# Cytotoxic and antimicrobial drimane meroterpenoids from a fungus of the *Stictidaceae* (Ostropales, Ascomycota)

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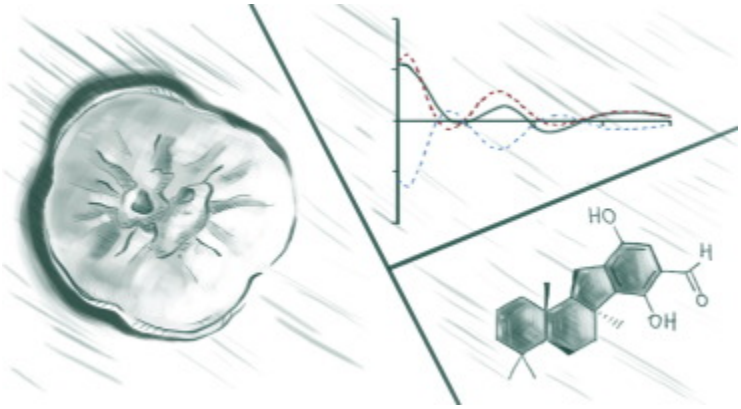
## Abstract:

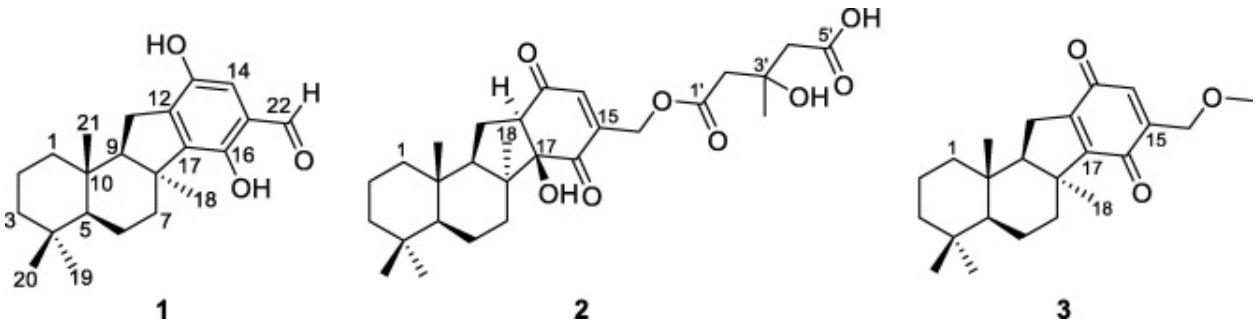
As part of our ongoing research on bioactive fungal metabolites, two new metabolites were isolated from a fungus of the *Stictidaceae* (strain MSX62440), dasyscyphins F and G (**1** and **3**), along with the known dasyscyphin C (**2**). Compound **1** was characterized by HRMS and 1D and 2D NMR data, and its absolute configuration established by ECD spectroscopy. A structural revision of dasyscyphin C (**2**) was based on NMR data and verified by ECD calculations. Compound **3** was previously reported as a synthetic product, and its identity confirmed by comparison with NMR data in the literature, and its absolute configuration was established by ECD spectroscopy. Compounds **1** and **2** showed moderate cytotoxicity and antimicrobial activity.




**Keywords:** Meroterpenoids | Dasyscyphins | *Stictidaceae* sp. | Cytotoxic | Antimicrobial

## Article:

As part of ongoing studies in pursuit of potential drug leads from the Mycosynthetix library of filamentous fungi [1], [2], [3], the organic extract from a fungus of the *Stictidaceae* (strain MSX62440, Fig. S1) displayed moderate activity against the human cancer cell lines MDA-MB-435 (melanoma), MDA-MB-231 (breast) and OVCAR3 (ovarian) (~65% inhibition of cell growth when tested at 20 µg/mL). Bioactivity-directed fractionation using flash chromatography followed by HPLC resulted in the isolation of two natural products, dasyscyphin F and G (**1** and **3**), along with the known dasyscyphin C (**2**) [4] (Fig. 1).



