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Cytotoxic indole alkaloids and polyketides produced by a marine-derived fungus *Aspergillus flavipes* DS720

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Marine-derived microorganisms possess the unique metabolic pathways to produce structurally novel secondary metabolites with potent biological activities. In this study, bioactivity-guided isolation of the marine deep-sea-derived fungus *Aspergillus flavipes* DS720 led to the characterization of four indole alkaloids (compounds **1–4**) and four polyketides (compounds **5–8**), such as two new indoles, flavonoids A (**1**) and B (**2**) with a C-6 reversed prenylation, and a new azaphilone, flaviazaphilone A (**5**). Their chemical structures were unambiguously established by an extensive interpretation of spectroscopic data, such as 1D/2D NMR and HRESIMS data. The absolute configurations of the new compound **5** were solved by comparing the experimental and calculated Electronic Circular Dichroism (ECD) spectra. Since sufficient amount of flavonoids A (**1**) was obtained, **1** was subjected to a large-scale cytotoxic activity screening against 20 different human tumor cell lines. The results revealed that **1** showed broad-spectrum cytotoxicities against HeLa, 5637, CAL-62, PATU8988T, A-375, and A-673 cell lines, with the inhibition rates of more than 90%. This study indicated that the newly discovered indole alkaloid **1** may possess certain potential for the development of lead compounds in the future.

KEYWORDS

indole alkaloids, polyketides, marine fungus, *Aspergillus flavipes*, cytotoxic activity

Introduction

Marine-derived microorganisms are widely distributed in the marine ecosystem. Marine microorganisms are subjected to various extreme environmental stresses, and therefore, they have evolved unique metabolic pathways to synthesize structurally novel secondary metabolites with potent biological activities (Jiang et al., 2020). Marine microorganisms are one of the most notable and prolific sources of bioactive natural products (Carroll et al., 2021). Although a large number of natural products have been discovered from marine microorganisms (Rateb and Ebel, 2011; Zhang et al., 2020), it is a matter of fact that, the trend toward finding new natural products is

approaching saturation due to the redundancy of the isolation and characterization of microorganisms. Therefore, the discovery of new compounds from unexplored environments has proven to be an alternative strategy to search for microbial novelty. Extremophiles, which were isolated from the deep-sea, hydrothermal vents, cold water, and polar region, are largely unexplored (Soldatou and Baker, 2017). These microorganisms are extraordinarily adapted and metabolically active under extreme environmental conditions, which promote them to produce abundant novel secondary metabolites (Obulisamy and Mehariya, 2021).

Marine-derived fungi belonging to the genus *Aspergillus* have been widely studied for their biosynthetic potential for generating bioactive secondary metabolites, such as diverse polyketides (macrolides, phenols, quinines, and lactones), heterocyclic alkaloids, terpenoids, steroids, and other miscellaneous compounds (Xu et al., 2020). In our ongoing research on bioactive secondary metabolites from the deep-sea-derived fungi, an *Aspergillus flavipes* DS720 (Figure 1) was isolated from a deep seawater sample, which was collected from the Mariana Trench at a depth of 2,000 m. Preliminary cytotoxic screening indicated that the extracts of this fungal strain possessed considerable inhibitory effects on various human tumor cell lines. Especially, the extracts showed strong activities against HeLa, PATU8988T, A-375, and A-673 cell lines at the concentration of 40 mg/ml, with inhibition rates of 75, 82, 83, and 86%, respectively. Based on prescreening results, a large fermentation was performed. Subsequent chromatographic purification of the ethyl acetate extracts yielded eight compounds, such as four indole alkaloids (compounds 1–4) and four polyketides (compounds 5–8) (Figure 2). Among them, flavonoids A (1) and B (2) with a C-6 reversed prenylation, and an azaphilone, flavia azaphilone A (5), are new compounds. Since a sufficient amount of flavonoids A (1) was obtained (45 mg/20 g, pure compound/crude extract), a large-scale cytotoxic activity screening of 1 against 20 different human tumor cell lines was performed. Interestingly, 1 showed broad-spectrum cytotoxicities against HeLa, 5637, CAL-62, PATU8988T, A-375, and A-673 cell lines, with the inhibition rates of more than 90%. In this study, the isolation, structural elucidation, and cytotoxic activities of the new compound 1 are discussed herein.

