the presence of carbonyl functionalities and aromatic ring system. The ^{1}H NMR spectrum (Table 1) of **1** illustrated resonances for four aromatic methines (δ_H 7.54, 2H, d, J = 8.5 Hz, H-8/12; 8.28, 2H, d, J = 8.5 Hz, H-9/11), three oxygenated or nitrogenous methines (δ_H 4.12, 1H, s, H-6; 6.31, 1H, d, J = 2.5 Hz, H-1; 4.15, 1H, m, H-2), and two methyl groups (δ_H 1.36, 3H, d, J = 6.5 Hz, H-13; 1.37, 3H, s, H-14). The ^{13}C NMR (Table 1) and DEPT spectra showed 13 carbon resonances including one carbonyl carbon (δ_C 170.1, C-4), two aromatic quaternary carbons (δ_C 147.6, C-10 and 140.1, C-7), four aromatic methine carbons (δ_C 127.2, C-8/12 and 123.2, C-9/11), one oxygenated quaternary carbon (δ_C 62.9, C-5), three oxygenated or nitrogenous methine carbons (δ_C 90.0, C-1; 57.8, C-2; 60.5, C-6), and two methyl groups (δ_C 13.7, C-14 and 12.9, C-13). In consideration of the ^{1}H and ^{13}C NMR data, combined with the molecular formula information, compound **1** was proposed as a symmetrical framework.

The planar structure of compound **1** was established by comprehensive analysis of 1D- and 2D NMR spectra. The COSY (Figure 2) correlations from H-8 (δ_H 7.54) to H-9 (δ_H 8.28), H-11 to H-12 and the HMBC correlations from H-8/H-12 to C-10 (δ_C 147.6), H-9/H-11 to C-7 (δ_C 140.1)/C-10 (δ_C 147.6) indicated the presence of a typical *p*-nitrophenyl moiety.[20] Subsequently, the chemical shifts of C-5 (δ_C 62.9) and C-6 (δ_C 60.5), along with the HMBC correlations from H_3-14 to C-4/C-5/C-6 and from H-6 to C-4/C-5, suggested a planar 2-methyl-2,3-epoxyamide moiety,[21] which was confirmed to be connected to C-7 of the *p*-nitrophenyl supported by the HMBC correlation from

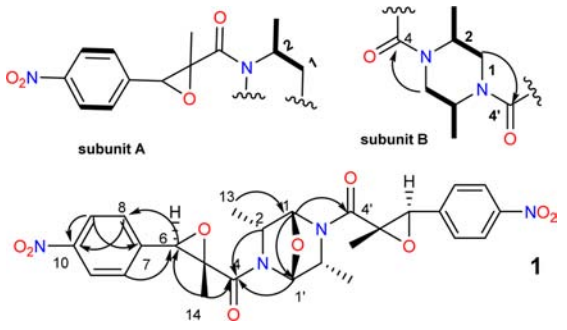

Figure 2. Key $^{1}H-^{1}H$ COSY (bold) and HMBC (arrows) correlations of **1**.

H-8/H-12 to C-6 and H-6 to C-7/C-8/C-12. Inn the $^{1}H-^{1}H$ COSY spectrum, correlations ranging from the methyl group H_3-13 to H-1 through H-2 revealed the existent of an isopropyl fragment, and this fragment was then found to be attached to N-3 on the basis of the HMBC correlation from H-2 to C-4. In view of the above evidence, the structure of subunit A (Figure 2) was established. Furthermore, taking the molecular formula and the chemical shifts of C-2/2′ (δ_C 57.8) into consideration, in conjunction with the key HMBC correlations between H-1′ and C-4 and H-1 and C-4′, two subunit A moieties were connected via two C−N bonds to form a symmetrical dimer skeleton. Thus, the presence of subunit B (Figure 2) can also be confirmed. Finally, combined with the downfield shifts of C-1/1′ (δ_C 90.0), the molecular formula, and the degrees of unsaturation, the remaining oxygen atom was further affirmed assign to bridge C-1 and C-1′ to form a novel 7-oxa-2,5-diazabicyclo[2.2.1]-heptane ring system, according to the key HMBC correlations between H-1 and C-1′/H-1′ and C-1.

