

LEADS SEARCH FOR ACETYLCHOLINESTERASE INHIBITORS DERIVED FROM SECONDARY METABOLITES OF ENDOPHYTIC FUNGI: A REVIEW

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ABSTRACT

Background: Acetylcholinesterase inhibitors (AChEIs) are substances that act by increasing acetylcholine levels in the brain to prevent neurotransmitter degradation. AChEIs are the most widely used agents for Alzheimer's disease (AD) therapy so far. **Objective:** This study aims to give insight into AChEIs produced by endophytic fungi through literature reviews, which are promising for further studies on their mode of action. **Methods:** Data search was conducted through scientific-based websites such as Google Scholar, Science Direct, and PubMed, which involved scientific publications from January 2000 to December 2022. **Results:** Fifteen genera, including *Aspergillus*, *Cladosporium*, *Colletotrichum*, and *Penicillium*, were reported to produce twenty-four secondary metabolites with AChEI activity. These compounds were classified based on their chemical skeleton into alkaloids, steroids, terpenoids, polyketides, and peptides. **Conclusion:** Endophytic fungi are promising sources of lead compounds possessing AChE inhibitory activity. Further research on molecular mechanisms of secondary metabolites from endophytic fungi with AChEI activity can provide new insight into the development of more potent AChEIs for AD treatment.

Keywords: Acetylcholinesterase inhibitors (AChEIs); Alzheimer's disease; Endophytic fungi; Secondary metabolites.

INTRODUCTION

The World Health Organization (WHO) estimated that 55 million people live with dementia around the world. One of the most common types of dementia, contributing to 60-70% of global dementia cases, is Alzheimer's disease (AD)^[1]. AD is caused by damage to neurons in the brain. The neuron

damage in AD patients affects their ability to perform normal activities and communication, such as losing the ability to remember names, current events, and conversations, which causes AD patients to experience confusion easily. There are no pharmacological drugs available to stop neuronal damage in the brain. Currently, the

treatment of AD patients is targeted at improving cognitive symptoms by increasing neurotransmitters in the brain^[2].

AChEIs are the most widely used agents for Alzheimer's disease (AD) therapy. AChE inhibitors are a group of drugs that can prevent the degradation of neurotransmitters by increasing acetylcholine levels in the brain and, therefore, improving neurotransmission in the brain^[2]. Rivastigmine, donepezil, galantamine, and tacrine are four AChEIs clinically used for AD treatment so far. Other than that, compounds with AChEI properties can be obtained either by chemical synthesis or by extracting secondary metabolites produced by plants and microorganisms^[3].

Endophytic fungi are a group of microorganisms with the capability of producing diverse secondary metabolites with various pharmacological properties, such as antimicrobial, anticancer, antioxidant, and soon. Moreover, through metabolic interaction between endophytes and their host, endophytic fungi, under evolutionary pressure, can produce analogous chemicals similar to their host based on chemical cues from the host plant^[4].

More than 400 natural products derived from endophytic fungi potentially active for anti-Alzheimer were also described in a recent review by Zhu *et al.* 2023^[5]. However, as the best-known target for AD therapy so far by inhibiting the acetylcholinesterase, in the present review, we focus on providing current evidence on secondary metabolites from endophytic fungi with remarkable AChEI properties in the search for new AChEI leads.

METHODS

Literature searches were conducted with the following inclusion criteria: 1) original research articles published from January 2000 to December 2022; 2) research aimed at investigating well-defined secondary

metabolites isolated from endophytic fungi, which exhibited acetylcholinesterase inhibitory activity; 3) AChEI compounds have IC₅₀ values below 10 μM. We limited our search to compounds with promising AChEI activity, indicated by their IC₅₀ values of less than 10 μM, as we intended to highlight compounds potentially developed as AChEI leads. Searches of the literature were conducted through science-based websites, such as Google Scholar, Science Direct, and PubMed. Only open-access articles were included in this review.

Keywords used in this literature review include acetylcholinesterase inhibitor, Alzheimer's disease, endophytic fungi, and secondary metabolites. Data collection was performed manually by authors, and information such as the name of the journal, year, title, host plant, endophytic fungi, secondary metabolites, and IC₅₀ values were extracted for this review.

RESULTS

Endophytic fungi are known for their capacity to produce enormous bioactive secondary metabolites, including those made by their hosts. In addition, they might be able to produce new lead compounds to tackle many incurable diseases, including secondary metabolites with AChEI activity. In this review, we highlight twenty-four compounds with activity as AChEIs derived from fifteen fungal genera, including *Aspergillus*, *Cladosporium*, *Colletotrichum*, and *Penicillium*, which were described herein. These bioactive natural products were grouped based on their core structures as alkaloids, terpenoids, steroids, polyketides, and peptides.

