© 2025 Journal of Pharmacy & Pharmacognosy Research, 13 (3), 757-773, 2025
ISSN 0719-4250
https://jppres.com

DOI: https://doi.org/10.56499/jppres24.2064_13.3.757

Original Article

In vitro and *in silico* cholinesterase inhibitory activities of aaptamine and derivatives from *Aaptos suberitoides*

[Actividades inhibidoras de la colinesterasa *in vitro* e *in silico* de la aaptamina y derivados de *Aaptos suberitoides*]

Suciati Suciati[1,2*], Hanifa Rahma Putri[3], Kishneth Palaniveloo[4,5], Ong Kuan Hung[4], Mohammed Rizman-Idid[4], Aty Widyawaruyanti[1,2], Nitra Nuengchamnong[6], Nungruthai Suphrom[7], Rhesi Kristiana[8], I Wayan Mudianta[9], Mary George[10], Edwin Setiawan[11]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia.
[2]Center for Natural Product Medicine Research and Development, Institute of Tropical Diseases, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia.
[3]Master Program in Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga, Surabaya, 60115, East Java, Indonesia.
[4]Institute of Ocean and Earth Sciences, Advanced Studies Complex, Universiti Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
[5]Center for Natural Products Research and Drug Discovery (CENAR), Level 3, Research Management & Innovation Complex, Universiti Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
[6]Science Laboratory Center, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand.
[7]Department of Chemistry, Faculty of Science and Center of Excellence for Innovation in Chemistry, Naresuan University, Phitsanulok 65000, Thailand.
[8]Indonesian Marine Education & Research Organisation (MERO) Foundation, Desa Tulamben, Karangasem 80853, Bali, Indonesia.
[9]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Ganesha, Singaraja 81116, Bali, Indonesia.
[10]Faculty of Law, Universiti Malaya, 50603 Wilayah Persekutuan Kuala Lumpur, Malaysia.
[11]Department of Biology, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh November, Surabaya 60111, East Java, Indonesia.
*E-mail: suciati@ff.unair.ac.id

Abstract

Context: Marine sponges from the genus *Aaptos* produce compounds, often alkaloids, with a wide range of bioactivities. *Aaptos suberitoides* exhibits anticancer, antioxidant, antibacterial proteasome inhibitory, and receptor activator of NF-kappaB ligand (RANKL) inhibitory activities. Alkaloids from marine sponges have shown potency as cholinesterase inhibitors, one of the targets in Alzheimer's disease treatment.

Aims: To isolate, identify, and investigate the potency of alkaloids from the water fraction of marine sponge *A. suberitoides* as cholinesterase inhibitors.

Methods: Bioassay-guided isolation was employed to obtain active alkaloids. The cholinesterase inhibitory assay was conducted based on Ellman's method with slight modification. Identification of isolated compounds was carried out based on NMR and MS data. The identified compounds were also subjected to molecular docking analysis as well as an ADMET study.

Results: Bioassay-guided isolation of water fraction yielded aaptamine (**1**) and a mixture containing five benzo[de][1,6]-naphthyridine derivatives, identified based on NMR and LC-MS/MS spectroscopy. Aaptamine **(1)** inhibited both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with IC_{50} values of 0.23 and 1.38 µg/mL, respectively. Likewise, the benzo[de][1,6]-naphthyridine mixture of N-demethylaaptanone (**2**), aaptanone (**3**), demethylaaptamine (**4**), aaptosine (**5**) and 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine (**6**) recorded IC_{50} values of 0.39 and 1.61 µg/mL, respectively. Computational molecular docking analysis calculated the S-score and RMSD values of aaptamine (**1**) to be the best compared to galantamine for both AChE (PBD: 4EY6) and BChE (PDB: 4BDS) with values of -6.65 kcal/mol, 0.57 Å and -5.98 kcal/mol, 1.05 Å, respectively.

Conclusions: These findings suggest that benzo[de][1,6]-naphthyridine alkaloids from *A. suberitoides* can be good candidates as cholinesterase inhibitors.

Keywords: *Aaptos suberitoides;* Alzheimer's disease; benzo[de][1,6]-naphthyridine alkaloids; cholinesterase inhibitor; marine sponge.

Resumen

Contexto: Las esponjas marinas del género *Aaptos* producen compuestos con una amplia gama de bioactividades. *Aaptos suberitoides* exhibe actividades anticancerígenas, antioxidantes, antibacterianas, inhibidoras del proteasoma y del activador del receptor del ligando NF-kappaB (RANKL). Los alcaloides de las esponjas marinas han demostrado potencia como inhibidores de la colinesterasa, uno de los objetivos en el tratamiento de la enfermedad de Alzheimer.

Objetivos: Aislar, identificar e investigar la potencia de los alcaloides de la fracción acuosa de la esponja marina *A. suberitoides* como inhibidores de la colinesterasa (iChE).

Métodos: Se empleó un aislamiento guiado por bioensayo para obtener alcaloides activos. El ensayo inhibidor de la colinesterasa se realizó según el método de Ellman con una ligera modificación. La identificación de los compuestos aislados se llevó a cabo según los datos de RMN y MS. Los compuestos identificados también se sometieron a un análisis de acoplamiento molecular, así como a un estudio ADMET.

Resultados: El aislamiento de la fracción acuosa mediante bioensayo permitió obtener aaptamina **(1)** y una mezcla que contenía cinco derivados de benzo[de][1,6]-naftiridina, identificados mediante espectroscopia RMN y LC-MS/MS. La aaptamina **(1)** inhibió tanto la acetilcolinesterasa (AChE) como la butirilcolinesterasa (BChE) con valores de CI_{50} de 0,23 y 1,38 µg/mL, respectivamente. Asimismo, la mezcla de benzo[de][1,6]-naftiridina de N-desmetilaaptanona **(2)**, aaptanona **(3)**, desmetilaaptamina **(4)**, aaptosina **(5)** y 8,9,9-trimetoxi-9H-benzo[de][1,6]-naftiridina **(6)** registró valores de CI_{50} de 0,39 y 1,61 µg/mL, respectivamente. El análisis computacional de acoplamiento molecular calculó que los valores de S-score y RMSD de aaptamina **(1)** eran los mejores en comparación con galantamina tanto para AChE (PBD: 4EY6) como para BChE (PDB: 4BDS) con valores de -6,65 kcal/mol, 0,57 Å y -5,98 kcal/mol, 1,05 Å, respectivamente.

Conclusiones: Estos hallazgos sugieren que los alcaloides benzo[de][1,6]-naftiridina de *A. suberitoides* pueden ser buenos candidatos como iChE.

Palabras Clave: *Aaptos suberitoides*; alcaloides benzo[de][1,6]-naftiridina; enfermedad de Alzheimer; esponja marina; inhibidor de la colinesterasa.

ARTICLE INFO
Received: April 26, 2024.
Accepted: November 22, 2024.
Available Online: December 17, 2024.

AUTHOR INFO
ORCID: 0000-0003-2436-4119 (SS) 0000-0002-2231-2502 (MRI) 0000-0002-6152-4305 (RK)
0000-0001-6016-8479 (HRP) 0000-0003-4639-1599 (AW) 0000-0002-0388-3828 (IWM)
0000-0003-0466-2602 (KP) 0000-0001-5659-9786 (NN) 0000-0003-1233-0835 (MG)
0000-0002-3108-7570 (OKH) 0000-0003-3767-8387 (NS) 0000-0003-3947-3334 (ES)

INTRODUCTION

Approximately 70% of the earth's surface is covered with oceans and seas, which is a natural habitat for almost 80% of plant and animal species in the world, therefore making it a natural source of bioactive compounds (Abdillah et al., 2013; Varijakzhan et al., 2021). Over 10,000 natural products have been isolated from marine organisms such as bacteria, fungi, micro and macroalgae, sponges, bryozoans, and sea cucumbers. Marine organisms that exist in extreme habitats produce bioactive secondary metabolites as part of their defensive or offensive mechanism. Currently, 38% of the identified natural products derived from bacteria, protozoa, fungi, and microalgae have reported pharmacological activities, and about 20% are associated with sponges and corals (Silva et al., 2021). In the last decades, marine sponges have attracted the attention of researchers for the diversity of new compounds with promising therapeutic potentials they synthesize (Esposito et al., 2022; Shaari et al., 2008). These marine invertebrates are known to produce alkaloids, terpenoids, polyketides, and glycosphingolipid, and these chemicals are known to exhibit various biological activities such as antitumor, antiviral, antibiotics, and anti-inflammatory properties (Aguiar et al., 2021).

Marine sponges from the genus *Aaptos* are reported to have many compounds with a wide range of bioactivity (He et al., 2020). The genus *Aaptos* is widely distributed in the South China Sea as well as the Caribbean and Indonesian waters (Carvalho et al., 2013). Aaptamine, a benzo[de][1,6]naphthyridine alkaloid, is a major compound from this genus (He et al., 2020). It was first isolated from the Japanese *Aaptos aaptos* by Nakamura et al. (1982). Several other derivatives of aaptamine have also been discovered from this genus (He et al., 2020; Pham et al., 2013), such as demethylaaptamine, demethyloxyaaptamine, isoaaptamine, along with a variety of other alkaloids (He et al., 2020; Nakamura et al., 1987).

Aaptos suberitoides is a widely studied member of the genus *Aaptos*. This species had previously been reported to contain the alkaloid aaptamine and its derivatives such as suberitines A-D (Liu et al., 2012), 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine, 11-methoxy-3H-[1,6]naphthyridino[6,5,4-*def*] quinoxaline-3-one, 2,11-dimethoxy-3H-[1,6] naphtyridino[6,5,4-*def*]-quinoxalin-3-one, 5-benzoyldemethylaaptamine, 3-aminodemethyl(oxy) aaptamine, 8-methoxybenzimidazo [6,7,1-*def*][1,6]-naphthyridine (Pham et al., 2013), aaptoline A (Tsukamoto et al., 2014), and aaptodines A-D (Wang et al., 2020). Extracts, fractions, and isolated compounds from *A. suberitoides* are known to exhibit anticancer, proteasome inhibitor, antioxidant, RANKL inhibitor, and antibacterial activities (Bashari et al., 2021; Nurhayati et al., 2018; 2019; Tsukamoto et al., 2010; Wang et al., 2020).

