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BRIEF COMMUNICATION

# A new *α*-pyrone from *Arthrinium pseudosinense* culture medium and its estrogenic activity in MCF-7 cells

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## Abstract

A new *α*-pyrone analog, arthrifuranone A (**1**) was isolated from an EtOAc-extract of *Arthrinium pseudosinense* culture medium. The isolation workflow was guided by a Molecular Networking-based dereplication strategy. The chemical structure of the new compound was elucidated using MS and NMR spectroscopic techniques, and the absolute configuration was established by the Mosher's method and gauge-including atomic orbital NMR chemical shift calculations, followed by DP4 + analysis. The isolated compound was evaluated for its estrogenic activity using the MCF-7 estrogen responsive human breast cancer cells. Compound **1** showed estrogenic activity by increasing the proliferation of MCF-7 cells at the concentration of 3.125 μM via phosphorylation of estrogen receptor-*α*.

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The genus *Arthrinium* Kunze (sexual morph: Apiospora) is a fungus belonging to the family *Apiosporaceae* in the *Ascomycota* and is widely distributed in various habitats including terrestrial and marine environments [1, 2]. It has been commonly reported as an endophyte in diverse plants, lichens, and marine algae [2]. *Arthrinium* species have been shown to produce various bioactive metabolites [3], including xanthones [4], pyridine alkaloids [5], and sesterterpenes [6] which possess anti-inflammatory [4], antibacterial [5], and antiviral activities [6].

Menopause is the end of women's menstrual cycles and occurs when the ovaries stop producing estrogen and progesterone in most elderly women [7]. Menopausal women usually experience various symptoms including cardiovascular diseases, cancers, psychiatric disorders, and sexual problems [8]. Hormone replacement therapy (HRT) is used to treat these menopausal symptoms; [9] however, it has been linked to a high risk of various cancers in women including endometrial, breast, and ovarian cancers [10]. Hence, developing an alternative to HRT without increasing the associated risk is required. In particular, isoflavones, coumestans, and stilbenes have been reported previously as an alternative to HRT with estrogenic activities [11], but there has been little investigation into the activities of *α*-pyrone derivatives.

In our ongoing project to discover bioactive metabolites from natural fungal sources [12], *Arthrinium pseudosinense* was studied. The analysis of Molecular Networking of

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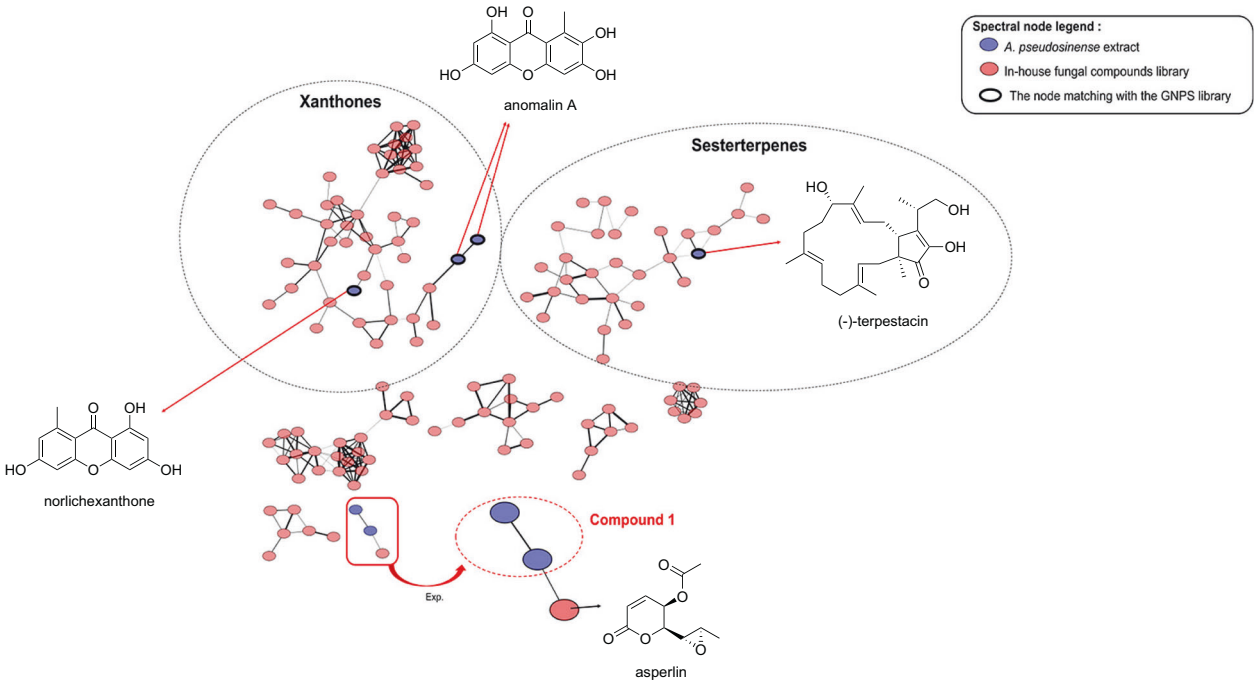