1. Alkaloids

Alkaloids are groups of secondary metabolites naturally produced by plants as a defense mechanism against predators.

Alkaloids are also known as poisonous compounds that may affect the central nervous system, gastrointestinal tract, and immune system^[6]. Alkaloids are easy to identify from their chemical structure. The nitrogen atom in its chemical structure is the key characteristic of alkaloid compounds that distinguishes them from other metabolites^[7]. In this review, five alkaloids were reported for their pronounced acetylcholinesterase inhibitory activity, and their structures are shown in Figure 1.

Endophytic fungus *Westerdykella nigra*, isolated from a mangrove species, *Avicennia marina* (Forssk.) Vierh. roots growing in Safaga, Red Sea, Egypt, were reported capable of producing alkaloids westalsan (1), phomacin B (2), and 19-hydroxy-19,20-dihydrophomacin C (3). These compounds showed potent activity as AChEIs, where 19-hydroxy-19,20-dihydrophomacin C showed the lowest IC₅₀ value of 0.056 μM, followed by westalsan and phomacin B with IC₅₀ values of 0.088 μM and 0.140 μM, respectively^[8].

Another alkaloid, 8-*O*-methylbostrycoidin (4) also showed prominent AChEI activity with an IC₅₀ value of 6.710 μM. This compound was produced by *Aspergillus terreus* (No. GX7-3B), an endophytic fungus isolated from mangrove *Bruguiera gymnoihiza* (Linn.) Savigny collected from the South China Sea area^[9]. A sulfur-containing metabolite, acrozine F (5) was an alkaloid isolated from *Acrostalagnus luteoalbus* TK-43, an endophytic fungus of fresh marine algae *Codium fragile* collected in Sinop, Turkey. Acrozine F showed an IC₅₀ value of 8.400 μM when tested for AChEI activity^[10].

2. Terpenoids

Terpenoids or terpenes are one of the largest groups of secondary metabolites with diverse molecular structures, either

containing oxygenated hydrocarbons or non-oxygenated derivatives.^[11] This class of metabolites is characterized by the presence of asymmetric carbon atoms which are mostly optically active on their skeletal structures^[11-12]. In this review, eight terpenoids were reported to act as acetylcholinesterase inhibitors (Figure 1).

Meroterpenes containing α-pyrone ring system, arigsugacins F (7) and I (8), along with territrem B (9) were obtained following chromatographic workup on the fungal extract of *Penicillium* sp. sk5GW1L. The fungus was isolated from leaves of the mangrove plant *Kandelia candel*, grown in Guangxi province, China. The isolated meroterpenes showed remarkable AChEI activity with IC₅₀ values from the nano Molar range to 0.64 μM. Territrem B showed the most potent inhibition among the tested compounds with an IC₅₀ value of 7.03 nM.^[13] Meanwhile, arigsugacins F and I showed weaker inhibition with IC₅₀ values of 0.37 and 0.64 μM. Further chemical investigation of this fungal strain by Ding et al. 2016 led to the isolation of other α-pyrone meroterpenes, arigsugacin B (6), territrem C (10), and terreulactone C (11). When tested for AChE inhibitory activity, all these compounds showed prominent inhibition with IC₅₀ values of 0.028; 0.230; and 3.030 μM, respectively^[13-14].

Two rare sesterterpenoids bearing 5/8/6/6 tetracyclic ring system identified as asperterpenols A (12) and B (13) were found as metabolites of endophytic *Aspergillus* sp. 085242. The fungus was isolated from a mangrove grown in the South China Sea. In the *in vitro* AChEI assay, both compounds exerted potent inhibitory activity with IC₅₀ values of 2.300 and 3 μM^[15].

3. Steroids

Steroids are a group of natural compounds with important regulatory roles

for various cellular functions in animals, plants, and fungi. In animals and fungi, steroids are derived from sterols and lanosterols, although steroids in plants are generally derived from cycloartenol^[16]. Steroid compounds can be recognized by the presence of sterane structures. The sterane structure is composed of three cyclohexane rings and one cyclopentane ring^[17]. Two steroids known as ergosterol (14) and NGA0187 (15) produced by endophytic fungi were reported to have AChEI activity in previous studies. The chemical structures of these compounds are shown in Figure 1.