The potency of alkaloids for Alzheimer's disease treatment, especially as cholinesterase inhibitors, is well documented, with some reports associated with marine sponges (Hafez Ghoran and Kijjoa, 2021; Lima and Medeiros, 2022). Alzheimer's disease (AD) is a neurodegenerative disease that mostly affects people above the age of 65. AD is characterized by the presence of Aβ peptides and neurofibrillary tangles within the patients' brains (Ahmed et al., 2021). The most popular hypothesis that causes AD is a decrease in levels of the neurotransmitter acetylcholine in the brain, which damages the cholinergic system, resulting in the loss of brain cell communication. Several studies have shown decreasing levels of acetylcholine in AD patients (Chen et al., 2022). Acetylcholine (ACh) levels can be increased by inhibiting the activity of the cholinesterase enzymes (Zilbeyaz et al., 2018). Two types of cholinesterase enzymes exist that play an important role in the regulation of acetylcholine, namely acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In the vertebrate's cholinergic pathways, AChE is the primary enzyme that controls neurotransmission; in the event that AChE activity is impaired, BChE assists and regulates cholinergic transmission (Moodie et al., 2019).

In our previous study, extract and fractions of *A. suberitoides* showed potency as cholinesterase inhibitors. Four known alkaloids, namely aaptamine, demethylaaptamine, isoaaptamine, 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine and demethyloxyaaptamine were detected in the ethyl acetate and *n*-hexane fractions of *A. suberitoides* (Putri et al., 2023). Although the potency of aaptamine as a cholinesterase inhibitor has been reported (Miao et al., 2022), there remain a considerable number of unexplored alkaloids derived from *A. suberitoides* that warrant further investigation. The objective of this study is to examine the potential of aaptamine derivatives obtained from *A. suberitoides* as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitors.

MATERIAL AND METHODS

Chemicals

Acetylcholinesterase from *Electrophorus electricus* (AChE type VI-S, C3389), butyrylcholinesterase from equine serum (BChE, C7512), acetylthiocholine iodide (ATCI, A5751), butyrylthiocholine iodide (BTCI,

20820), 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB, D8130), bovine serum albumin (BSA, A2153), and trisma base-HCl buffer (Tris-buffer) were obtained from Merck, USA.

Sample collection

The sponge specimen, *A. suberitoides,* was collected from the coast of Tulamben, Bali, Indonesia, by SCUBA diving in December 2020 at a depth of 5-20 m (8° 17'23.9994"S; 115° 36'44.0634"E). The sample was promptly frozen at -20°C until extraction. The specimen, voucher number SPT-11, was deposited at the Department of Biology, ITS, Surabaya, Indonesia. The identification of the sponge used in this study has been reported (Putri et al., 2023).

Extraction and isolation

The sponge extraction and extract fractionation were done based on the protocol described in our previous work (Putri et al., 2023) to obtain methanol extract and 3 fractions, namely n-hexane, ethyl acetate, and water fractions. The water fraction (4.2 g) was subjected to flash chromatography on a Sepacore® BUCHI. A total of 2 g of water fraction was pre-mixed with 2 g of silica gel 60 (0.040-0.063 mm) and dried. The stationary phase used was silica gel 60 inserted into a column with a length of 230 mm and diameter of 26 mm and with nitrogen gas. A gradient mobile phase elution of ethyl acetate/methanol (7:3 20 min, 3:2 50 min, 100% methanol for 90 min) at a flow rate of 10 mL/min was used. Detection was performed under UV at 254 nm to obtain seven fractions (F1-F7). Fraction F3 (60 mg) was purified by HPLC (Shimadzu LC-6AD, Japan) using isocratic methanol/water (40:60) elution for 30 mins in a C18 semi-preparative column (Phenomenex Gemini, 250 × 10 mm) to yield aaptamine (**1**) (10 mg). Fraction F4 was separated over ACN/water 30:70 *v/v* at 1.0 mL/min for 30 mins to yield three fractions, namely, F.4.1-F4.3. The major peak at F4.3 was then subjected to identification.

Compound identification

Compounds were identified based on NMR and LC-MS/MS spectroscopic data. ^{1}H and ^{13}C-NMR experiments were carried out on a JEOL ECS-400 using a 5 mm SEI probe. Measurements were made in deuterated methanol (MeOH-d_4) referenced at δ_H 3.31 ppm and δ_C 49.00 ppm. High-resolution mass spectrometry was conducted on a QTOF 6540 UHD accurate mass spectrometer. Analysis parameters were established for both negative and positive ionization modes, with spectra collected across a mass range of *m/z* 100-1,000 amu. The ESI-MS and MS/MS experiments were set up as has been described in our previous publication (Putri et al., 2023).

Cholinesterase inhibitory assay

The assay was conducted based on a modified Ellman's method (Aristyawan et al., 2022; Ellman et al., 1961; Putri et al., 2023). The sample solutions (25 µL) at a concentration ranging from 0.0001 to 50 µg/mL were prepared in 10% methanol. Samples were mixed with 25 µL ATCI (1.5 Mm) as a substrate for the AChE enzyme or 25 µL of BTCI (1.5 Mm) as a substrate for the BChE enzyme. Next, 125 µL DTNB (3 mM), 50 µL of Tris buffer, and 25 µL of AChE or BChE enzymes at a concentration of 0.22 U/mL were added. The absorbances were then measured in a microplate reader (Thermo Scientific Multiskan FC) at 405 nm every 5 seconds for 2 minutes before and after the addition of the enzyme. Each experiment was conducted three times. A concentration of 100 µg/mL of galantamine served as the positive control, while 10% methanol was employed as the negative control. The inhibitory activity of the samples was determined using the following equation [1].

$$\%Inhibition = \frac{mean\ velocity\ of\ control - mean\ velocity\ of\ sample}{mean\ velocity\ of\ control} \times 100 \quad [1]$$

Molecular docking analysis

Molecular docking studies were conducted to investigate the cholinesterase inhibitory properties of sponge compounds based on the initial enzymatic screening against the AChE and BChE proteins, as described in our previous research (Palaniveloo et al., 2023). A rule of thumb for an optimally docked metabolite would be an RMSD value and S-score less than 2Å and −7 kcal/mol, respectively, as compared to the reference used.

Preparation of receptor proteins and ligands

The reference ligand-protein combination of (1) galantamine and crystal structure of recombinant human acetylcholinesterase (AChE, 4EY6); and (2) tacrine and human butyrylcholinesterase (BChE, 4BDS) (Cheung et al., 2012; Nachon et al., 2013) were selected for the neurotransmitter choline inhibition modeling. The receptor model, binding sites, ligand-protein interaction validation, energy convergence, and hydrogen coordinates of receptors were prepared as described in our previous publication (Palaniveloo et al., 2023).

The six sponge metabolites reported were all subjected to molecular docking simulation using structural data from PubChem. The compound processing, forcefield module, RMS gradient, selection of binding

sites, and validation protocols were identical to our previous publication. Additionally, the binding sites for AChE and BChE were reported (Palaniveloo et al., 2023). Docking was performed using the induced fit method, where the best out of 100 docking was generated.

Docking conformational analysis

The free binding energy (S-score) and root mean square deviation (RMSD) are critical in the determination of optimum docking. A combination of values of these two components, the parameters, along with the docked conformations 2D diagrams, visualized using Discovery Studio 2016 Client software, was analyzed for determination of candidate of potential (Palaniveloo et al., 2023).

In silico drug-likeness analysis

The six sponge metabolites and control, galantamine and tacrine, were all subjected to *in silico* drug-likeness analysis using ADMETlab 3.0 (Dong et al., 2018). Key molecular features, including molecular weight (MW), hydrogen bond donors (HBD) and acceptors (HBA), the logarithm of compound partition coefficient between n-octanol and water (logP), the number of rotatable bonds (n-ROTB), and topological polar surface area (TPSA), were computed and assessed in accordance with Lipinski's rule of five. Additionally, pharmacokinetic properties—such as human colon adenocarcinoma cell line permeability (Caco-2), P-glycoprotein inhibitor/substrate (pgp_inh/sub), human intestinal absorption (HIA), blood-brain barrier (BBB) penetration, plasma protein binding (PPB), cytochrome P450 enzymes inhibitor/substrate (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP2B6), plasma clearance (cl-plasma), and half-life (T0.5)—were calculated. Toxicological profiles, including human Ether-à-go-go-related gene blocker (hERG) inhibition, drug-induced liver injury (DILI), Ame's mutagenicity, rat oral acute toxicity (ROA), carcinogenicity, human hepatotoxicity (H-HT), drug-induced neurotoxicity, hematotoxicity, immunotoxicity (RPMI-8226), and human embryonic kidney cell cytotoxicity (HEK293), were evaluated.

Statistical analysis

The IC_{50} value is defined as the concentration of samples that inhibited 50% of AChE or BChE enzyme activity. The values were calculated from the dose-response curves using nonlinear regression analysis, with $r^2 > 0.95$. The statistical analyses were performed by using Prism software (Graph Pad 8 Inc, San Diego, USA). The IC_{50} values were expressed as a mean ± standard error of the mean (SEM) from three experiments, each carried out in triplicates.