Key NOESY interactions observed between H-1,1′/H_3-13,13′ indicated that H-1/1′ and H_3-13/13′ were located at the same

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side of the ring and assigned to be α-oriented. Additional NOESY correlations of H_3-14,14′/H-8,8′ suggested H_3-14,14′/H-6,6′ were on a difference face of the oxirane ring (Supporting Information). Finally, the structure was confirmed by X-ray single-crystal diffraction analysis (Figure 3), and the final

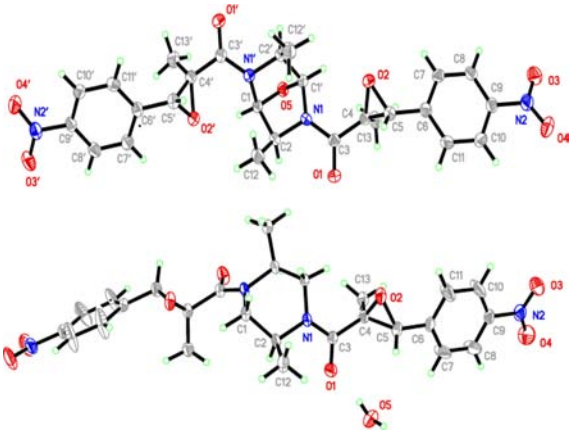

Figure 3. X-ray structures of **1** and **2**.

refinement on the Cu Kα data had a good Flack parameter 0.06 (5),[22] which unambiguously determined the absolute stereochemistry to be 1*R*,1′*R*,2*R*,2′*R*,5*S*,5′*S*,6*R*,6′*R*. It is noteworthy that compound **1**, featuring a novel centrosymmetric dimer skeleton, represents the first example of nitrophenyl *trans*-epoxyamide dimer in nature.

The molecular formula of compound **2** was determined as $C_{26}H_{28}N_4O_8$ on the basis of the HRESIMS peak at *m*/*z* 547.1801 $[M + Na]^+$, implying 14 amu less than compound **1** and 15 degrees of unsaturation. The UV spectrum of **2** was similar to that of **1**, indicating that two compounds shared the same skeleton. However, comparison of the 1D NMR data of compounds **1** and **2** (Table 1) revealed the differences occurred at the piperazine ring. Two methylene signals at δ_C 44.0 for C-1/1′ were observed in **2** instead of the two oxygenated methines between C-2/2′ and N-3/3′ in **1** because of the absence of the oxygen bridge between C-1 and C-1′. The deduction was further supported by the COSY correlations from H_a-1, H_b-1 to H-2 and consistent with the molecular formula and the degrees of unsaturation. The relative configurations of C-5, C-5′, C-6, and C-6′ were established to be the same as those of **1** in light of NOESY experiments (Supporting Information). However, the NOESY spectrum did not provide sufficient information to determine the configuration of C-2/C-2′. To our delight, compound **2** was crystallized from methanol to obtain white crystals. Subsequently, compound **2** was subjected to the X-ray diffraction experiment. The result gave the absolute configuration (Flack parameter −0.01 (7)) as 2*R*,2′*R*,5*S*,5′*S*,6*R*,6′*R* (Figure 3). Finally, the structure of **2** was established as depicted in Figure 1.

Compound **3** was obtained as yellowish powder. The molecular formula was determined to be $C_{26}H_{26}N_4O_{10}$ on the basis of the HRESIMS peak at *m*/*z* 577.1544 $[M + Na]^+$, with one oxygen atom more than **1**. The UV spectrum of **3** was similar to that of **1**, suggesting that two compounds shared the same chromophores. By comparison of the ^1H and ^{13}C NMR data (Table 1), **3** was found to similar to **1**. However, the most obvious deviation was that the high-field shift effect of C-13′ (+14.4 ppm) and a carbonyl group at δ_C 204.0 replaced the C-2′, indicating the presence of an acetyl moiety located at carbon C-1′. The deduction was further supported by the HMBC correlations from H_3-13′ to C-1′, C-2′, from H-1′ to C-2′. An exchangeable proton at δ_H 7.13 in the ^1H NMR spectrum was attributed to amide proton (NH), supported by the coupling between H-1 and 3′-NH ($J_{H\text{-}1,\ 3'\text{-}NH}$ = 10.0 Hz) and the COSY correlation from H-1 to 3′-NH.

In the NOESY spectrum of **3**, the relative configurations of the epoxypropane moieties were in good agreement with those for **1** and **2**. The absolute configurations of the epoxypropanes were believed to be the same as those of **1** and **2** in a biogenetic view. The cross-peak observed between H_3-13 and NH-3′ and between H-2 and H-1′ in the NOESY spectrum revealed that H-1, H-1′, and H-2 were on the same face of the oxazolidine ring and tentatively assigned as α-oriented (Supporting Information). Subsequently, determination of the absolute configurations of the oxazolidine ring moiety investigation was carried out by theoretical computations. Two feasible stereoisomers at C-1, C-1′, and C-2 of the 5*S*,5′*S*,6*R*,6′*R* structure were built and separately subjected to the MMFF conformational search followed by geometry optimization using the density functional theory (DFT) method. The resulting low energy conformers (see the Supporting Information) were applied to TDDFT calculations to reproduce the ECD spectra. Finally, the absolute configurations of the oxazolidine ring moiety were determined as 1*S*,1*S*′,2*S* by the comparison of the measured ECD spectrum with the PBE1PBE/TZVP calculated spectra of (1*S*,2*S*,1*S*′)- and (1*R*,2*R*,1*R*′)-**3** in MeOH since the calculated ECD spectra (1*S*,2*S*,1*S*′) of **3** fit well with the experimental spectrum (Figure 4). Thus, the complete structure of **3** was established and depicted in Figure 1.