**Fig. 1** Molecular Networking of EtOAc extract of *A. pseudosinense* culture medium

EtOAc-extract of *A. pseudosinense* culture medium led to the isolation of a new α-pyrone derivative (**1**). The chemical structure of the isolated compound was established using spectroscopic and chemical techniques and it was tested for estrogenic and related activities. In this study, we describe the isolation, structural determination, and biological evaluation of the compound.

The MS/MS data of EtOAc-extract of *A. pseudosinense* culture medium were subjected to the Global Natural Products Social Molecular Networking (GNPS, www.gnps.ucsd.edu) [12]. The analysis of Molecular Networking revealed that the nodes at *m/z* 275.2 and 259.2 consist of the largest cluster which is indicated as a xanthone molecular family and is annotated as anomalin A [4] and norlichexanthone [13], respectively. Likewise, the node at *m/z* 403.6 consists of the other cluster which is observed as a sesterterpene molecular family and is annotated by terpestacin [6] (Fig. 1). However, the spectral nodes at *m/z* 307.0 and 305.3 could not be annotated in neither in-house fungal compounds library nor the GNPS library and had a strong spectral similarity score with the node at *m/z* 213.2 as asperlin which is a pyrone analog (Fig. 1). This led to the isolation of a new *α*-pyrone derivative (**1**) from the EtOAc-extract of *A. pseudosinense* culture medium (Fig. 2a).

Compound **1** was obtained as a pale brown oil and assigned the molecular formula $C_{18}H_{26}O_4$ via HRESIMS data, suggesting that it had 6 degrees of unsaturation. The IR spectrum exhibited peaks at 3425 $cm^{-1}$ and 1640 $cm^{-1}$, which were indicative of the presence of hydroxy and carbonyl groups, respectively. The $^1$H NMR spectral data displayed three olefinic groups at $\delta_H$ 6.10 (1H, br s, H-5), 5.87 (1H, br s, H-10) and 5.04 (1H, d, $J = 9.4$ Hz, H-12), two methylene groups at $\delta_H$ 2.62 (2H, m, H-7) and 1.37 (1H, m, H-14$_a$)/1.25 (1H, m, H-14$_b$), a methine group at $\delta_H$ 2.34 (1H, m, H-13), an oxygenated methine group at $\delta_H$ 4.36 (1H, dd, $J = 5.0$ Hz, $J = 5.0$ Hz, H-8), and five methyl groups at $\delta_H$ 0.85 (3H, t, $J = 7.4$ Hz, H-15), 1.83 (3H, s, H-16), 1.79 (3H, s, H-17), 1.68 (3H, s, H-18) and 0.94 (3H, d, $J = 6.6$ Hz, H-19). The $^{13}$C NMR spectral data exhibited 18 resonances for three olefinic, two methylene, two methine, including an oxygenated, five methyl, and six quaternary carbons. The $^1$H and $^{13}$C NMR spectral data obtained for **1** were similar to those for diaporpyrone A [14], which has an *α*-pyrone carbon framework, except for the additional signals of a methyl group, as evidenced by the appearance of the signals at $\delta_H$ 1.79 (H-17) and $\delta_C$ 12.6 (C-17) and the HMBC correlations of H-17/C-8, H-17/C-9, and H-17/C-10. The planar structure of **1** was associated with a detailed analysis of the 2D NMR data (Fig. 2a). The geometries of $\Delta^{9,10}$ and $\Delta^{11,12}$ were assigned to be *E* based on the ROE correlations from H-10/H-12, H-12/H-14, H-17/H-18, and H-18/H-19 (Fig. 2a). The absolute configurations of C-8 and C-13 were determined through the modified Mosher's method and gauge-including atomic orbital NMR chemical shift calculations, followed by DP4 + analysis [15], respectively (Fig. 2b and Fig. 2c). Based on the absolute configuration of 8 *R* by the modified Mosher's method, computational calculations of the electronic circular dichroism (ECD) of the two possible diastereomers, **1c** (8 *R*,13 *R*) and **1d** (8 *R*,13 *S*), were