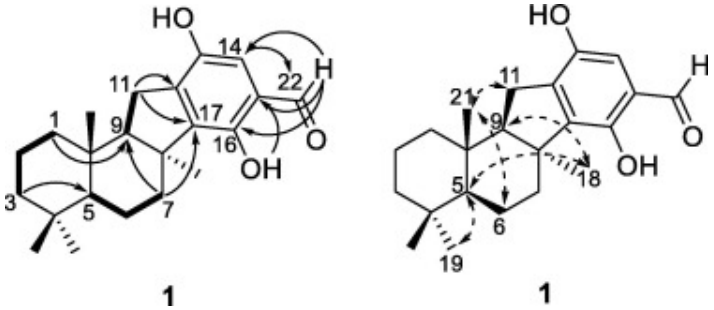

**1** **2** **3**

**Fig. 1.** Compounds isolated from a fungus of the *Stictidaceae* (strain MSX62440).

Compound **1** [5] was isolated as an optically active powder ([α]D20= +27, *c* = 0.1, $CH_3OH$). The molecular formula was established as $C_{22}H_{30}O_3$ by HRESIMS (Fig. S2), indicating an index of hydrogen deficiency of 8. Analysis of the $^1H$, $^{13}C$, and HSQC NMR data (Fig. S3, S4, and Table 1) indicated 22 carbons, consisting of four methyls ($\delta_H/\delta_C$ 0.49/15.7, 0.80/21.9, 1.25/30.4, and 0.89/33.4), six methylenes ($\delta_H/\delta_C$ 0.92 and 1.68/41.3, 1.39 and 1.49/18.5, 1.15 and 1.41/42.1, 1.63 and 1.32/19.6, 1.75 and 2.82/32.8, and 2.73 and 2.90/29.4), three methines ($\delta_H/\delta_C$ 0.98/51.9, 1.75/62.0, and 6.75/116.8), one aldehyde ($\delta_H/\delta_C$ 9.72/195.9), and eight non-protonated carbons ($\delta_C$ 33.4, 37.3, 48.5, 120.1, 140.1, 142.0, 144.6, and 153.0). These data were similar to those reported for dasyscyphin B [4], where the main difference was the lack of a methoxymethyl group attached to the aromatic ring at position C-15 (i.e., $\delta_{H\text{-}22}/\delta_{C\text{-}22}$ 4.60/74.3 and $\delta_H/\delta_C$ 3.42/58.0 as reported [4]), which was replaced by an aldehyde ($\delta_H/\delta_C$ 9.72/195.9), whose position was verified by the HMBC correlations between H-22 with C-14, C-15, and C-16 (Fig. 2). The skeleton for the tricyclic-ring system for the sesquiterpene portion of **1** was confirmed by analysis of the COSY and HMBC experiments (Fig. 2). Specifically, the COSY spectrum (Fig. S5) indicated three isolated spin systems $H_2$-1/$H_2$-2/$H_2$-3, H-5/$H_2$-6/$H_2$-7, and H-9/$H_2$-11 (Fig. 2); key HMBC correlations were used to connect these, particularly H-1 with C-9, H-3 with C-5, H-7 with C-9, H-11 with C-17 and C-12, and H-7 with C-17 (Fig. S6). The relative configuration was assigned through NOESY correlations (Fig. 2 and S7) between $H_3$-21/$H_2$-6/$H_2$-11, $H_3$-18/H-5/H-9, and H-5/$H_3$-19; the relative configuration was identical to that of other dasyscyphin analogues [4], [6]. The absolute configuration of **1** was determined by comparison of the experimental and calculated ECD spectra. TDDFT calculations were used to predict the ECD spectrum for the proposed relative configuration, using the B3LYP/G-31 + G(d,p) level of theory, and the experimental data matched the calculated spectrum for the 5*S*,8*S*,9*R*,10*S* configuration (Fig. 3). Compound **1** was ascribed the trivial name dasyscyphin F.