Ergosterol (14) afforded from the endophytic fungus *Curvularia* sp. T12 exhibited strong AChEI activity with an IC₅₀ value of 1.520 μM. The fungal endophyte was isolated from the medicinal plant *Rauvolfia macrophylla* stem bark growing in Mount Kalla, Cameroon^[18]. In addition to the capability to produce alkaloid, 8-*O*-methylbostrycoidin (4) with AChEI activity, a mangrove-derived endophytic *A. terreus* (No. GX7-3B), was found to produce a steroid compound, anicequol denoted by developmental code NGA0187 (15)^[19]. Anicequol revealed potent AChEI activity with an IC₅₀ value of 1.890 μM^[9].

4. Polyketides

Polyketides are one of the largest categories of secondary metabolites with a wide variety of structures and various biological activities. Polyketides can be isolated from diverse microorganisms^[20]. Structurally, polyketides are a group of natural products that contained many carbonyls and alcohols, generally separated by methylene carbons^[21]. In this review, eight polyketides were reported to possess AChEI activity as chemical structures are shown in Figure 1.

In a study performed by Kaaniche *et al.* 2018 on an endophytic fungus *Curvularia* sp.

T12 from Cameroonian *R. macrophylla*, bioactive polyketides, hexylitaconic acid (16) and 2'-deoxyribolactone (19) were also isolated from the fungal cultures. These polyketides showed prominent AChEI activity with IC₅₀ values of 1.540 and 1.930 μM^[18]. Moreover, polyketides 6'-*O*-desmethylcandidusin B (17) and 3''-deoxy-6'-*O*-desmethylcandidusin B (18) were also afforded upon chromatographic procedure on *Penicillium chermesinum* (ZH4-E2) extract. This fungal endophyte was isolated from *Kandelia candel* stems, and the host plant was collected in the South China Sea. Both isolated polyketides showed strong AChEI activity with IC₅₀ values of 5.200 and 7.800 μM^[22]. Another polyketide, anhydrojavanicin (20) isolated from endophytic *A. terreus* (No. GX7-3B) revealed potent AChEI activity as well, with an IC₅₀ value of 2.010 μM^[7]. Chemical investigation on endophytic *Chaetomium* sp. NF00754 associated with *Pharbitis nil* grown in Morocco resulted in bioactive polyketides, orsellides A (21) and C (22), together with globosumone C (23). These polyketides displayed inhibition towards the activity of acetylcholinesterase with IC₅₀ values of 7.340; 5.190; and 7.670 μM, respectively^[23].

5. Peptides

Peptides are biological products that consist of repeating amino acid units bound together by a peptide bond^[24]. Many peptides have been reported for various pharmacological activities^[25]. A polypeptide beauvericin (24), was reported to have strong inhibition towards acetylcholinesterase with an IC₅₀ value of 3.090 μM. The compound was isolated from *A. terreus* (No. GX7-3B), an endophytic strain that was also shown to produce other secondary metabolites with AChEI activity belonging to the alkaloid, polyketide and steroid-type of compounds^[9].