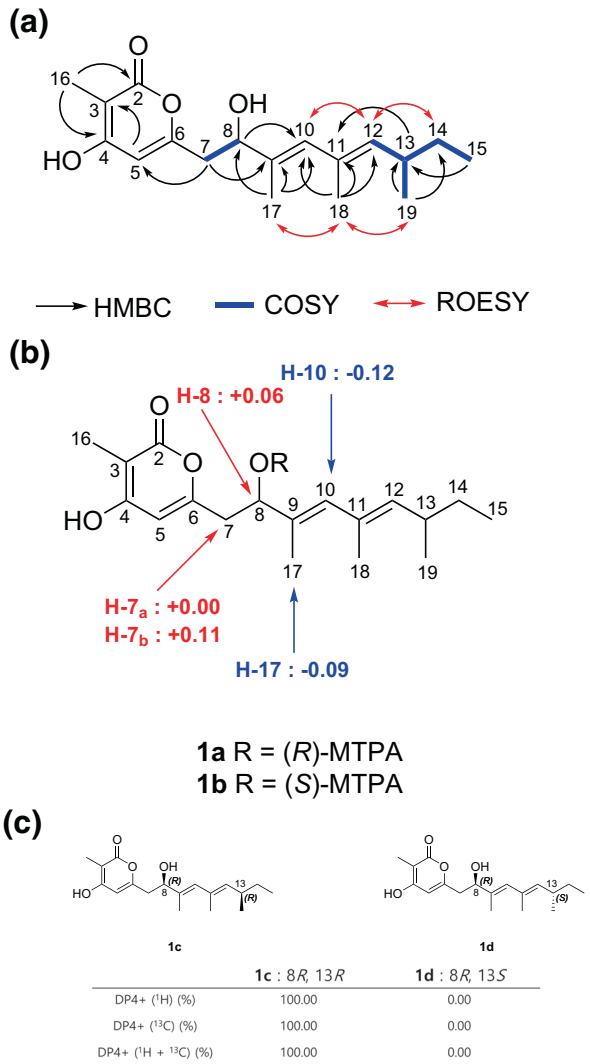


**Fig. 2** **a** Key HMBC (single-headed), COSY (bold) and ROESY (double-headed) correlations 1; **b** Values of $\delta_S$-$\delta_R$ (in Hz) of the MTPA esters (1a and 1b); **c** DP4 + analysis of 1c and 1d

attempted [16]; however, the calculated ECD spectra of the two diastereomers were very similar, implying that the determination of neither **1c** nor **1d** has a limitation. Therefore, the gauge-including atomic orbital NMR chemical shifts calculation including DP4 + probability analysis was applied and the calculated $^{1}$H and $^{13}$C NMR chemical shifts of the two diastereomers, **1c** and **1d**, were compared with the experimental values of **1** via a DP4 + probability analysis [17]. The statistical analysis showed that **1c** (8*R*,13*R*) and **1** are structurally coincident, with a DP4 + probability score of 100% [17, 18]. Accordingly, the new compound **1** was elucidated as shown and it has been assigned the trivial name, arthrifuranone A.

Compound **1** has been evaluated for its estrogenic activity using the MCF-7 estrogen responsive human breast cancer cells. It showed estrogenic activity from the concentration of 3.125 μM by increasing the proliferation of the MCF-7 cells. Estrogenic activity was evaluated after treatment with the compound **1** for 96 h and 17*β*-estradiol was used as a positive control (Fig. 3a). The cell proliferation of the compound-treated group continuously increased in a concentration-dependent manner to the highest concentration of 25 μM. However, the proliferation of cells was suppressed by co-treatment of compound **1** and 17*β*-estradiol with 500 nM ICI 182.780, a well-known estrogen receptor (ER) antagonist. Thus, the effect of compound **1** on the cell proliferation may be ER-dependent, and compound **1** may be an ER agonist.

The mechanism of the estrogenic activity of compound **1** was assessed by western blotting analysis of ER-*α* protein, a major sub-type of ERs in MCF-7cells, and its phosphorylated form (p-ER-*α*) [19]. After 96 h of treatment with compound **1**, ER-*α* protein expression was decreased, whereas the expression of p-ER-*α* was increased in a concentration-dependent manner compared to the non-treated group (Fig. 3b). These data indicated that compound **1** stimulated the phosphorylation of ER-*α*, which led to the induction of the cell proliferation in ER-positive breast cancer MCF-7 cells.

Estrogen not only controls the reproductive system, but also regulates immune system and glucose metabolism in elderly women [20]. The immunodulatory assay in mouse splenocytes (Fig. S12), anti-inflammatory assay in mouse macrophage RAW 264.7 cells (Fig. S13), and α-glucosidase inhibitory assay (Fig. S14) were performed. However, compound **1** did not exhibit any activities up to the concentration of 25 μM.

In conclusion, the present work demonstrates the isolation and structural characterization of the new *α*-pyrone derivative, arthrifuranone A (**1**) from an EtOAc-extract of *A. pseudosinense* culture medium. Arthrifuranone A (**1**) increased the proliferation of MCF-7 cell lines by phosphorylating ER-*α*, suggesting that compound **1** could be a potential therapeutic candidate for the relief of menopausal symptoms in elderly women.