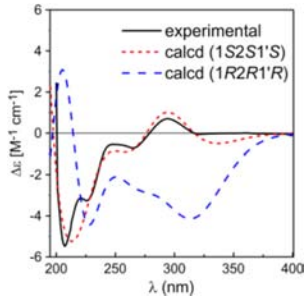

Figure 4. Comparison of the measured CD spectrum with the PBE1PBE/TZVP calculated spectra of (1*S*,2*S*,1′*S*)- and (1*R*,2*R*,1′*R*)-**3** in MeOH.

Structurally, compounds **1** and **2** demonstrate a unique symmetrical skeleton, and compound **1** represents the first example of dimeric nitrophenyl *trans*-epoxyamide in nature, featuring a piperazine ring conjugate with an oxygen bridge forming a novel 7-oxa-2,5-diazabicyclo[2.2.1]heptane ring system. The biogenetic pathway was proposed in Scheme 1. L-Phenylalanine and D-alanine are the likely biosynthetic precursors of **1**−**3**. L-Phenylalanine underwent a series of subsequent conversions, including deamination, oxidation, methylation, and aryl nitration,[23−25] to generate **a**, which was acylated with D-alanine to produce product **b**. Subsequently, two **b** underwent a condensation reaction to form a dioxopiperazine whose amide carbonyls were then reduced to give the key intermediate **c**. The important intermediate **c** further suffered several transformations to generate compounds **1**−**3**. It was noted that compound **3**, derived from intermediate **c**, underwent a series of subsequent conversions (Scheme 1), which may be the reason that its

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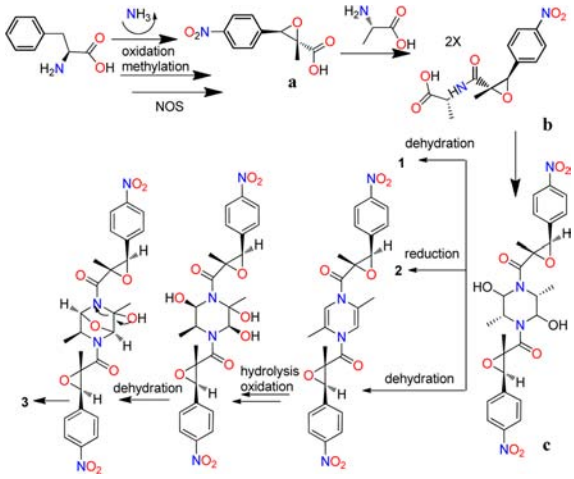
Scheme 1. Plausible Biosynthesis of Compounds 1−3



configuration of C-1, C-2, C-1′ switched to 1*S*,2*S*,1′*S*, the stereoisomer of compound **1**.

The cytotoxicity of **1**−**3** was tested against K562, A549, and HUH7 cancer cell lines using the CCK-8 method, but none of them exhibited activity at 30 μM. Antibacterial activity was screened against three bacteria, *Staphylococcus aureus, Escherichia coli, and Salmonella* sp., but none of them was active. In the antiinflammatory assay, compound **3** exhibited inhibitory effect on the production of proinflammatory cytokine IL-17, the inhibitory rate on the production of IL-17 was 40.06% at 1.0 μM (SR2211 as positive control, inhibitory rate was 62.86% at 1.0 μM), while compounds **1** and **2** did not show any inhibitory effects at 50 μM (see the Supporting Information). In view of the antiinflammatory results for compounds **1**−**3**, the oxazolidine ring in compound **3** which was different from compounds **1** and **2** was considered to contribute antiinflammatory activity. However, the structure−activity relationship of compounds **1**−**3** still needs to be studied further.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications Web site. NMR and HRESIMS spectra of **1**-**3** as well as their biological activities. Computational details of **3**, experimental section, X-ray crystallographic data for **1** and **2**. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01699.

NMR and HRESIMS spectra of **1**−**3** as well as their biological activities; computational details of **3**; experimental procedures; X-ray crystallographic data for **1** and **2** (PDF)

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Notes

The authors declare no competing financial interest.

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