Materials and methods

General

Specific rotation values were recorded on a JASCO P-1020 digital polarimeter (Tokyo, Japan). UV spectra were obtained with a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer, Waltham, United States). Scientific LTQ Orbitrap XL spectrometer (Thermo Scientific, Waltham) was applied to

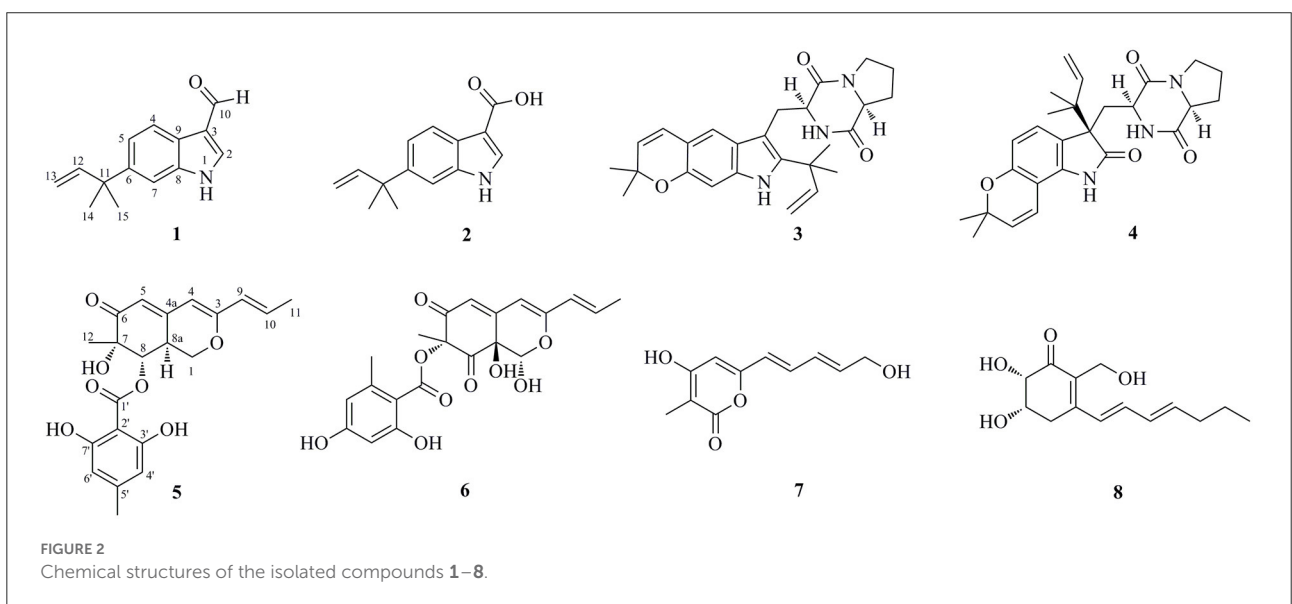
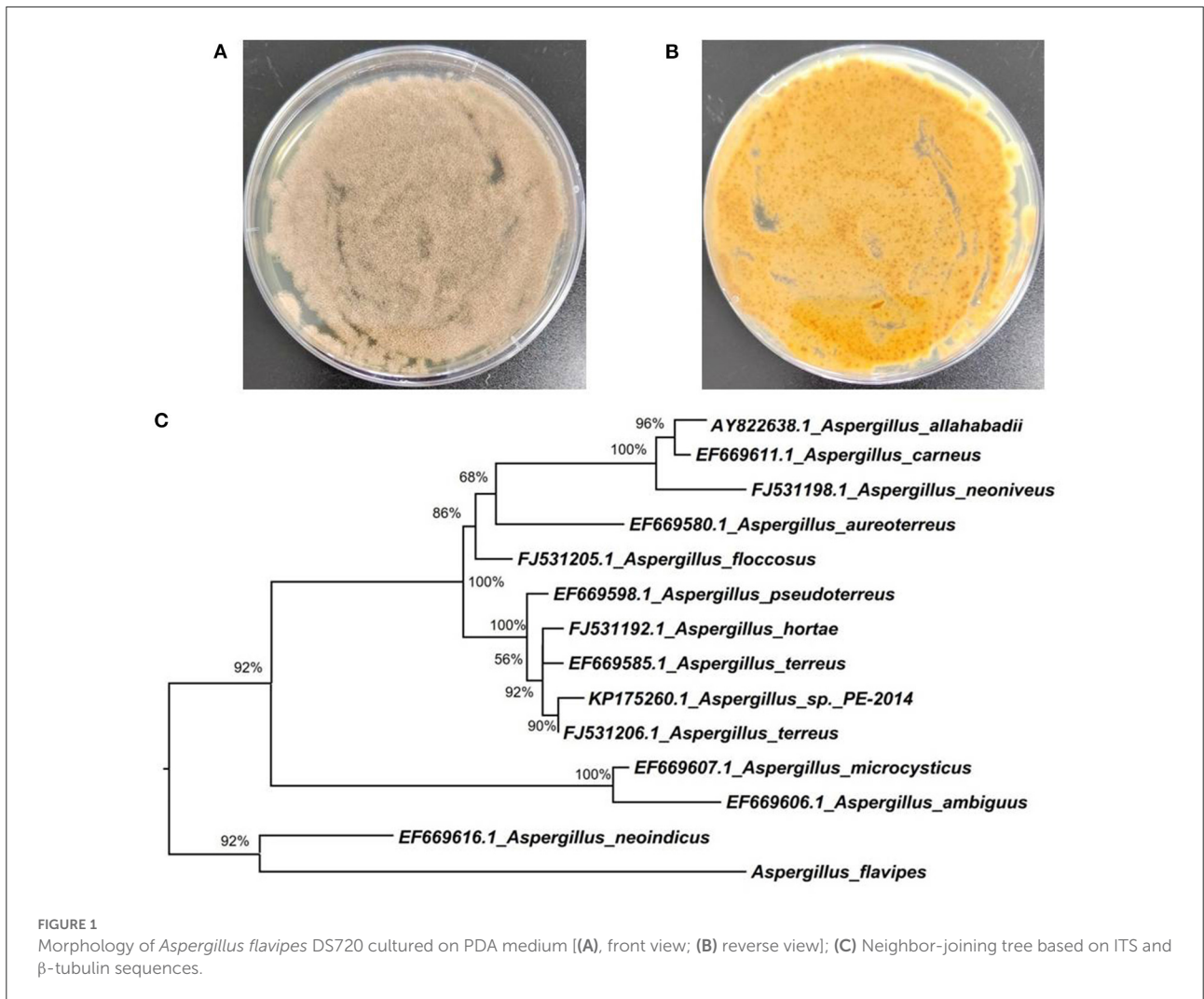
measure the mass spectra of the new compounds. The 1D and 2D NMR spectra were measured with an Agilent DD2 spectrometer (Agilent Technologies, Santa Clara, United States, 500 MHz for ^1H and 125 MHz for ^{13}C). Chemical shifts (δ) were referenced to DMSO- d_6 at 2.50 for ^1H and 39.5 for ^{13}C . Open column chromatography (CC) was performed by silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), octadecylsilyl (ODS) reversed-phase gel (30–50 μm , YMC CO., LTD., Japan), and Sephadex LH-20 (GE Healthcare, United States). All solvents used were of either analytical grade or filtered prior to use.