RESULTS AND DISCUSSION

Activity-guided fractionation of compounds

The goal of bioassay-guided fractionation is to separate molecules with the bioactivity of interest from complicated mixtures using separation and analytical techniques. The material undergoes sequential fractionation and purification in conjunction with biological activity assessment (van Beek et al., 2009). The sponge extract and fractions were screened against AChE and BChE enzymes. The cholinesterase inhibitory activity of the extract, *n*-hexane, and ethyl acetate fractions have been reported in our previous publications (Putri et al., 2023). In the current study, bioassay-guided fractionation was carried out on the water fraction, followed by identification of the components. The water fraction showed inhibition against AChE and BChE with IC_{50} values of 1.95 and 19.37 µg/mL, respectively. The sample was then subjected to flash column chromatography to obtain seven fractions, namely F1-F7, and later screened for the ChE inhibitory activities. The results (Fig. 1) indicated that fractions F3 and F4 showed higher inhibition against AChE and BChE enzymes than other fractions.

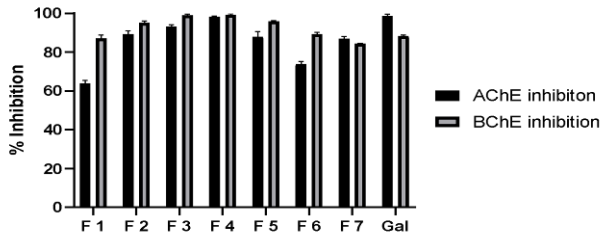

Figure 1. The AChE and BChE inhibitory activities of fractions F1-F7 and control (galantamine).
All groups were screened at 100 µg/mL.

Purification and identification of compounds

Based on the results of the bioassay, the chromatographic profile and the weight of the samples, fractions F3 and F4 were targeted for purification by using HPLC. The chromatography separation of F3 yielded a peak at 18.8 minutes (Fig. 2). Thin layer chromatography (TLC) of the isolated compound (**1**) appeared as an orange spot upon derivatization with Dragendorff reagent, which indicated the presence of an alkaloid. The mass spectrometry of compound **1** recorded a molecular ion at *m/z* $[M+H]^+$ 229.0971, corresponding to the molecular formula of $C_{13}H_{12}N_2O_2$ with 9 degrees of unsaturation. The ^1H NMR spectrum of compound **1** recorded in CD_3OD exhibited four doublets at δ_H 7.69, 7.21, 6.79, and 6.27 ppm, a singlet at δ_H 6.96 ppm, and two methoxy signals at δ_H 3.99 and 3.89 ppm, which suggested the presence of benzo[de][1,6]-naphthyridine. The ^1H and

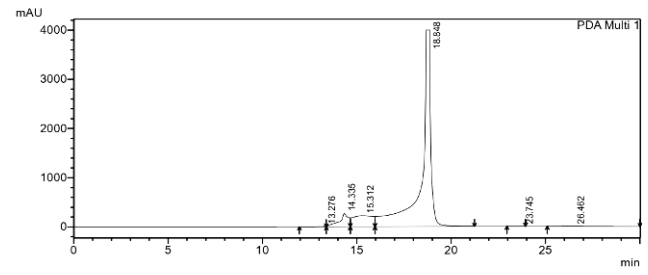

Figure 2. HPLC Profile of fraction F3 (detection with UV 254 nm).

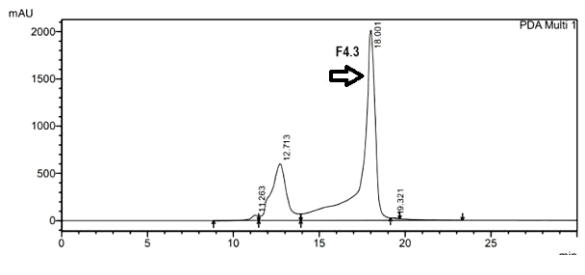

Figure 3. HPLC Profile of fraction F4 (Detection with UV 254 nm).

Table 1 ^{1}H and ^{13}C NMR data of compound (**1**).

Position	δH (ppm)	δC (ppm)
2	7.69 (d, 7.0 Hz)	140.9
3	6.27 (d, 7.0 Hz)	98.0
3′		150.5
5	7.21 (d, 7.2 Hz)	129.6
6	6.79 (d, 7.2 Hz)	112.9
6′		132.9
7	6.96 (s)	100.6
8		157.4
9		131.9
9′		133.9
9"		116.8
8-O-CH_3	3.99 (s)	55.6
9-O-CH_3	3.89 (s)	59.8

*MeOH-*d4* peak was referenced at 3.31 ppm for ^{1}H NMR and 49.0 ppm for ^{13}C NMR; coupling constant (*J*) are reported in Hz.

^{13}C NMR spectra of compound **1** (Table 1) resembled aaptamine that has been isolated from *A. suberitoides* and several other *Aaptos* species, such as *A. aaptos*, *A. lobata*, and *A. nigra* (He et al., 2020; Pham et al., 2013).

Fraction F4 was subjected to HPLC, and three fractions were collected (F4.1-F4.3). The major peak, fraction F4.3, at 18.0 mins (Fig. 3) was subjected for identification. Five compounds were identified in fraction F4.3 based on LC-MS/MS spectrometry data, namely N-demethylaaptanone (**2**), aaptanone (**3**), demethylaaptamine (**4**), aaptosine (**5**), and 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine (**6**) (Table 2, Fig. 4). Compounds **1**, **4**, and **6** were also detected in the ethyl acetate fraction of *A. suberitoides*, as reported in our previous publication (Putri et al., 2023). All identified compounds were benzo[de][1,6]-naphthyridine alkaloids, which is the characteristic of the genus *Aaptos*. Utkina and Denisenko (2016) isolated N-demethylaaptanone (**2**) along with aaptanone (**3**) and 9-demethylaptamine (**4**) from a Vietnamese sponge *A. aaptos* (Utkina et al., 2009; Utkina and Denisenko, 2016). Demethylaaptamine (**4**) was first reported by Nakamura et al. (1987) from the Okinawan marine sponge *A. aaptos*, from which the same group discovered aaptamine (**1**). Due to the limited yield of the sample, an attempt to isolate the individual compounds in fraction F4.3 was not carried out

In vitro cholinesterase inhibitory activity of isolated compounds

The isolated compound, aaptamine and fraction F4.3 containing a mixture of five compounds were subjected to *in vitro* cholinesterase inhibitory assay towards AChE and BCHE. The results presented in Fig. 5 suggested that both samples inhibited AChE and BCHE in a dose-dependent manner. The isolated aaptamine exhibited significant cholinesterase inhibitory activities with IC_{50} values of 0.23 and 1.38 µg/mL against AChE and BChE, respectively. Likewise, fraction F4.3 demonstrated similar inhibitory effects against AChE and BChE with IC_{50} values of 0.39 and 1.61 µg/mL, respectively (Table 3). These data suggested that aaptamine and a mixture of benzo[de][1,6]-naphthyridine alkaloids in fraction F4.3 gave stronger inhibition towards AChE as compared to BChE. Our findings are in agreement with those

Table 2. LC-MS/MS data for identified alkaloids from fraction F4.3.

Compound	RT[a] (min)	$[M+H]^-$	Product ions *m/z*[b]	Formula	Exact mass	Mass error (ppm)
2	7.844	243.0411	228.0161, 200.0214, 172.0269, 144.0321, 116.0377, 88.0146, 64.0194	$C_{12}H_8N_2O_4$	243.0411	0.12
3	8.334	257.0556	242.0314, 227.0081, 199.0136, 171.0191, 143.0246, 115.0295, 87.0083	$C_{13}H_{10}N_2O_4$	257.0568	4.59
4	8.748	213.0668	198.0423, 185.0327, 169.0398, 153.0452, 141.0440	$C_{12}H_{10}N_2O_2$	213.0670	0.71
5	9.477	227.0827	212.0573,197.0342,169.0396,153.0441,141.0452	$C_{13}H_{12}N_2O_2$	227.0826	-0.44
6	11.074	257.0921	227.0445, 212.0207, 200.0205, 184.0255, 125.0583, 84.0216	$C_{14}H_{14}N_2O_3$	257.0932	4.15

[a]RT: Retention Time, [b]Product ions at collision energy 20 eV.

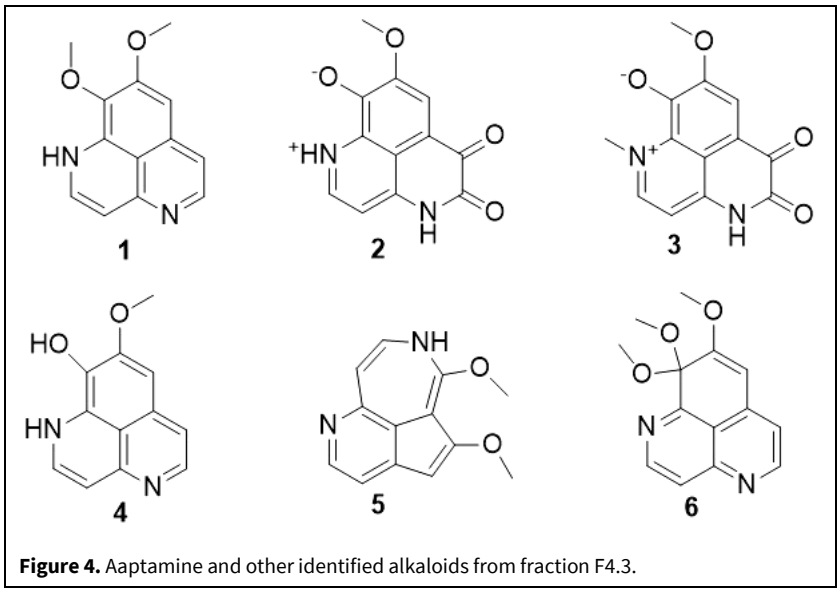

Figure 4. Aaptamine and other identified alkaloids from fraction F4.3.

