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ASPERGILLUS FUNGI ARE A SOURCE OF BIS-INDOLE ALKALOIDS WITH ANTICANCER ACTIVITY*

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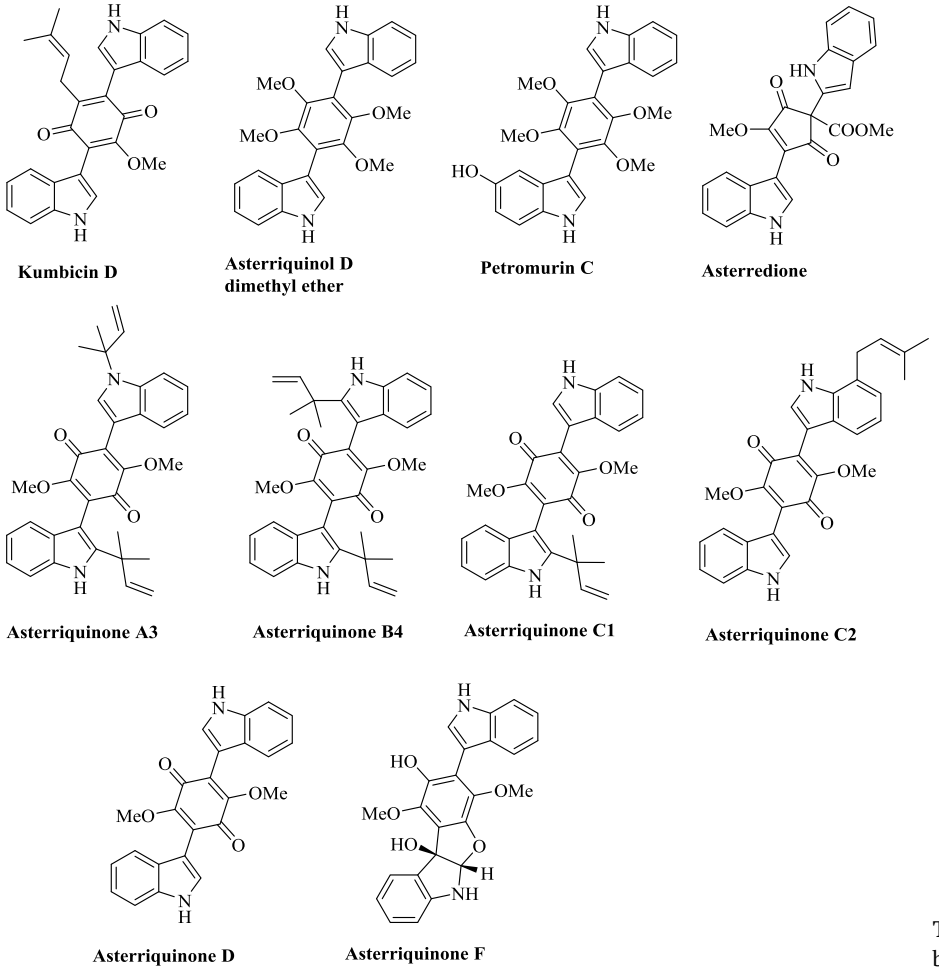
Abstract

Marine-derived *Aspergillus* strains produce bis-indole alkaloids, some of which (petromurin C, asterriquinones B4 and C1) exhibit selective cytotoxicity against MCF-7 breast cancer cells in monolayer and 3D, inhibiting proliferation via the ER-dependent pathway. The biotechnological potential of these strains was confirmed by the high yield of compounds, reaching 2.7 mg/g in the fungal extract.

Bis-indole alkaloids are structurally complex natural compounds with two indole groups moieties attached to pyrrole, piperazine, quinone, and other moieties which provide various bioactivities. For example, staurosporine and its derivatives exhibit potent antiproliferative activity [1]. A total of 425 microorganism-derived bis-indole alkaloids have been reported between 1962 and 2023 [2]. Bacteria are the source of bis-indole pyrroles and dixiamycins, while indole piperazines and asterriquinones have been isolated from various fungi, mainly from the *Aspergillus* genus, including marine isolates [3].