Fungal material

The fungal strain DS720 used in this study was isolated from a deep seawater sample, which was collected from the Mariana Trench at a depth of 2,000 m (N 11°21.738', E 142°29.307'). This fungus was preliminarily identified as *Aspergillus flavipes* by a standard molecular biological protocol. The sequence analysis of the internal transcribed spacer (ITS) region of the rDNA shared a 99% match to *A. flavipes* NRRL 5175 (Accession No. EF661428) in the BLASTn search. Then, sequences of the ITS (GenBank Accession No. ON340751) and β -tubulin genes from *Aspergillus* species were aligned by MEGA version 6.0 and manually improved when necessary. Subsequently, the phylogenetic tree of the combined dataset was made on the basis of maximum-likelihood (ML) analysis with MEGA version 6.0 with 1,000-generation bootstrap values, for which a value $\geq 50\%$ was considered significantly (Figure 1). A voucher strain of this fungus was deposited at School of Life Sciences, Nanjing University.

Cultivation and extraction

The cultivation of the fungal strain DS720 was performed in 1 L Erlenmeyer flasks containing commercially available PDB medium (potato dextrose broth, Solarbio Life Sciences CO., LTD., Beijing, China). The mycelium from each culture plate was inoculated in a 500 ml Erlenmeyer flask, which was filled with 200 ml of PDB medium supplemented with 3% sea salt. Then, the flask culture was subjected to a rotary shaker at 200 rpm as seed cultures. Following cultivation for 5 days, the seed cultures were transferred into autoclaved 1 L Erlenmeyer flasks with PDB medium. The fermentation process was carried out under static conditions and daylight for 30 days. After the fermentation, the cultures (~ 30 L) were filtered to separate the broth and mycelia layer. The broth was extracted adequately with EtOAc for three times, while the mycelia were crushed and extracted with EtOAc. The combined EtOAc extracts were evaporated under reduced pressure to yield 20 g of a crude gum.