**Table 1.** NMR data for compound **1** in $CDCl_3$ (400 and 100 MHz for $^1H$ and $^{13}C$, respectively).

| position | $\delta_C$ | type | $\delta_H$, mult (*J* in Hz) |
|---|---|---|---|
| 1 | 41.3 | $CH_2$ | 1.68, m, 0.92, m |
| 2 | 18.5 | $CH_2$ | 1.49, m, 1.39, m |
| 3 | 42.1 | $CH_2$ | 1.15, td (14.2, 13.5, 4.4) 1.41, m |
| 4 | 33.4 | C | |
| 5 | 51.9 | CH | 0.98 dd (11.4, 4.4) |
| 6 | 19.6 | $CH_2$ | 1.63, 1.32m |
| 7 | 32.8 | $CH_2$ | 2.82 dt (13.8, 5.5) 1.75m |
| 8 | 48.5 | C | |
| 9 | 62.0 | CH | 1.75, m |
| 10 | 37.3 | C | |
| 11 | 29.4 | $CH_2$ | 2.90, dd (17.6, 7.9)2.73, d (17.5) |
| 12 | 142.0 | C | |
| 13 | 153.0 | C | |
| 14 | 116.8 | CH | 6.75, s |
| 15 | 120.1 | C | |
| 16 | 144.6 | C | |
| 17 | 140.1 | C | |
| 18 | 30.4 | $CH_3$ | 1.25, s |
| 19 | 33.4 | $CH_3$ | 0.89, s |
| 20 | 21.9 | $CH_3$ | 0.80, s |
| 21 | 15.7 | $CH_3$ | 0.49, s |
| 22 | 195.9 | CH | 9.72, s |
| 16-OH | | | 10.82, s |




**1** **1**

**Fig. 2.** Key COSY (bold lines), HMBC (solid arrows) and NOESY (dashed arrows) correlations of **1**.

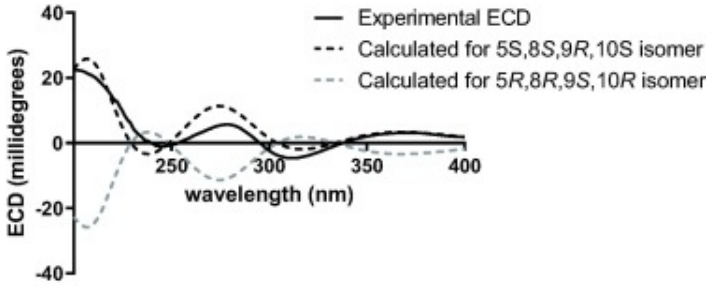
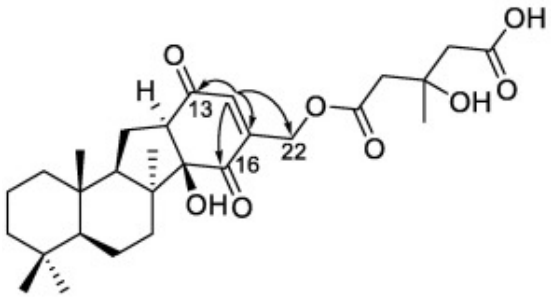



**Fig. 3.** Comparison of the ECD spectra of **1** in $CH_3OH$. Experimental spectrum recorded at 0.2 mg/mL (solid line), and calculated spectra for the two possible enantiomers (dotted and dashed lines).

Compound **2** [7] was isolated as an optically active power ([α]D20 = −28, *c* = 0.1, $CH_3OH$). The HRMS data and the $^{1}H$ and $^{13}C$ NMR spectra (Fig. S9) of **2** matched those reported for dasyscyphin C. In that publication, the structure of **2** corresponded to a meroterpenoid with a 2-cyclohexene-1,2-dione in the fourth ring; however, the $^{13}C$ NMR data did not seem consistent with such an arrangement of the ketones. Indeed, based on the NMR data for several natural products with a 2-cyclohexene-1,2-dione system, the reported chemical shifts for the ketones are typically in the range of 181–192 and 190–197 ppm [8], [9], [10], [11]. On the other hand, in a 2-cyclohexene-1,4-dione system the electron density of the two ketones is more evenly distributed, and thus, the chemical shifts will be more similar to each other, as in the case of the ketone moieties in **2** (i.e., 196.9 and 201.0 ppm for positions 13 and 16) and in many other examples in the literature [9], [12], [13], [14]. Additionally, the HMBC correlations between H-14 with C-13, C-15, C-16, and C-22 supported the 2-cyclohexene-1,4-dione system in **2** (Fig. 4). The ECD spectrum for **2** was used as an orthogonal method to confirm the 2-cyclohexene-1,4-dione moiety. Specifically, the chromophores in **2a** and **2b** (Fig. 5) are not identical, resulting in different UV absorptions, and thus, different Cotton effects. This was demonstrated by calculating the ECD spectra for the two possible molecules, **2a** and **2b**, and comparing those with the measured spectrum. As predicted, the calculated ECD spectra for **2a** and **2b** were not identical. Compound **2a** displayed two negative Cotton effects at 210 and 286 nm and two positive Cotton effects at 234 and 340 nm; **2b** had a positive Cotton effect at 250 nm and a negative Cotton effect at 290 nm. The experimental ECD data matched with the spectrum calculated for **2a**, confirming the 2-cyclohexene-1,4-dione system, serving to refine the structure of dasyscyphin C and establishing its absolute configuration as 5*S*,8*S*,9*R*,10*S*,12*R*,17*R*.