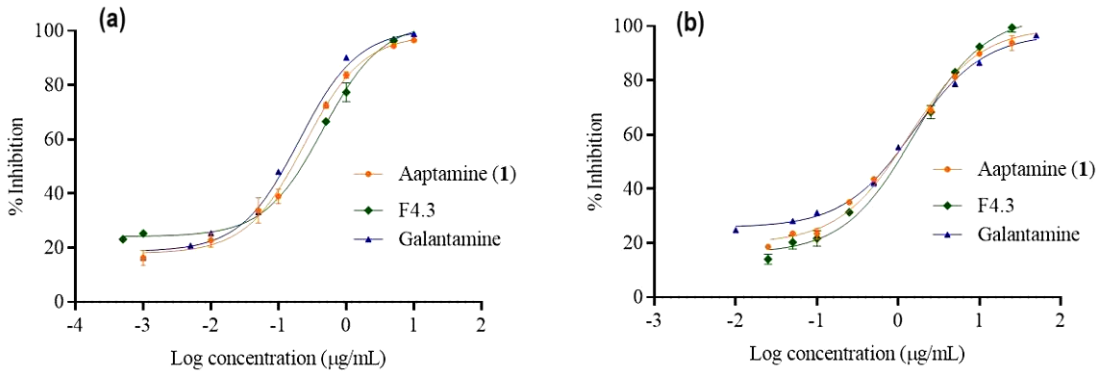

Figure 5. Dose-dependent response of aaptamine (1), fraction F4.3, and galantamine against **(a)** AChE and **(b)** BChE.

Table 3. Cholinesterase inhibitory activity of fractions, aaptamine (**1**), and galantamine.

Sample	IC_{50} value (µg/mL)[a]	
	AChE	**BChE**
Water fraction	1.95 ± 0.04	19.37 ± 0.59
Fraction F3	0.14 ± 0.01	3.11 ± 0.03
Fraction F4	0.74 ± 0.02	3.37 ± 0.15
Fraction F4.3	0.39 ± 0.04	1.61 ± 0.14
Aaptamine (**1**)	0.23 ± 0.01	1.38 ± 0.06
Galantamine	0.20 ± 0.01	1.49 ± 0.05

[a]Data presented as mean ± SEM of three experiments, each carried out in triplicates.

reported by Miao et al. (2022), which outline the cholinesterase inhibitory of aaptamine against AChE and BChE. To the best of our knowledge, the cholinesterase inhibitory activity of N-demethylaaptanone (**2**), aaptanone (**3**), demethylaaptamine (**4**), aaptosine (**5**), and 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine (**6**) has not been reported.

Alkaloids have been recorded as the major class of compounds with cholinesterase inhibitory activities. A well-known example of terrestrial origin is galantamine, an isoquinoline alkaloid first isolated from *Galanthus nivalis* and *Galanthus woronowii* (Kim and Park, 2017). The discovery of galantamine led to the findings of other alkaloids as potent cholinesterase inhibitors, including from marine sources (Ahmed et al., 2021; Berkov et al., 2022; Moodie et al., 2019). Cholinesterase inhibitors have been reported from sponges, algae, cnidarians, molluscs, bryozoans, echinoderms, tunicates, and microorganisms.

Molecular docking analysis

A metabolite that is optimally docked was identified with the best combination of S-score (<2Å) and RMSD value (<−7 kcal/mol). Table 4 lists the docking results for all analyzed molecules.

Protein-ligand interaction with AChE

The reference ligand, galantamine, displayed the lowest S-score (-7.73 kcal/mol), suggesting a strong binding affinity for AChE. In comparison, the six analyzed compounds showed S-scores between -5.64 and -6.65 kcal/mol, with aaptamine **(1)** having the lowest score (-6.65 kcal/mol) among N-demethylaaptanone **(2)**, aaptanone **(3)**, demethylaaptamine **(4)**, aaptosine **(5)**, and 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine **(6)**. This indicates that, while aaptamine **(1)** binds well to AChE, its affinity is lower than that of galantamine. The RMSD value for all six compounds and reference ligand (<2Å) showed the accurate prediction of the ligand binding model between compounds and AChE.

The active site of acetylcholinesterase (AChE) is composed of four key subunits: The esteratic site, anionic subsite, oxyanion hole, and acyl pocket. The esteratic site housed the catalytic triad (Ser203, His447, and Glu334) that catalyzes the hydrolysis of ACh (Peitzika and Pontiki, 2023; Rosales Hernández et al., 2022). The peripheral anionic site consisted of key residues, including Trp286, Tyr124, Asp74, Ser125, and Phe295. Additionally, the active site features the oxyanion hole, an anionic subsite, and the acyl pocket, which includes residues Gly448, Glu202, Ile451, Tyr133, and Trp86 (Rosales Hernández et al., 2022). These subunits regulate the catalysis of AChE. The binding site of the AChE includes amino acid residues Trp86, Gly120, Gly121, Gly122, Glu202, Phe295, Phe297, Tyr337 and His447. Galantamine formed four conventional hydrogen bonds, 12 van der Waals interactions, and two carbon-hydrogen bonds with AChE, as shown in Table 4. In Fig. 6, galantamine formed four conventional hydrogen bonds with Glu202, Ser203 and Gly121, Tyr337 from the binding site. Galantamine formed 12 van der Waals interactions at Gly120, Gly122, Tyr124, Tyr133, Ala204, Trp236, Phe295, Phe338, Tyr341, Gly448, Ile451 and formed two carbon-hydrogen bonds at Ser125 and His447. The docking conformation (Fig. 6) also showed galantamine occupied the key amino acid residues of the active site to prevent the substrate from interacting with the enzyme. Comparatively, aaptamine (**1**) formed a conventional hydrogen bond at Tyr337 from the binding site. The compound formed 12 van der Waals interactions at Gly121, Ser125, Gly126, Ala127, Leu130, Tyr133, Ser203, Tyr341, Trp439, Gly448, Tyr449 and Ile451 while displaying three carbon-hydrogen bonds at Gly120, Glu202 and His447 with AChE. Aaptamine **(1)** also interacted with Asp74, Trp86, Glu202, and His447, at the active site. It potentially obstructs the active site by forming interactions with binding site residues Tyr337 and Gly120. All docking interactions indicated that aaptamine may exhibit inhibitory properties similar to galantamine. The docking conformation of

aaptamine (**1**) with AChE is shown in Fig. 7. The detailed computational analysis values for all compounds are recorded in Table 4.

Protein-ligand interaction with BChE

Table 4 shows the binding affinity of all six compounds with the reference ligand, tacrine. Tacrine recorded an S-score of -6.05 kcal/mol with BChE. The compounds analyzed had S-scores ranging from -5.60 to -6.40 kcal/mol. Galantamine, as an approved AD drug, was also docked with BChE to evaluate its binding affinity for comparison against the six sponge compounds. The docking experiment recorded a combination of S-score and RMSD of -6.07 kcal/mol and 1.68Å with BChE. As recorded in Table 4, two compounds, namely 8,9,9-trimethoxy-9H-benzo[de][1,6]naphthyridine (**6**) and aaptanone (**3**), exhibited S-scores of -6.22 and -6.09 kcal/mol, respectively which is greater in binding affinity compared to the reference ligand, tacrine. However, as previously highlighted, an RMSD value of lower than 2Å is recommended in considering potential of a compound. Therefore, from this perspective, both 8,9,9-trimethoxy-9H-benzo[de][1,6]naphthyridine (**6**) and aaptanone (**3**) did not fulfill the requirements between the predicted and expected binding poses. On the other hand, aaptamine (**1**) had an S-score value almost comparable to both tacrine and galantamine with an RMSD value lower than 2Å, suggesting that aaptamine (**1**) has a similar binding affinity to both the controls with accurate model prediction.

The active site of BChE is composed of five key subunits: the catalytic triad (Glu325, Ser198, and His438), an anionic site (Trp82, Tyr128, and Phe329), a peripheral anionic site (Asp70 and Tyr332), an oxyanion hole (Gly116, Gly117, and Ala199), and an acyl pocket (Val288 and Leu286). Each of these regions plays a distinct role in substrate recognition and catalysis (Ji et al., 2022; Wajid et al., 2019). The binding site of BChE includes amino acid residues Trp82, Glu197, Ala328, Tyr332, Trp430, and His438. Tacrine formed one conventional hydrogen bond and thirteen (13) van der Waals interactions with BChE, as shown in Table 4.

Table 4. Molecular docking results of selected compounds with acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) protein.

Name	S score (kcal/mol)	RMSD (Å)	Hydrogen bond	Van der Waals	Carbon hydrogen bond	Amino acid residue (H bond)
Acetylcholinesterase (AChE)						
Galantamine	-7.73	1.74	4	12	2	*GLY121; SER203; *TYR337
Aaptamine (**1**)	-6.65	0.57	1	12	3	*TYR337
Aaptanone (**3**)	-6.32	1.04	1	12	6	*GLU202
8,9,9-Trimethoxy-9H-benzo[de][1,6]naphthyridine (**6**)	-6.04	1.32	0	17	1	-
N-Demethylaaptanone (**2**)	-6.03	1.39	3	13	0	*GLY120; *GLU202; *TYR337
Demethylaaptamine (**4**)	-5.67	0.84	2	11	3	*GLY120; SER125
Aaptosine (**5**)	-5.64	1.06	2	12	4	TYR133; *HIS447
Butyrylcholinesterase (BChE)						
8,9,9-Trimethoxy-9H-benzo[de][1,6]naphthyridine (**6**)	-6.22	2.31	0	14	5	-
Aaptanone (**3**)	-6.09	2.69	1	13	1	*HIS438
Galantamine	-6.07	1.68	1	15	2	ASP70
Tacrine	-6.05	1.58	1	13	0	*HIS438
Aaptamine (**1**)	-5.98	1.05	1	13	3	GLY115
Aaptosine (**5**)	-5.87	1.20	0	14	3	-
Demethylaaptamine (**4**)	-5.64	2.04	2	13	1	GLU197; *HIS438
N-Demethylaaptanone (**2**)	-5.60	0.85	2	12	1	GLY115; *HIS438