Isolation and purification

The obtained crude gum was subjected to open silica gel CC with a stepwise mixed CH₂Cl₂/MeOH solvent system with the ratios of 100:1, 50:1, 25:1, 10:1, 5:1, and 1:1 (v/v) to yield six fractions (Fr.1–6). Fr.2, eluted with CH₂Cl₂/MeOH 50:1, was fractionated by ODS reversed-phase gel column with a stepwise solvent system of MeOH/H₂O (from 20 to 90%) to give subfractions Fr.2.1–2.6. The Fr.2.2 was purified by semi-preparative HPLC eluting with 50% MeOH–H₂O to obtain compound **3** (*t*_R 12.6 min; 2.1 mg), while Fr.2.4 was applied to a Sephadex LH-20 (MeOH) to give compound **4** (1.8 mg). Fr.3, eluted with CH₂Cl₂/MeOH 25:1, was afforded to silica gel CC (CH₂Cl₂/MeOH, from 30:1 to 5:1) to give two subfractions, Fr.3.1 and Fr.3.2. Compound **1** (*t*_R 16.0 min; 45 mg) was isolated from Fr.3.1 as the main ingredient components by semi-preparative HPLC (60% MeOH–H₂O). Purification of Fr.3.2 by semi-preparative HPLC using 55% MeOH–H₂O obtained compound **2** (*t*_R 10.5 min; 3.2 mg). Fr.4 (eluted with CH₂Cl₂/MeOH 10:1) and Fr.5 (eluted with CH₂Cl₂/MeOH 5:1) were combined and then fractionated with silica gel CC (CH₂Cl₂/MeOH, from 20:1 to 1:1) to obtain three subfractions Fr.4.1–4.3. Compound **7** (2.6 mg) was isolated from Fr.4.1 by preparative TLC (pTLC) eluting with MeOH/H₂O 20:1, whereas compound **8** (2.0 mg) was obtained from Fr.4.2 by pTLC eluting with CH₂Cl₂/MeOH/acetic acid 20:1:0.5. Fr.4.3 was subjected to semi-preparative HPLC (55% MeOH–H₂O) to give compounds **5** (*t*_R 10.0 min; 1.9 mg) and **6** (*t*_R 15.2 min; 1.2 mg).

Flavonoid A [**1**, 6-(2-methylbut-3-en-2-yl)-1*H*-indole-3-carbaldehyde]: colorless oil; UV (MeOH) λ_{max} (log ε) 204 (4.28), 229 (4.24), 292 (3.54) nm; ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 214.1223 [M + H]⁺ (calcd for C₁₄H₁₆NO, 214.1226); *m/z* 236.1044 [M + Na]⁺ (calcd for C₁₄H₁₅NONa, 236.1046).

Flavonoid B [**2**, 6-(2-methylbut-3-en-2-yl)-1*H*-indole-3-carboxylic acid]: colorless oil; UV (MeOH) λ_{max} (log ε) 203 (4.32), 231 (4.22), 296 (3.49) nm; ¹H and ¹³C NMR data, see Table 1; HRESIMS *m/z* 230.1179 [M + H]⁺ (calcd for C₁₄H₁₆NO₂, 230.1176).

Flaviazaphilone A [**5**, (7*S*,8*S*,8*aS*)-7-hydroxy-7-methyl-6-oxo-3-((*E*)-prop-1-en-1-yl)-6,7,8,8*a*-tetrahydro-1*H*-isochromen-8-yl 2,6-dihydroxy-4-methylbenzoate]: colorless oil; [α]_D²⁰ + 72.5 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.36), 268 (3.96), 317 (3.85), 340 (3.84) nm; ¹H and ¹³C NMR data, see Table 2; HRESIMS *m/z* 387.1433 [M + H]⁺ (calcd for C₂₁H₂₃O₇, 387.1438).

Computational section

The conformer rotamer ensemble sampling tool (crest) was utilized to generate candidate conformers and DFT calculations were performed using the Gaussian 16 program (Frisch

et al., 2016). The conformers within an energy window of 10 kcal/mol were optimized at B3LYP/6-31G(d) level of theory with Grimme's D3 dispersion correction ("EmpiricalDispersion = GD3" key words in input files). Frequency analysis of all optimized conformations was undertaken at the same level of theory to ensure they were true local minima on the potential energy surface. Then, energies of all optimized conformations were evaluated by M062X/6-311+G(2d,p) with D3 dispersion correction. Gibbs free energies of each conformer were calculated by adding "Thermal correction to Gibbs Free Energy" obtained by frequency analysis to electronic energies obtained at M062X/6-311+G(2d,p). Room-temperature (298.15 K) equilibrium populations were calculated according to the Boltzmann distribution law. Those conformers accounting for over 2% population were subjected to subsequent calculations. Time-dependent density-functional theory (TDDFT) ECD calculations were run at Cam-B3LYP/TZVP level of theory in MeOH with IEFPCM solvent model, respectively. For each conformer, 30 excited states were calculated. The calculated ECD curves were generated using Multiwfn 3.6 software.