**2**

**Fig. 4.** Key HMBC correlations for compound **2**.

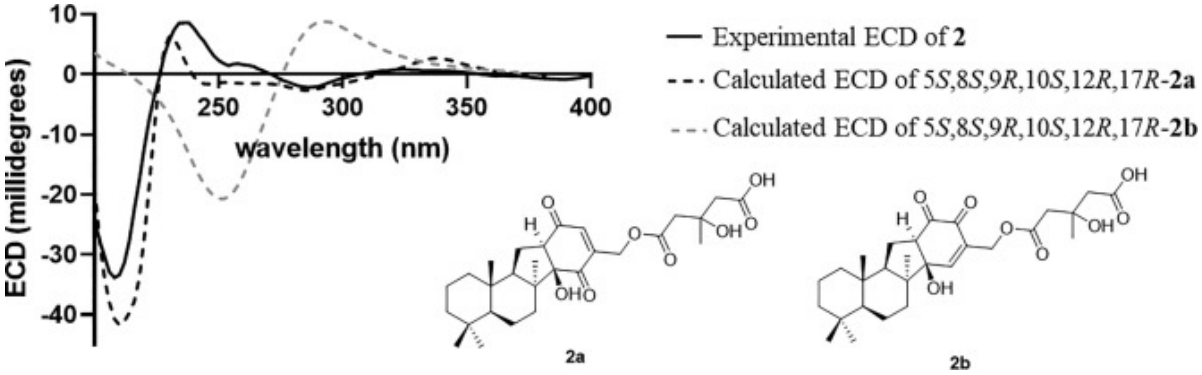



**Fig. 5.** ECD spectrum for **2** and the calculated spectra of **2a** and **2b**.

Compound **3** [15] was isolated as an optically active powder ([α]D20= +13, *c* = 0.1, $CH_3OH$). The molecular formula was established as $C_{23}H_{32}O_3$ based on the protonated molecule observed in the HRESIMS spectrum. Analysis of the $^{1}H$, $^{13}C$ and HSQC NMR spectra (Fig. S13) supported the presence of 23 carbons, including four methyl groups ($\delta_H/\delta_C$ 0.70/16.0, 0.84/21.5, 0.85/32.9, and 1.25/29.8), seven methylene groups ($\delta_H/\delta_C$ 0.90 and 1.59/41.8, 1.13 and 1.41/42.3, 1.34 and 1.66/19.0, 1.37 and 1.37 and 1.50/18.5, 1.80 and 2.17/30.8, 2.60 and 2.73/29.4, and 4.27/68.1), three methine groups ($\delta_H/\delta_C$ 1.00/49.9, 1.64/59.9, and 6.63/131.0), one methoxy ($\delta_H/\delta_C$ 3.44/59.3), and eight non-protonated carbons, including two ketones ($\delta_C$ 33.5, 36.9, 49.6, 146.2, 147.5, 153.6, 186.1, and 187.3); these data were in agreement with the structure of an intermediate in the synthesis of dasyscyphin B [16] (Table S2). The reported relative configuration for **3** is the same as compound **2**, and its ECD spectrum showed the same Cotton effects observed in **2** (Fig. S16). This evidence, along with biogenetic considerations, suggests the absolute configuration for **3** as 5*S*,8*S*,9*R*,10*S*. This is the first-time compound **3** has been isolated as a natural product, and it was ascribed the trivial name dasyscyphin G.