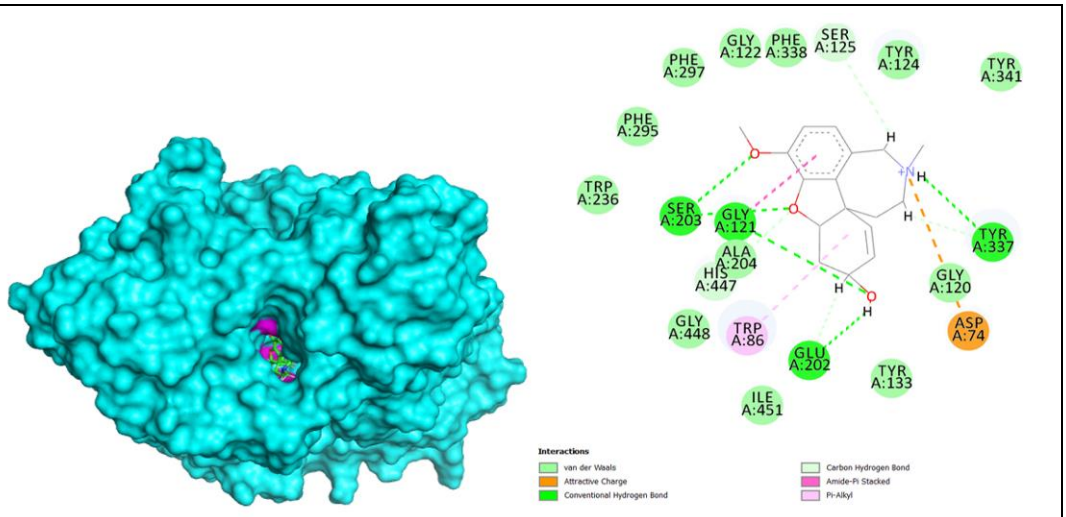

Figure 6. The docking conformation of AChE with galantamine.

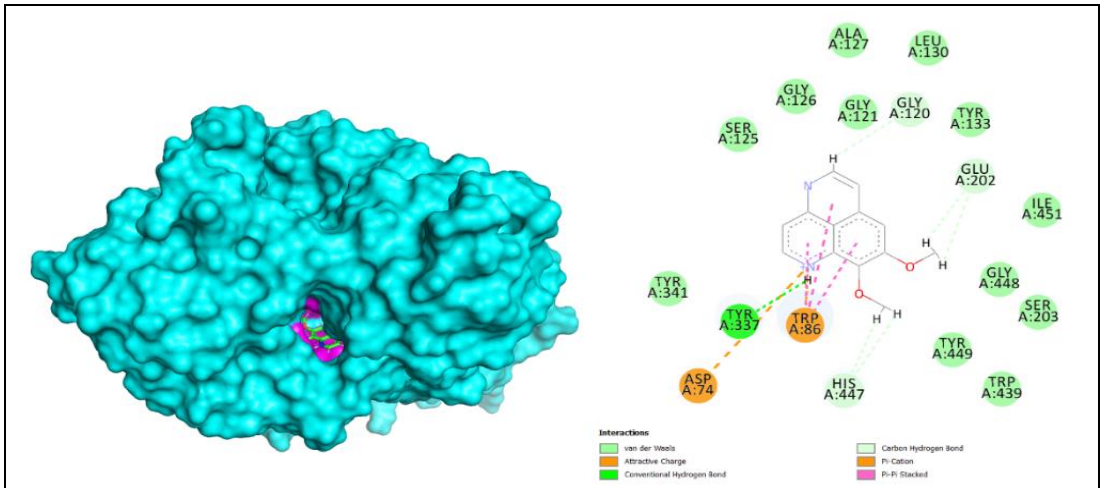

Figure 7. The docking conformation AChE with aaptamine (**1**).

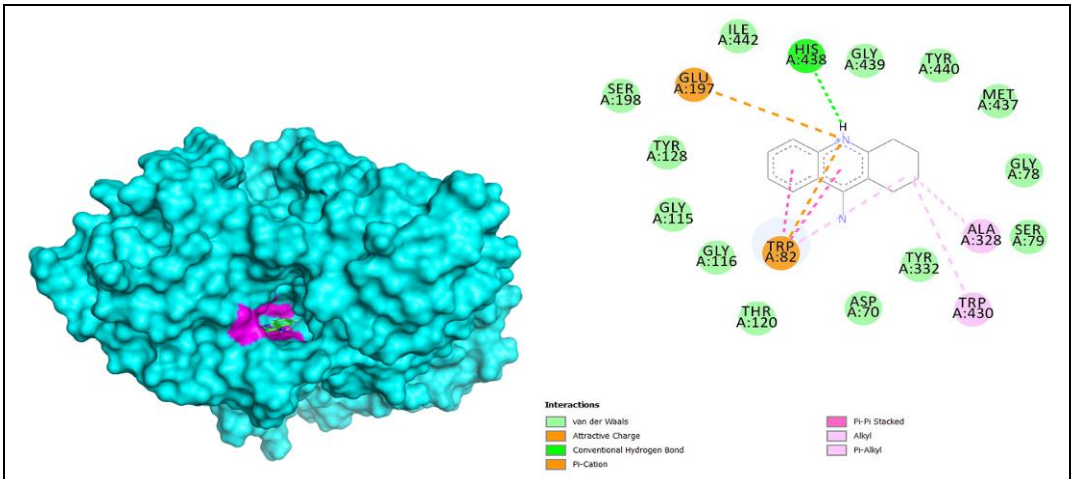

Figure 8. The docking conformation of BChE with tacrine.

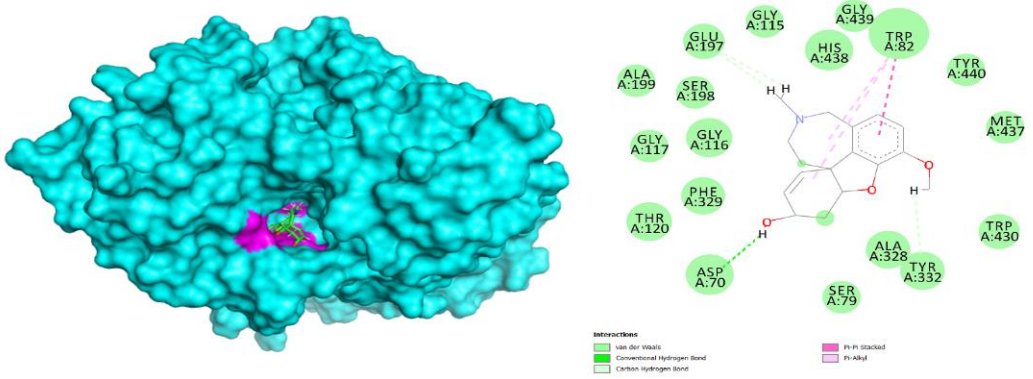

Figure 9. The docking conformation of BChE with galantamine.

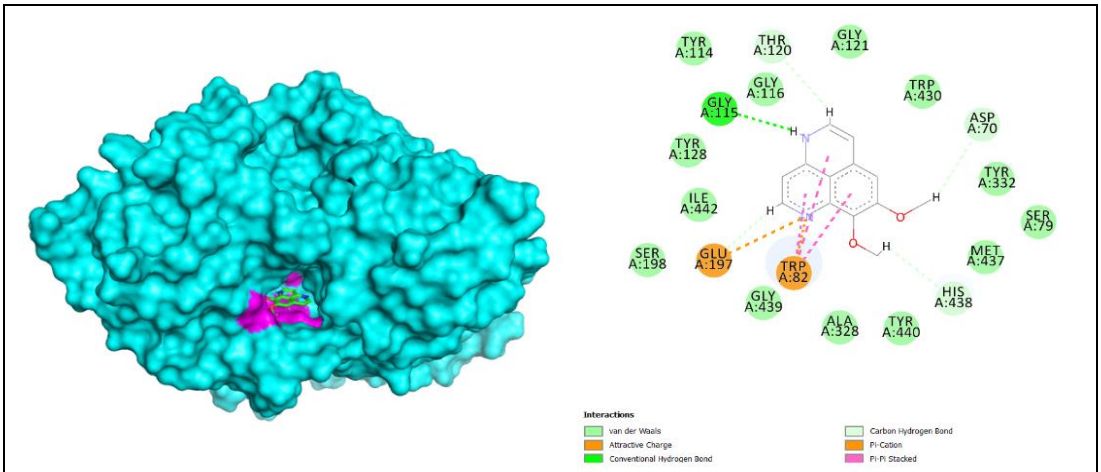

Figure 10. The docking conformation BChE with aaptamine (**1**).

As shown in Fig. 8, tacrine formed the conventional hydrogen bond with amino residue His438 from the binding site while showing thirteen (13) van der Waals interactions at Asp70, Gly78, Ser79, Gly115, Gly116, Thr120, Tyr128, Ser198, Tyr332, Met437, Gly439, Tyr440 and Ile442. On the other hand, galantamine formed the conventional hydrogen bond with Asp70, fifteen (15) van der Waals reactions at Ser79, Trp82, Gly115, Gly116, Thr120, Ser198, Phe329, Trp430, Met437, His438, Gly439 and Tyr440, and two carbon-hydrogen bonds at Trp82 and Tyr332, as shown in Fig. 8. Compared to both tacrine and galantamine, aaptamine (**1**) displayed one conventional hydrogen bond, thirteen (13) van der Waals interactions, and three carbon-hydrogen bonds with BChE. Aaptamine (**1**) formed the conventional hydrogen bond at Gly115 while formed van der Waals interactions at Ser79, Tyr114, Gly116, Gly121, Tyr128, Ser198, Ala328, Tyr332, Trp430, Met437, Gly439, Tyr440 and Ile442. Aaptamine (**1**) also formed carbon-hydrogen bonds at Asp70, Thr120 and His438. Tacrine interacted with the active site of BChE at Trp82 and His438 while aaptamine **(1)** occupied the amino acid residues at the catalytic triad (His 438), anion site (Trp82), and peripheral anionic site (Asp70), as shown in Figs. 8 and 10. The analysis of amino acid interactions indicates that aaptamine **(1)** shares similar binding characteristics with tacrine, interacting specifically at residues Trp82, Glu197, and His438. This observation suggests that aaptamine **(1)** exhibited greater BChE inhibitory properties more closely aligned with tacrine than with galantamine. The protein-ligand interactions of BChE with tacrine, galantamine, and aaptamine (**1**) are shown in Figs. 8-10.