Cytotoxic assay

Cytotoxic activities of the crude extracts and the new compound **1** against 20 different human tumor cells (human lung cancer cells A549, human breast cancer cells MCF7, human gastric carcinoma cells MKN-45, human colon cancer cells HCT 116, human hepatoma cell lines HepG2, human cervical carcinoma cells HeLa, human chronic myelogenous leukemia cells K-562, human brain tumor stem cells SF126, human ovarian teratoma cells PA-1, human renal clear cell adenocarcinoma cells 786-O, human esophageal cancer cells TE-1, human bladder cancer cells 5,637, human prostatic cancer cells DU 145, human thyroid cancer cells CAL-62, human pancreatic cancer cells PATU8988T, human osteosarcoma cells HOS, human malignant melanoma cells A-375, human rhabdomyosarcoma cells A-673, human pharyngeal squamous cells FaDu, and human gallbladder carcinoma cells GBC-SD) were evaluated by the CCK-8 method (Chen et al., 2021). The tested cells were treated with 40 mg/ml of compound samples. A total of 10 μl of 5 g/L CCK-8 solutions were added to each well at 48 h. The cell lines were then incubated at 37°C for 1.5 h. Absorbance data were obtained with a microplate spectrophotometer reader at 490 nm. Commercial doxorubicin was used as the positive control.

Statistical analysis

The data were statistically analyzed using SPSS software (Version 18.0, Chicago, IL, USA) and were expressed as the means ± SD.

TABLE 1 ^1H NMR (500 MHz, δ in ppm) and ^{13}C NMR Data (125 MHz, δ in ppm) for **1** and **2** (measured in DMSO- d_6).

Position	Compound 1		Compound 2	
	δ_{H} (J in Hz)	δ_{C} , type	δ_{H} (J in Hz)	δ_{C} , type
1-NH	11.25, br s	–	10.84, br s	–
2	8.17, s	138.1, CH	7.83, d (2.1)	131.6, CH
3		117.8, C		107.5, C
4	7.18, d (8.5)	120.5, CH	7.09, s	119.4, CH
5	8.05, dd (8.5, 1.8)	119.5, CH	7.96, dd (8.4, 1.9)	119.1, CH
6		132.1, C		131.7, C
7	7.18, br s (overlapped)	122.2, CH	7.10, s	120.9, CH
8		134.2, C		133.5, C
9		125.2, C		127.1, C
10	9.95, s	185.0, CH		166.1, C
11		40.2, C		40.2, C
12	6.15, dd (17.5, 10.6)	146.3, CH	6.12, dd (17.5, 10.6)	146.4, CH
13	5.08, d (10.6) 4.98, d (17.5)	112.4, CH ₂	5.07, d (10.6) 5.00, d (17.5)	112.2, CH ₂
14	1.50, s	27.5, CH ₃	1.48, s	27.4, CH ₃
15	1.50, s	27.5, CH ₃	1.48, s	27.4, CH ₃

Results and discussion

Structural elucidation of the new compounds

Flavonoid A (**1**) was obtained as colorless oil. The molecular formula of **1** was deduced as C₁₄H₁₅NO by the observation of [M + H]⁺ and [M + Na]⁺ ion peaks in the HRESIMS spectrum at *m/z* 214.1223 and 236.1044, respectively. The ^1H NMR spectroscopic data (Table 1) exhibited the presence of one isolated NH signal at δ_{H} 11.25 (1H, br s, 1-NH), two overlapped methyl groups at δ_{H} 1.50 (6H, s, H₃-14, and H₃-15), a set of terminal methylene signal at δ_{H} 5.08 (1H, d, *J* = 10.6 Hz, H-13 α) and 4.98 (1H, d, *J* = 17.5 Hz, H-13 β), six methines such as three aromatic at δ_{H} 7.18 (1H, d, *J* = 8.5 Hz, H-4), 8.05 (1H, dd, *J* = 8.5, 1.8 Hz, H-5), and 7.18 (1H, br s, H-7), two olefinic at δ_{H} 8.17 (1H, s, H-2) and 6.15 (1H, dd, *J* = 17.5, 10.6 Hz, H-12), and one aldehyde group at δ_{H} 9.95 (1H, s, H-10). The ^{13}C NMR combined with DEPT spectra revealed 14 carbon signals, which were classified into one aldehyde carbon at δ_{C} 185.0 (C-10), one terminal methylene group at δ_{C} 112.4 (C-13), five aromatic/olefinic methines, five quaternary carbons, and two methyl groups. Eight characteristic aromatic/olefinic carbons (C-2–C-9) along with the NH signal (1-NH) hinted at the presence of an indole moiety containing a 1,2,5-trisubstituted benzene ring. The key heteronuclear Multiple-Bond Correlation (HMBC) correlations from H₂-13 to C-11 and C-12, from H-12 to C-6, and from H₃-14 to C-12 revealed a *trans*-isopentene group. The definite HMBC correlations from the two *gem*-methyl groups of the isopentene (H₃-14 and H₃-15) to C-6 as

well as correlations from H-5 and H-7 to C-11 indicated that the prenylation was occurred at C-6 of the benzene (Figure 3). Moreover, the aldehyde group was attached to C-3 as evidenced from the HMBC correlation from H-2 to C-10 (Figure 3). Accordingly, the structure of compound **1** was established.