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**Author contributions** HK and QNN performed the experiments, with the assistance of MWN, KHK, YG, and SHS. JJK offered the fungal source. DL and KSK designed the research. HK, QNN, DL, and KSK wrote the paper.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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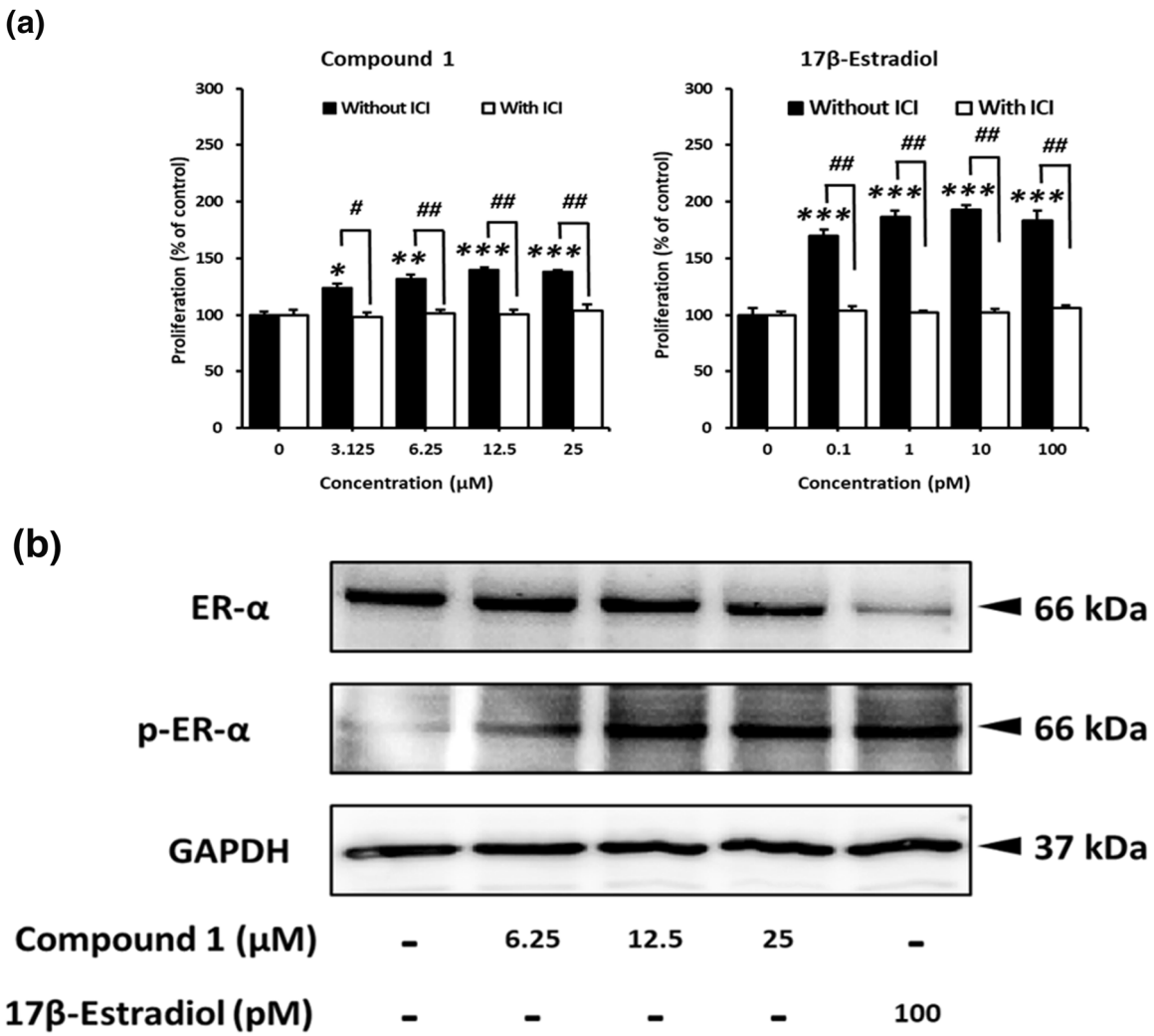


**Fig. 3** **a** Proliferation of MCF-7 cells after 96 h treatment. Cell viability is presented as mean ± standard error of mean (SEM). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus the non-treated group without ICI 182,780. $^{\#}p < 0.01$, $^{\#\#}p < 0.001$ versus the ICI 182,780-treated groups (500 nM); **b** Western blotting of ER-$\alpha$ and phosphorylated ER-$\alpha$ (Ser118) proteins

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