Our data well complements the only available study by Miao et al. (2022), who confirmed that aaptamine **(1)** interacted with key amino acid residues in the active sites of AChE and BChE through hydrogen bonds or π-π interaction. However, this research conducted docking simulation using the recombinant human AChE in complex with donepezil (4EY7) and BuChE with N-[1-(2,3-dihydro-1H-inden-2-yl) piperidin-3-yl)methyl]-N-(2-(dimethylamino)ethyl)-2-naph thamide (4TPK) which slightly varies with our data.

In silico drug-likeness analysis

Drug-likeness analysis is an important screening tool in early drug development to develop a hit compound into a lead compound. Lipinski's rule of five described the important compound properties such as molecular weight, lipophilicity, hydrogen bonds donors/acceptors, and number of rotatable bonds for an orally bioavailable lead (Lipinski et al., 2012). Additionally, the number of rotatable bonds and topological polar surface area are criteria for a high probability of good oral bioavailability compound (Veber et al., 2002). Violations of any of the criteria mentioned above may cause problems with bioavailability. ADMETlab 3.0 was used to screen the molecular properties of the six compounds, galantamine and tacrine based on Lipinski's rule of five and Veber's variation. Drug-likeness analysis (Table 5) showed none of the six compounds violated the criteria.

In silico studies on the absorption, distribution, metabolism, excretion, and toxicity (ADME/T) properties of the compounds can provide insights into their pharmacokinetics and pharmacodynamics, which contribute to their safety and efficacy. These studies can also provide predictive structural information to improve the compounds during drug design and development (Zhong, 2017). Therefore, the ADME/T properties of the compounds were evaluated and compared with galantamine and tacrine *in silico* using ADMETlab 3.0 (Table 6). Absorption screening provided absorption and permeation information on the safety and efficacy of the compounds. Absorption screening showed that, except for demethylaaptanone and aaptanone, the other four sponge compounds showed overall good intestinal absorption and permeation that was similar to galantamine.

BBB permeation and PPB are two critical molecular properties that facilitate drug distribution in the body for anti-Alzheimer compound screening (Ji et al., 2022; Zhong, 2017). High BBB permeability demonstrated the compound's capacity to effectively penetrate the BBB and exert a localized anti-Alzheimer's effect within the brain, while low PPB indicated high compound availability and its ability to cross cellular membranes, engage with target receptors, and undergo metabolism. 8,9,9-Trimethoxy-9H-benzo[de][1,6]-naphthyridine showed a favorable distribution profile with both BBB permeation and PPB, while aaptanone and aaptosine only showed low PPB.

The cytochrome P450 (CYP) enzyme family plays a crucial role in the metabolism of xenobiotics and pharmaceuticals. Compounds that inhibit or bind to these enzymes can lead to compound-induced toxicity or result in compound inactivation (Foti and Dalvie, 2016; Zhong, 2017). Aaptamine is an inhibitor of CYP1A2 and serves as a substrate for both CYP1A2 and CYP2D6. Demethylaaptanone is an inhibitor of CYP1A2, whereas aaptanone is a substrate of CYP1A2. Similarly, demethylaaptamine is an inhibitor of CYP1A2 and a substrate for both CYP2C9 and CYP2D6. Aaptosine inhibits multiple CYP enzymes, specifically CYP1A2, CYP2C19, CYP3A4, and CYP2B6, while is also a substrate for CYP1A2 and CYP2D6. Lastly, 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine is an inhibitor of CYP1A2 and CYP2B6, and acts as a substrate for CYP1A2, CYP2C19, and CYP2C9.

Plasma clearance and half-life indicated the excretion of the compound from the body. Generally, plasma clearance rates higher than 15 mL/min/kg are considered high; 5-15 mL/min/kg is moderate clearance; lower than 5 mL/min/kg is low plasma clearance. Ultra-short half-life drugs have a half-life of less than 1 hour; short half-life drugs have a half-life between 1 and 4 hours; intermediate half-life drugs have a half-life between 4 and 8 hours; and long half-life drugs, with a half-life greater than 8 hours (Dong et al., 2018; Zhong, 2017). All six sponge compounds showed moderate plasma clearance and short half-life. Toxicological evaluation was predicted using the ADMET3.0 lab, and the potential pharmacodynamic risk of the compounds was shown. Galantamine posed a risk of ROA, carcinogenicity, H-HT, and neurotoxicity-DI. Meanwhile, tacrine showed Ame's toxicity, carcinogenicity, H-HT, neurotoxicity-DI, and hematotoxicity. All six sponge compounds posed a risk of Ames toxicity, DILI, carcinogenicity, H-HT, and neurotoxicity-DI. Besides that, demethylaaptanone and aaptanone also showed hematotoxicity, while 8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine showed ROA. Toxicological evaluation of six sponge compounds showed to have a high risk of mutagenicity, liver toxicity, carcinogenicity, and neurotoxicity in general.

In silico drug-likeness analysis of six sponge derived compounds revealed favorable oral and intestinal absorption profiles, a weak distribution profile, and moderate metabolism and excretion properties. However, these compounds exhibited a high risk of toxicity. Given their natural origins, further optimization through drug design is warranted to enhance their ability to cross the blood-brain barrier and to mitigate their toxicity risks. This approach could improve the therapeutic potential of these compounds, making them more suitable for clinical applications.

Table 5. Drug-likeness analysis of six sponge compounds, galantamine, and tacrine.

Compound	MW [a]	LogP [b]	HBD [c]	HBA [d]	n-ROTB [e]	TPSA (Å^2) [f]	Lipinski's Violation
Rule	<500	≤5	≤5	≤10	≤10	≤140	≤1
Aaptamine	228.09	2.39	1	4	2	43.38	0
demethylaaptanone	244.05	0.87	2	6	1	88.52	0
aaptanone	258.06	0.24	1	6	1	82.34	0
demethylaaptamine	214.07	1.97	2	4	1	54.38	0
aaptosine	228.09	1.62	1	4	2	43.38	0
8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine	258.10	1.88	0	5	3	53.47	0
Galantamine	287.15	0.80	1	4	1	41.93	0
Tacrine	198.12	2.43	2	2	0	38.91	0

[a]Molecular weight; [b]Logarithm of compound partition coefficient between n-octanol and water; [c]Hydrogen bond donors; [d]Hydrogen bond acceptors; [e]Number of rotatable bonds; [f]Topological polar surface area.

Table 6. Adsorption, distribution, metabolism, excretion, and toxicity profile of six sponge compounds, galantamine, and tacrine.

Molecule	Aaptamine	Demethylaaptanone	Aaptanone	Demethylaaptamine	Aaptosine	8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine	Galantamine	Tacrine
Absorption								
Caco2, log unit	-4.59	-5.16	-5.80	-5.02	-4.44	-4.75	-4.31	-4.68
pgp_inh*	0.24	0.04	0.05	0.03	0.29	0.26	0.06	0.06
pgp_sub*	0.65	0.01	0.16	0.27	0.64	0.65	0.67	0.18
hia*	0.93	0.29	0.05	0.76	0.87	0.96	1.00	1.00
Distribution								
BBB*	0.22	0.00	0.00	0.08	0.12	0.72	1.00	0.98
PPB,% [a]	92.91	96.77	54.37	98.03	82.70	73.49	21.32	59.57
Metabolism								
CYP1A2-inh*	0.96	0.92	0.07	1.00	0.99	0.89	0.00	1.00
CYP1A2-sub*	0.87	0.21	0.83	0.41	0.75	0.92	0.43	0.99
CYP2C19-inh*	0.27	0.05	0.00	0.39	0.82	0.65	0.00	0.03

Table 6. Adsorption, distribution, metabolism, excretion, and toxicity profile of six sponge compounds, galantamine, and tacrine (continued...)