The structures of investigated bis-indole alkaloids

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The aim of the present study was to investigate the cytotoxic and anticancer activities of several bis-indole alkaloids isolated from marine-derived *Aspergillus subramanianii* 1901NT-1.40.2 and *A. terreus* KMM 5900 strains. Kumbicin D, asterriquinol D dimethyl ether, petromurin C, and asterredione were isolated from Vietnamese strain *A. subramanianii* 1901NT-1.40.2. Asterriquinones A3, B4, C1, C2, D, and F were obtained from a Vietnamese isolate of *A. terreus* [4].

The cytotoxic activities of all compounds on breast cancer MCF-7 and normal H9c2 and MCF-10A cells were investigated.

Petromurin C and asterriquinones B4 and C1 ($\leqslant$ 10 μM) significantly decreased MCF-7 cell viability and inhibited colony formation, proliferation, and migration in monolayer culture. They exhibited specific cytotoxicity against breast cancer MCF-7 cells compared to normal cells. Petromurin C significantly decreased the area of MCF-7 spheroids in 3D culture by approximately 30 %. Competitive test with 4-hydroxytamoxifen and molecular docking showed that estrogen receptors (ERβ more than ERα) have been involved in the anticancer effect of petromurin C. RT-PCR assay confirmed that petromurin C affects the transcriptional activity of ER-dependent *CCNG2*, *REA*, *GREB1*, and *IGFBP4* genes, resulting in cell cycle disturbance.

Approaches to the synthesis of prenylated asterriquinones have been developed [5], but they have encountered a number of difficulties, and attempts to develop a synthesis, for example, of petromurin C, continue [6]. In this regard, the biotechnological method of obtaining these compounds from fungal cultures is relevant. In our investigation, the marine stain *A. subramanianii* 1901NT-1.40.2 produced petromurin C with a yield of 2.7 mg/g of crude EtOAc dried extract. The yields of asterriquinones B4 and C1 from *A. terreus* KMM 5900 culture were 1.0 mg/g and 0.9 mg/g of crude EtOAc dried extract, respectively.

Thus, the Vietnamese marine fungal strains *A. subramanianii* 1901NT-1.40.2 and *A. terreus* KMM 5900 are a source of bis-indole benzoquinone alkaloids with significant activity against estrogen-dependent breast cancer MCF-7 cells.

References

1. Gupta L., Talwar A., Chauhan P. M. S. Bis and Tris Indole Alkaloids from Marine Organisms: New Leads for Drug Discovery // Frontiers in Medicinal Chemistry. 2012. Vol. 6.

2. Zhang Z.-L., Xu H.-N., Gong C.-M. et al. Microorganism-Derived Bisindole Alkaloids With Anticancer Potential and Their Mechanisms: A Comprehensive Review // Chemistry & Biodiversity. 2025. Vol. 22, No. 5.

3. Wibowo J. T., Ahmadi P., Rahmawati S. I. et al. Marine-Derived Indole Alkaloids and Their Biological and Pharmacological Activities // Marine Drugs. 2022. Vol. 20, No. 1.

4. Girich E. V., Yurchenko A. N., Smetanina O. F. et al. Neuroprotective Metabolites from Vietnamese Marine Derived Fungi of Aspergillus and Penicillium Genera // Marine Drugs. 2020. Vol. 18, No. 12.

5. Lindel T., Marsch N., Adla S. K. Indole Prenylation in Alkaloid Synthesis // Alkaloid Synthesis / H.-J. Knölker. Berlin, Heidelberg. 2012. P. 67–129.

6. Zi-Hao Jia, Qing-Chun Song, Pei-Hua Zhao et al. Organocatalytic Substrate-Controlled Regioselective Reactions of 2-Indolylmethanols with Phenols And Alcohols Access 3-Aryl Indoles and Indole-Derived Triaryl Ethers // 2025.