Compounds **1**–**3** were tested against the human cancer cells lines MDA-MB-435 (melanoma), MDA-MB-231 (breast) and OVCAR3 (ovarian). The results showed that **1** and **2** were moderately active with $IC_{50}$ values ranging from 4 to 16 μM against the three cell lines while compound **3** was effectively inactive (Table 2). These data were consistent with the literature on related dasyscyphin analogues, where cytotoxic activities ranged from 1 to 8 μM [17].

**Table 2.** Cytotoxic activity of compounds **1**–**3** against MDA-MB-435 (melanoma), MDA-MB-231 (breast) and OVCAR3 (ovarian) cancer cell lines.

| | **$IC_{50}$ values (μM)** | | |
|---|---|---|---|
| | **MDA-MB-435** | **MDA-MB-231** | **OVCAR3** |
| 1 | 4.1 | 8.2 | 16.2 |
| 2 | 14.1 | 12.2 | 10.4 |
| 3 | 19.7 | >25 | >25 |
| taxol | 0.007 | 0.002 | 0.003 |

**Table 3.** Antimicrobial activities of compounds **1** and **2** against a suite of microorganisms.

| | **Minimal inhibitory concentration (μg/mL)** | | |
|---|---|---|---|
| **Microorganisms** | **1** | **2** | **Erythromycin** |
| *E. coli* | >125 | >125 | 8 |
| *S. aureus* | >125 | >125 | 0.125 |
| MRSA | 63 | 16 | >25 |
| *P. aeruginosa* | 125 | 63 | 32 |
| *M. smegmatis* | >125 | >125 | 125 |
| *B. subtilis* | >125 | >125 | >125 |
| *B. anthracis* | 31 | 2 | 0.25 |
| *S. cerevisiae* | >125 | >125 | >125 |
| *C. albicans* | >125 | >125 | >125 |
| *A. niger* | >125 | >125 | >125 |

Since the dasyscyphins have been studied as antifungal and antibacterial agents [6], [17] we evaluated the antimicrobial activity of **1** and **2** against *Escherichia coli*, *Staphylococcus aureus*,

Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Mycobacterium smegmatis*, *Bacillus* subtilis, *Bacillus anthracis*, *Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus niger*. Compounds **1** and **2** (Table 3) inhibited the growth of *P. aeruginosa,* Methicillin-resistant *Staphylococcus aureus* (MRSA), and *B. anthracis,* and of those, the MIC of **2** against *B. anthracis* (MIC value of 2 μg/mL) was the most potent.

In conclusion, we report the isolation and elucidation of three drimane meroterpenoids (**1**–**3**) from a fungus of the *Stictidaceae*. This includes the description of two natural products, dasyscyphin F (**1**) and G (**3**), and a structural revision of dasyscyphin C (**2**), demonstrating that it has a 2-cyclohexene-1,4-dione ring, instead of a 2-cyclohexene-1,2-dione, as noted from analysis of both NMR chemical shifts and ECD calculated spectra. The structural characterizations of **1**–**3** were established using a combination of conventional spectrometric (HRESIMS) and spectroscopic (NMR) techniques, while their absolute configurations were determined by analysis of measured vs calculated ECD spectra. Moreover, we report that dasyscyphin F and C (**1** and **2**) had moderate cytotoxic activity, and the potent MIC of compound **2** vs *B. anthracis* merits further study.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.152896.

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[5] Dasyscyphin F (1): Yellow powder; = +27 (c = 0.1, CH3OH); UV (CH3OH) λmax (log ε) 254 (3.63); 1H NMR (CDCl3, 400 MHz) and 13C NMR (CDCl3, 100 MHz) see Table 1 and Fig. S2; HRESIMS m/z 341.2125 [M-H]- (calcd. for C22H29O3, m/z 341.2122, 0.8 ppm).

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[15] Dasyscyphin G (3): yellow oil; = +13 (c = 0.1, CH3OH); UV (CH3OH)λmax (log ε) 289 (2.79); 1H NMR (DMSO-d6, 400 MHz) and 13C NMR (DMSO-d6, 100 MHz), see Table S2 and Fig. S13; HRESIMS m/z 357.2416 [M+H]+ (calcd. for C23H33O3, 357.2424, 2.6 ppm)

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