Flavonoid B (**2**) was also obtained as colorless oil and its molecular formula was determined by HRESIMS data. The HRESIMS spectrum of **2** showed a prominent pseudomolecular ion peak at *m/z* 230.1179 [M + H]⁺, which was attributed to the molecular formula of C₁₄H₁₅NO₂. With compound **1** in hand, the structural elucidation of **2** was straightforward. Investigation of the 1D NMR data (Table 1) confirmed that **2** possessed high structural similarity with **1**. Compared with the ^1H and ^{13}C NMR data of **1**, the main difference was that **2** had an additional ester carbonyl at δ_{C} 166.1 (C-10), rather than the aldehyde group in **1**. In addition, the chemical shifts at C-2 (δ_{C} 131.6) and C-3 (δ_{C} 107.5) in **2** were changed significantly. The ester carbonyl group was deduced to be located at C-2 based on the HMBC correlation from H-2 to C-10 (Figure 3). With the aid of detailed analysis of 1D and 2D NMR data, compound **2** was characterized as a new indole with a C-6 reversed prenylation.

Flaviazaphilone A (**5**) was isolated as colorless oil. The HRESIMS of **5** gave a molecular formula of C₂₁H₂₂O₇ (*m/z* 387.1433 [M + H]⁺, calcd for C₂₁H₂₃O₇, 387.1438). The NMR data of **5** (Table 2) indicated the presence of one ketone carbonyl at δ_{C} 195.1 (C-6), one ester carbonyl at δ_{C} 169.2 (C-1'), seven quaternary carbons such as six sp² and one oxygenated sp³ at δ_{C} 73.8 (C-7), six aromatic/olefinic methines at δ_{C} 133.4 (C-10), 126.0 (C-9), 116.7 (C-5), 110.1 (C-6'), 103.4 (C-4), and 100.9 (C-4'), two sp³ methines such as one oxymethine at δ_{C} 75.5

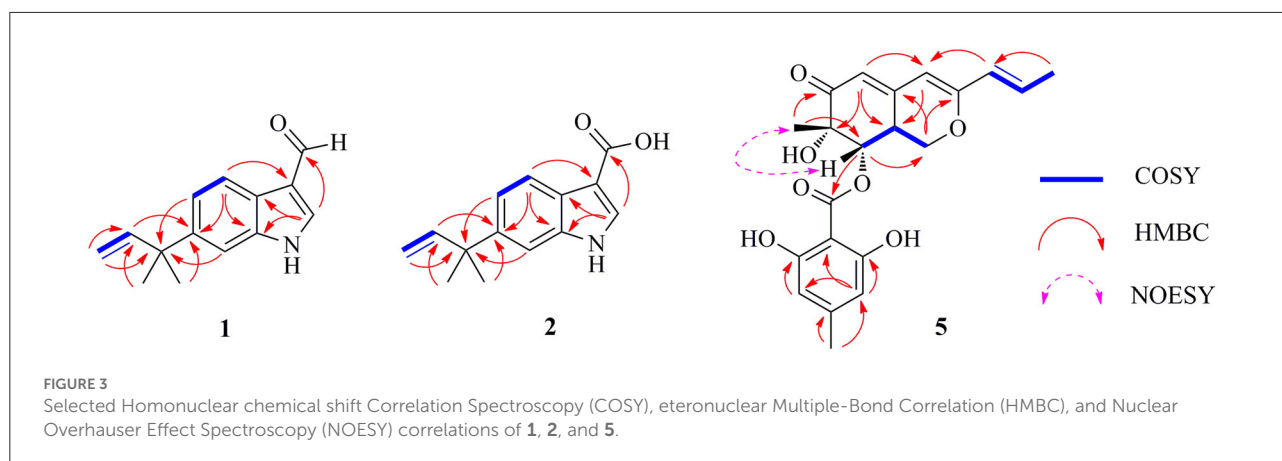
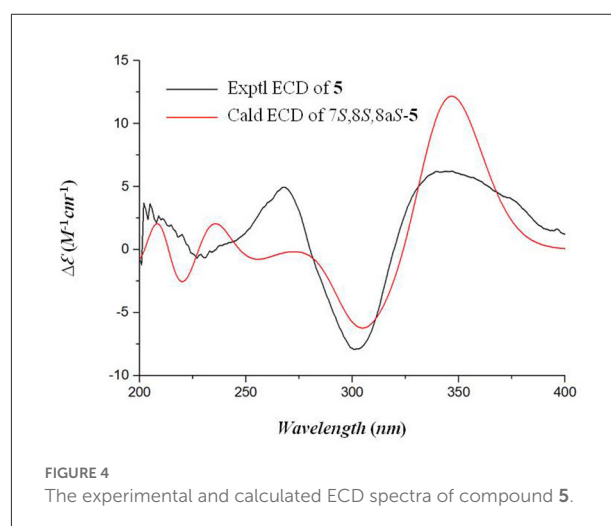
(C-8), one oxygenated methylene at δ_C 68.0 (C-1), and three methyl groups at δ_C 21.8 (C-8'), 19.8 (C-12), and 18.6 (C-11). The ^1H - ^1H COSY correlations indicated the presence of two spin systems, CHO-CH-CH₂O and CH=CH-CH₃ (Figure 3). The mutual HMBC correlations shown in Figure 3 revealed that **5** possessed an azaphilone skeleton. These spectroscopic features suggested the presence of a similar azaphilone skeleton with that of berkazaphilone C, which was previously isolated from an extremophilic fungus *Penicillium rubrum* (Stierle et al., 2012).