Molecule	Aaptamine	Demethylaaptanone	Aaptanone	Demethylaaptamine	Aaptosine	8,9,9-trimethoxy-9H-benzo[de][1,6]-naphthyridine	Galantamine	Tacrine
CYP2C19-sub*	0.59	0.00	0.00	0.03	0.39	0.96	0.87	0.89
CYP2C9-inh*	0.11	0.19	0.00	0.28	0.59	0.09	0.00	0.00
CYP2C9-sub*	0.58	0.00	0.00	0.71	0.41	0.75	0.00	0.00
CYP2D6-inh*	0.09	0.12	0.00	0.18	0.53	0.05	0.12	0.23
CYP2D6-sub*	0.83	0.00	0.00	0.93	0.90	0.49	0.98	0.83
CYP3A4-inh*	0.52	0.05	0.00	0.11	0.81	0.24	0.00	0.00
CYP3A4-sub*	0.63	0.01	0.00	0.01	0.06	0.36	0.40	0.97
CYP2B6-inh*	0.55	0.00	0.00	0.13	0.86	0.90	0.01	0.02
CYP2B6-sub*	0.07	0.00	0.00	0.00	0.01	0.98	0.00	0.00
Excretion								
cl-plasma,ml/min/kg	5.81	5.31	5.72	6.23	8.48	6.49	6.18	5.29
T0.5	1.25	1.16	1.44	1.40	0.96	1.33	5.09	0.99
Toxicity								
hERG*	0.18	0.07	0.09	0.11	0.17	0.21	0.36	0.37
hERG-10um*	0.46	0.37	0.24	0.40	0.45	0.52	0.44	0.66
DILI*	0.87	0.98	0.96	0.86	0.90	0.86	0.46	0.60
Ames*	0.81	0.84	0.75	0.86	0.82	0.82	0.56	0.88
ROA*	0.59	0.51	0.64	0.61	0.55	0.71	0.71	0.33
Carcinogenicity*	0.81	0.76	0.75	0.77	0.81	0.75	0.73	0.73
H-HT*	0.72	0.89	0.87	0.72	0.73	0.71	0.80	0.75
Neurotoxicity-DI*	0.93	0.87	0.48	0.88	0.89	0.88	0.74	0.92
Hematotoxicity*	0.50	0.91	0.84	0.41	0.49	0.60	0.51	0.72
RPMI-8226*	0.16	0.17	0.08	0.18	0.18	0.14	0.10	0.10
HEK293*	0.42	0.60	0.61	0.46	0.36	0.47	0.24	0.60

*The output value indicates the probability range from 0 to 1; [a]The value is the degree of plasma protein binding fraction, optimal <90%. Red colored are unfavorable properties.

Conservation of natural resources

In the context of AD, there has been a 117% rise in the global estimates of dementia patients from 1990 to 2016 (GBD 2016 Dementia Collaborators, 2019). By 2050, it is projected that 152 million individuals will be affected by Alzheimer's disease and other forms of dementia (GBD 2019 Dementia Forecasting Collaborators, 2022). In 2019, Southeast Asia had a notable rise in instances, with figures showing 400,000 cases, which is more than quadruple the number reported in 1990. This enhances the dependence on natural products (Li et al., 2022).

Human reliance on marine resources for sustenance and medicinal purposes dates back centuries, making the conservation of these resources as paramount. Marine organisms are invaluable sources of bioactive compounds with pharmaceutical applications and are a largely untapped reservoir. In recent years, the degradation of marine ecosystems has raised global concerns. As part of the Coral Triangle Region, Indonesia is located in the center of worldwide maritime biodiversity. Coastal populations depend on marine environments for sustenance, livelihoods, coastal protection, and cultural practices (Amkieltiela et al., 2022). The loss of marine species and habitat through natural and anthropogenic means could hinder new discoveries, resulting in the development of legal frameworks aimed at ensuring their sustainable use (Romanelli et al., 2015).

The importance of conserving marine resources in Indonesia is clearly in line with international agenda with ratification of conventions such as the 1982 United Nations Convention on the Law of the Sea (UNCLOS), the 1992 Convention on Biological Diversity (CBD), the 1995 Code of Conduct for Responsible Fisheries (CCRF) for fisheries protection, and the Nagoya Protocol that emphasize the fair and equitable sharing of benefits (ABS) arising from the utilization of genetic resources (Buck and Hamilton, 2011). The ABS system provides for the way in which biological resources may be accessed, and how users who access biological resources perform scientific research to develop pharmaceuticals and how providers of biological resources who grant the users access to the biological resource in exchange for monetary or non-monetary benefits from their use, reach agreement on the fair and equitable sharing of benefits that might arise from their utilization.

Domestically, Indonesia has protected its coastal ecosystems and biodiversity through customary means called *adat* (e.g., periodic fisheries closures and marine protected areas managed by fishers and communities formed through customary law) and formalized conservation through Marine Protected Areas (MPA) managed under (i) the Ministry of Marine Affairs and Fisheries (MMAF) and (ii) the Ministry of Environment and Forestry (MoEF) with the intention to conserve biodiversity and sustainable fisheries as stated in the 1945 Constitution of the Republic Indonesia Article 33(4) (Amkieltiela et al., 2022; Mardiastuti, 2019; Widayanti et al., 2022). This is further guided by national legislations: Law Number 5 of 1994 to implement the 1992 CBD, Law Number 32 of 2009 concerning Environmental Protection and Management, Law Number 32 of 2014 concerning Marine Affairs, Presidential Regulation No 56 of 2019 concerning the National Action Plan for Integrated Management of National Areas and National Water Conservation Areas for 2018-2025, and Presidential Regulation No 34 of 2022 concerning Action Plans for Indonesian Marine Policy, for ensuring that marine genetic resources are ethically and sustainably accessed for pharmaceutical research (Widayanti et al., 2022).

As such, Indonesia is well-poised from a policy perspective to protect the marine ecosystem but has governance challenges for biodiversity conservation, ranging from a lack of specialized courts to inadequate sentencing issues and profit-based motives that do not support the SDG 14 goals. Sponges are invertebrates that are widely distributed worldwide from shallow to deep waters. Knowledge of sponge biodiversity is still substantially inadequate. Approximately 11,000 species have been officially described, with around 8,500 being recognized as legitimate and their status in IUCN, the red list is probably not updated (Van Soest et al., 2012). Though it may seem abundant, they are being affected by climate change, where warming oceans kill off the microorganisms and crustaceans reliant on sponges. Given the importance of sponges in the treatment of AD, as revealed in this study, mass production of the active ingredient, in this case aaptamine (**1**), through synthetic means is the best solution. Alternatively, the cultivation of *A. suberitoides* through aquaculture is the way forward. The marine sponge was first farmed with bath sponges over 5000 years ago and has been experimentally cultivated for over 20 years now for their biologically active metabolites (Duckworth, 2009; Maslin et al., 2021). Sponge aquaculture is not complicated and requires simple tools and low investment to perform. To date, it is proven that both sea- and land-based studies have shown yield in months. Therefore, it is the best solution to address depleting sources of sponge stocks in the wild (Duckworth, 2009).

CONCLUSION

The results obtained in this study demonstrated the potency of *Aaptos suberitoides* aqueous fraction and its secondary metabolites as cholinesterase inhibitors. This bioactivity is related to the presence of aaptamine and other benzo[de][1,6]-naphthyridine alkaloids. Further research is needed to isolate the alkaloids from the mixture and evaluate the bioactivity of the isolated compounds to confirm their activity in the *in vitro* study. This indeed warrants more study on the benzo[de][1,6]-naphthyridine for Alzheimer's disease.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the SATU President forum for SATU Joint Research Scheme (JRS) grants between Universitas Airlangga (Project No. 1259/UN3.15/PT/2022) and Universiti Malaya (Project No. ST093-2022).

REFERENCES

Abdillah S, Nurhayati, Dyah AP, Nurhatika S, Setiawan E, Heffen WL (2013) Cytotoxic and antioxidant activities of marine sponge diversity at Pecaron Bay Pasir Putih Situbondo East Java, Indonesia. J Pharm Res 6: 685–689. https://doi.org/10.1016/j.jopr.2013.07.001

Aguiar ACC, Parisi JR, Granito RN, de Sousa LRF, Renno ACM, Gazarini ML (2021) Metabolites from marine sponges and their potential to treat malarial protozoan parasites infection: A systematic review. Mar Drugs 19: 134. https://doi.org/10.3390/md19030134

Ahmed S, Khan ST, Zargaham MK, Khan AU, Khan S, Hussain A, Uddin J, Khan A, Al-Harrasi A (2021) Potential therapeutic natural products against Alzheimer's disease with reference of acetylcholinesterase. Biomed Pharmacother 139: 111609. https://doi.org/10.1016/j.biopha.2021.111609

Amkieltiela, Handayani CN, Andradi-Brown DA, Estradivari, Ford AK, Beger M, Hakim A, Muenzel DK, Carter E, Agung F, Veverka L, Iqbal M, Lazuardi ME, Fauzi MN, Tranter SN, Ahmadia GN (2022) The rapid expansion of Indonesia's marine protected area requires improvement in management effectiveness. Mar Policy 146: 105257. https://doi.org/10.1016/j.marpol.2022.105257

Aristyawan AD, Setyaningtyas VF, Wahyuni TS, Widyawaruyanti A, Ingkaninan K, Suciati S (2022) *In vitro* acetylcholinesterase inhibitory activities of fractions and iso-agelasine C isolated from the marine sponge *Agelas nakamurai*. J Res Pharm 26: 279–286. https://doi.org/10.29228/jrp.126

Bashari MH, Arsydinilhuda FZ, Ilhamsyah RS, Nugrahani AD, Nurdin RA, Kartika A, Huda F, Abdurahman M, Putri T, Qomarilla N, Atmaja H, Sudji IR, Subhan B, Usman HA, Pamela Y, Ariyanto EF, Meiyanto E (2021) The ethanol extract of marine sponge *Aaptos suberitoides* suppress cell viability, cell proliferation and cell migration in HER2-positive breast cancer cell line. Asian Pac J Cancer Prev 22: 25–32. https://doi.org/10.31557/APJCP.2021.22.S1.25

Berkov S, Atanasova M, Georgiev B, Bastida J, Doytchinova I (2022) The Amaryllidaceae alkaloids: an untapped source of acetylcholinesterase inhibitors. Phytochem Rev 21: 1415–1443. https://doi.org/10.1007/s11101-021-09790-0

Buck M, Hamilton C (2011) The Nagoya protocol on access to genetic resources and the fair and equitable sharing of benefits arising from their utilization to the convention on biological diversity. Rev Eur Community Int Environ Law 20: 47–61. https://doi.org/10.1111/j.1467-9388.2011.00703.x

Carvalho MDS, Da Silva SM, Pinheiro U (2013) Two new species of *Aaptos* (Demospongiae, Hadromerida) from Brazil (western Atlantic). Zootaxa 3750: 357–366. https://doi.org/10.11646/zootaxa.3750.4.4