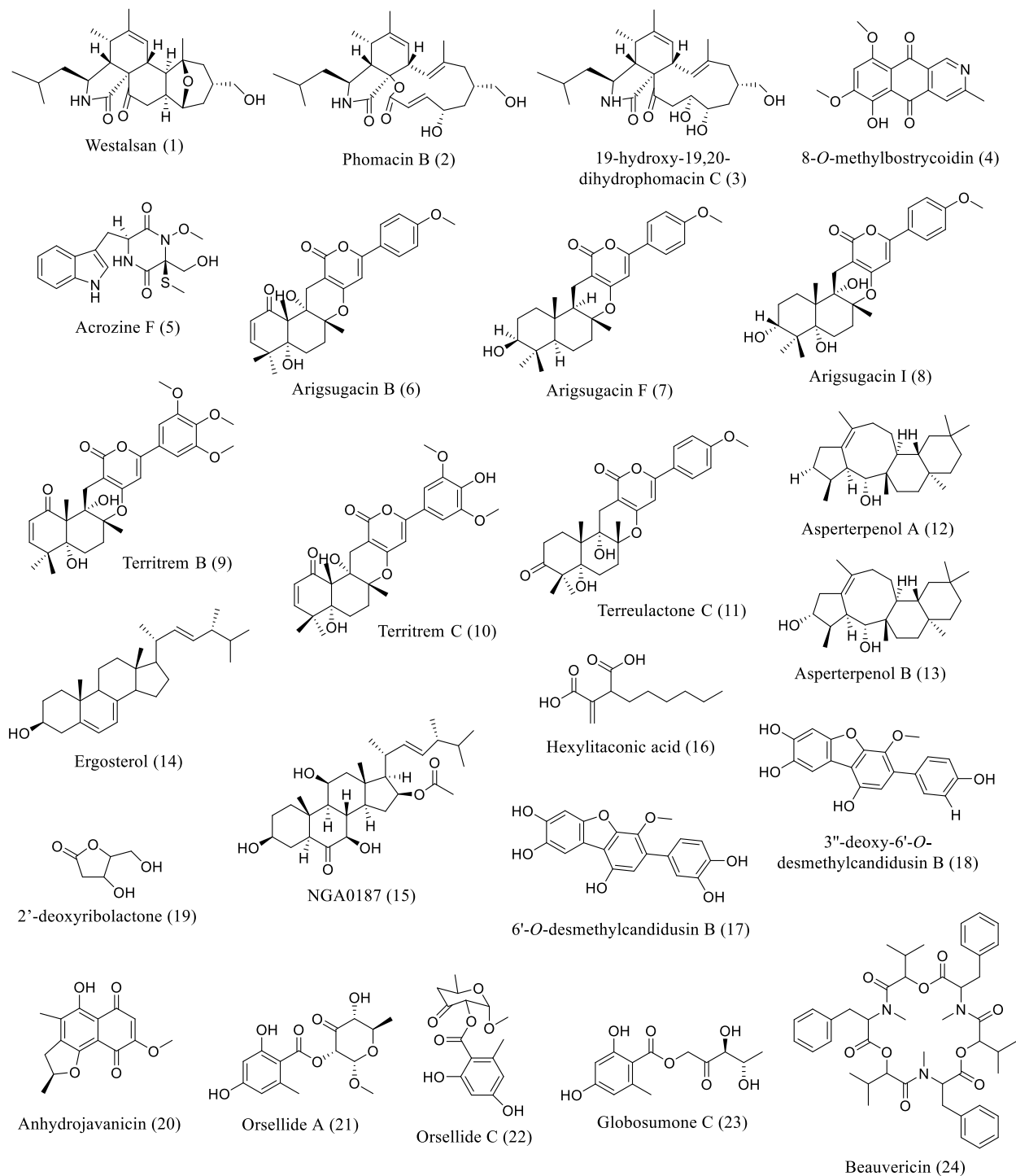


Figure 1. Structures of secondary metabolites with prominent AChEI activity from fungal endophytes

DISCUSSION

Endophytic fungi are one of the important resources as natural products in the discovery and development of new drug candidates due to their capacity to produce diverse bioactive compounds. Fifteen genera of endophytic fungi from various host plants were described herein to produce secondary metabolites with acetylcholinesterase inhibitory activity. These genera include *Aspergillus*, *Cladosporium*, *Colletotrichum*, and *Penicillium*. Among those genera, there are twenty-four compounds shown in this review that have AChEI properties with IC_{50} values ranging from 0.028-8.400 μ M.

Nine compounds such as westalsan, phomacin B, 19-hydroxy-19,20-dihydrophomacin C, arigsugacins F and I, territrems B and C, as well as terreulactone C showed remarkable activity as acetylcholinesterase inhibitors with IC_{50} values below 1 μ M. These results suggest the potential of endophytic fungi as sources of compounds with acetylcholinesterase inhibitory activity.

Apart from an increasing number of naturally occurring AChEIs reported from endophytic fungi, the re-discovery of known secondary metabolites has become one of the major challenges in the search for new leads. Further research on how to optimize endophytic fungi potential is needed to find lead compounds suitable for the development of new and more effective Alzheimer's drugs. The application of approaches such as the One Strain Many Compounds (OSMAC) strategy, fungal-bacteria or fungal-fungal co-cultivation on talented endophytic fungal strain might be beneficial to uncover the biosynthetic capacity of the corresponding fungal strain, which possibly leads to wider chemically diverse secondary metabolites with more potent AChEI activity. Moreover, the scarcity of toxicological studies available for compounds possessing remarkable AChEI activity and molecular mechanisms

also needs particular attention for in-depth studies in the future.

CONCLUSION

Twenty-four compounds from fifteen genera of endophytic fungi were found to have promising AChEI activity. Among them, nine compounds show remarkable activity with IC_{50} values below 1 μ M, which highlights the potential of utilizing endophytic fungi as producers of compounds with AChEI activity. These promising AChEIs-derived endophytic fungi include westalsan, phomacin B, 19-hydroxy-19,20-dihydrophomacin C, arigsugacins F and I, territrems B and C, as well as terreulactone C.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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