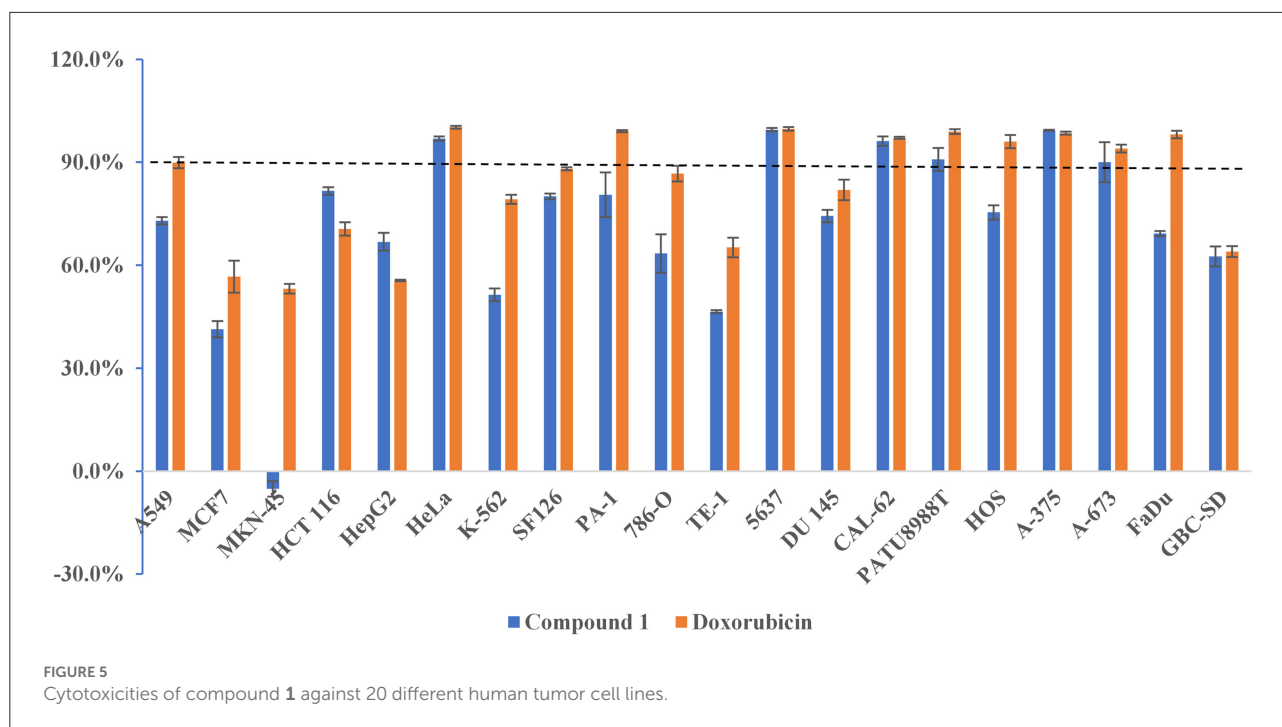
TABLE 2 ^1H NMR (500 MHz, δ in ppm) and ^{13}C NMR Data (125 MHz, δ in ppm) for **5** (measured in DMSO-*d*₆).

Position	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1	4.62, dd (10.6, 5.2)	68.0, CH ₂
3		159.7, C
4	5.74, s	103.4, CH
4a		150.8, C
5	5.68, d (1.5)	116.7, CH
6		195.1, C
7		73.8, C
8	5.02, d (10.0)	75.5, CH
8a	3.18, m	35.1, CH
9	6.00, d (15.9)	126.0, CH
10	6.29, m	133.4, CH
11	1.79, d (7.4)	18.6, CH ₃
12	1.10, s	19.8, CH ₃
1'		169.2, C
2'		109.9, C
3'		161.1, C
4'	6.16, d (2.3)	100.9, CH
5'		140.1, C
6'	6.14 d (2.3)	110.1, CH
7'		159.6, C
8'	2.25, s	21.8, CH ₃

The main differences were the substituents of the benzene ring. Key HMBC correlations from H-8' to C-4' and C-5'; from H-6' to C-7'; from H-4' to C-2' and C-3' constructed the substructure of 1,2,3,5-tetrasubstituted benzene ring.

The relative stereochemistry of **5** was established by interpretation of NOESY spectrum and 3J -coupling data. The NOE correlation between H₃-12 and H-8 indicated a cofacial relationship between H₃-12 and H-8 (Figure 3). In addition, 3J -coupling data showed *ax/ax* interactions between H-8 and H-8a (*J* = 10.0 Hz) (Stierle et al., 2012). Furthermore, the large coupling constant between H-9 and H-10 (*J* = 15.9 Hz) illustrated that the double bond at C-9 and C-10 was in the *E* configuration. To determine the absolute configurations of **5**, ECD computations for B3LYP/6-311+G(d)-optimized conformers were carried out at Cam-B3LYP/TZVP level. The experimental and calculated ECD spectra of **5** exhibited high consistency (Figure 4), and thus finally determined the absolute configurations of **5** as 7*S*, 8*S*, 8a*S*.





In addition to the new compounds 1, 2, and 5, five related known compounds (3, 4, 6–8) were obtained from this fungal strain. Based on comparison of their spectroscopic data with those in the literatures, they were identified as dihydrocarneamide A (3) (Zhang et al., 2015), notoamide C (4) (Kato et al., 2007), purpurquinone A (6) (Wang et al., 2011), saturnispol G (7) (Meng et al., 2018), and palitantin B (8) (Yang et al., 2020), respectively.

Cytotoxic activity

The new compounds 1, 2, and 5 were evaluated to determine their cytotoxic activity against HeLa cell lines. Compound 1 exhibited obvious cytotoxicity with the inhibition rate of (96.94 ± 0.62) % at the concentration of $20 \mu\text{M}$. Since sufficient amount of 1 was obtained (45 mg/20 g, pure compound/crude extract), a large-scale cytotoxic activity screening of 1 against 20 different human tumor cell lines was performed. As a result, 1 showed high and broad-spectrum cytotoxicities against HeLa, 5637, CAL-62, PATU8988T, A-375, and A-673 cell lines, with the inhibition rates of (96.94 ± 0.62) %, (99.49 ± 0.50) %, (96.16 ± 1.34) %, (90.83 ± 3.31) %, (99.32 ± 0.11) %, and (90.01 ± 5.81) %, respectively (Figure 5). In particularly, since thyroid cancer is one of the leading cancers worldwide, chemotherapy is currently needed. Compound 1 showed strong activity against human thyroid cancer cells CAL-62 (96.16%), with an IC_{50} value of $10.4 \mu\text{M}$, indicating that it may possess certain potential for the development of antitumor lead compounds. Moreover, further studies should focus on cytotoxicity assay on normal cell lines

to check the specificity of the cytotoxicity. The safety index for cytotoxicity assay will reveal the true cytotoxic potential of the isolated compounds.

Conclusions

In conclusion, four indole alkaloids (compounds 1–4) and four polyketides (compounds 5–8) were isolated and identified from the deep-sea-derived fungus *Aspergillus flavipes* DS720. Among them, the indoles flavonoids A (1) and B (2) and the azaphilone flaviazaphilone A (5) are new compounds. Flavonoids A (1) and B (2) represent rare examples with a C-6 reversed prenylation. The structures of the new compounds were determined by analysis of NMR data, HRESIMS, and TDDFT ECD calculations. In the screening of cytotoxicities of 1 against 20 different human tumor cell lines, 1 showed high and broad-spectrum cytotoxicity against HeLa, 5637, CAL-62, PATU8988T, A-375, and A-673 cell lines. This study indicated that the deep-sea-derived microbes were considered to be valuable resources for the development of new drugs. Meanwhile, the newly discovered indole alkaloid 1 may be a promising antitumor lead compound.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AX and X-NX: conceptualization and writing—original draft preparation. AX, X-NX, MZ, and C-LL: experiment implementation. LL: data processing. D-YF: writing—review and editing. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships

that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2022.959754/full#supplementary-material>

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