Chen Z-R, Huang J-B, Yang S-L, Hong F-F (2022) Role of cholinergic signaling in Alzheimer's disease. Molecules 27: 1816. https://doi.org/10.3390/molecules27061816

Cheung J, Rudolph MJ, Burshteyn F, Cassidy MS, Gary EN, Love J, Franklin MC, Height JJ (2012) Structures of human acetylcholinesterase in complex with pharmacologically important ligands. J Med Chem 55: 10282–10286. https://doi.org/10.1021/jm300871x

Dong J, Wang NN, Yao ZJ, Zhang L, Cheng Y, Ouyang D, Lu AP, Cao DS (2018) ADMETlab: A platform for systematic ADMET evaluation based on a comprehensively collected ADMET database. J Cheminform 10(1): 29. https://doi.org/10.1186/s13321-018-0283-x

Duckworth A (2009) Farming sponges to supply bioactive metabolites and bath sponges: A review. Mar Biotechnol 11: 669–679. https://doi.org/10.1007/s10126-009-9213-2

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7: 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Esposito R, Federico S, Bertolino M, Zupo V, Costantini M (2022) Marine Demospongiae: A challenging treasure of bioactive compounds. Mar Drugs 20(4): 244. https://doi.org/10.3390/md20040244

Foti RS, Dalvie DK (2016) Cytochrome P450 and non–cytochrome P450 oxidative metabolism: Contributions to the pharmacokinetics, safety, and efficacy of xenobiotics. Drug Metab Dispos 44(8): 1229-1245. https://doi.org/10.1124/dmd.116.071753

GBD 2016 Dementia Collaborators (2019) Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 18(1): 88-106. https://doi.org/10.1016/S1474-4422(18)30403-4

GBD 2019 Dementia Forecasting Collaborators (2022) Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health 7(2): e105-e125. https://doi.org/10.1016/S2468-2667(21)00249-8

Hafez Ghoran S, Kijjoa A (2021) Marine-derived compounds with anti-Alzheimer's disease activities. Mar Drugs 19: 410. https://doi.org/10.3390/md19080410

He Q, Miao S, Ni N, Man Y, Gong K (2020) A Review of the secondary metabolites from the marine sponges of the genus *Aaptos*. Nat Prod Commun 15: 1934578X20951439. https://doi.org/10.1177/1934578X20951439

Rosales Hernández MC, Fragoso Morales LG, Correa Basurto J, Olvera Valdez M, García Báez EV, Román Vázquez DG, Anaya García AP, Cruz A (2022) *In silico* and *in vitro* studies of benzothiazole-isothioureas derivatives as a multitarget compound for Alzheimer's disease. Int J Mol Sci 23(21): 12945. https://doi.org/10.3390/ijms232112945

Ji D, Ma J, Dai J, Xu M, Harris PW, Brimble MA, Agyei D (2022) Anticholinesterase inhibition, drug-likeness assessment, and molecular docking evaluation of milk protein-derived opioid peptides for the control of Alzheimer's disease. Dairy 3(3): 422-437. https://doi.org/10.3390/dairy3030032

Kim JK, Park SU (2017) Pharmacological aspects of galantamine for the treatment of Alzheimer's disease. EXCLI J 16: 35–39. https://doi.org/10.17179/excli2016-820

Li X, Feng X, Sun X, Hou N, Han F, Liu Y (2022) Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2019. Front Aging Neurosci 14: 937486. https://doi.org/10.3389/fnagi.2022.937486

Lima E, Medeiros J (2022) Marine organisms as alkaloid biosynthesizers of potential anti-Alzheimer agents. Mar Drugs 20: 75. https://doi.org/10.3390/md20010075

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 64: 4-17. https://doi.org/10.1016/j.addr.2012.09.019

Liu C, Tang X, Li P, Li G (2012) Suberitine A–D, four new cytotoxic dimeric aaptamine alkaloids from the marine sponge *Aaptos suberitoides*. Org Lett 14: 1994–1997. https://doi.org/10.1021/ol3004589

Mardiastuti A (2019) Implementation of access and benefit sharing in Indonesia: Review and case studies. J Manaj Hutan Trop 25: 35–43.

Maslin M, Gaertner-Mazouni N, Debitus C, Joy N, Ho R (2021) Marine sponge aquaculture towards drug development: An ongoing history of technical, ecological, chemical considerations and challenges. Aquac Rep 21: 100813. https://doi.org/10.1016/j.aqrep.2021.100813

Miao S, He Q, Li C, Wu Y, Liu M, Chen Y, Qi S, Gong K (2022) Aaptamine – A dual acetyl – and butyrylcholinesterase inhibitor as potential anti-Alzheimer's disease agent. Pharm Biol 60: 1502–1510. https://doi.org/10.1080/13880209.2022.2102657

Moodie LWK, Sepčić K, Turk T, Frangež R, Svenson J (2019) Natural cholinesterase inhibitors from marine organisms. Nat Prod Rep 36: 1053–1092. https://doi.org/10.1039/C9NP00010K

Nachon F, Carletti E, Ronco C, Trovaslet M, Nicolet Y, Jean L, Renard P-Y (2013) Crystal structures of human cholinesterases in complex with huprine W and tacrine: Elements of specificity for anti-Alzheimer's drugs targeting acetyl- and butyryl-cholinesterase. Biochem J 453: 393–399. https://doi.org/10.1042/BJ20130013

Nakamura H, Kobayashi J, Ohizumi Y, Hirata Y (1982) Isolation and structure of aaptamine a novel heteroaromatic substance possessing α-blocking activity from the sea sponge. Tetrahedron Lett 23: 5555–5558. https://doi.org/10.1016/S0040-4039(00)85893-1

Nakamura H, Kobayashi J, Ohizumi Y, Hirata Y (1987) Aaptamines. Novel benzo[de][1,6]naphthyridines from the Okinawan marine sponge Aaptos aaptos. J Chem Soc, Perkin Trans 1: 173–176. https://doi.org/10.1039/P19870000173

Nurhayati, Dyah AP, Prastiwi R, Sukardiman, Wahyuningsih T (2018) Cytotoxic activity of ethanolic extract of the marine sponge *Aaptos suberitoides* against T47D cell. AIP Conf Proc 1945: 020013. https://doi.org/10.1063/1.5030235

Nurhayati, Dyah AP, Pratiwi R, Sukardiman (2019) Total and differential cells of leukocyte mice (*Mus musculus*) on evaluation *in vivo* anticancer extracts ethanol marine sponges *Aaptos suberitoides*. J Phys Conf Ser 1374: 012031. https://doi.org/10.1088/1742-6596/1374/1/012031

Palaniveloo K, Ong KH, Satriawan H, Abdul Razak S, Suciati S, Hung H-Y, Hirayama S, Rizman-Idid M, Tan JK, Yong YS, Phang S-M (2023) *In vitro* and *in silico* cholinesterase inhibitory potential of metabolites from *Laurencia snackeyi* (Weber-van Bosse) M. Masuda. 3 Biotech 13: 337. https://doi.org/10.1007/s13205-023-03725-6

Peitzika SC, Pontiki E (2023) A review on recent approaches on molecular docking studies of novel compounds targeting acetylcholinesterase in Alzheimer disease. Molecules 28(3) 1084. https://doi.org/10.3390/molecules28031084

Pham C-D, Hartmann R, Müller WEG, de Voogd N, Lai D, Proksch P (2013) Aaptamine derivatives from the Indonesian sponge *Aaptos suberitoides*. J Nat Prod 76: 103–106. https://doi.org/10.1021/np300794b

Putri HR, Kristiana R, Mudianta IW, Setiawan E, Widyawaruyanti A, Nuengchamnong N, Suphrom N, Suciati S (2023) Metabolite profile and *in vitro* cholinesterase inhibitory activity of extract and fractions of *Aaptos suberitoides*. J Pharm Pharmacogn Res 11: 129–136. https://doi.org/10.56499/jppres22.1511_11.1.129

Romanelli C, Cooper D, Campbell-Lendrum D, Maiero M, Karesh WB, Hunter D, Golden CD (2015) Connecting Global Priorities: Biodiversity and Human Health. A State of Knowledge Review. Switzerland: World Health Organization and Secretariat of the Convention on Biological Diversity.

Shaari K, Ling KC, Rashid ZM, Jean TP, Abas F, Raof SM, Zainal Z, Lajis NH, Mohamad H, Ali AM (2008) Cytotoxic aaptamines from Malaysian *Aaptos aaptos*. Mar Drugs 7(1): 1-8. https://doi.org/10.3390/md7010001

Silva M, Seijas P, Otero P (2021) Exploitation of marine molecules to manage Alzheimer's disease. Mar Drugs 19: 373. https://doi.org/10.3390/md19070373

Tsukamoto S, Kudo Y, Kato H, Rotinsulu H, Losung F, Mangindaan REP, de Voogd N (2014) Aaptoline A, a new quinoline alkaloid from the marine sponge *Aaptos suberitoides*. Heterocycles 88: 591. https://doi.org/10.3987/COM-13-S(S)3

Tsukamoto S, Yamanokuchi R, Yoshitomi M, Sato K, Ikeda T, Rotinsulu H, Mangindaan REP, de Voogd NJ, van Soest RWM, Yokosawa H (2010) Aaptamine, an alkaloid from the sponge *Aaptos suberitoides*, functions as a proteasome inhibitor. Bioorg Med Chem Lett 20: 3341–3343. https://doi.org/10.1016/j.bmcl.2010.04.029

Utkina NK, Denisenko VA (2016) N-Demethylaaptanone, a new congener of aaptamine alkaloids from the vietnamese marine sponge *Aaptos aaptos*. Nat Prod Commun 11: 1259–1260. https://doi.org/10.1177/1934578X1601100916

Utkina NK, Denisenko VA, Pushilin MA (2009) Aaptanone, a novel zwitterionic metabolite of the aaptamine class with an oxygenated 1,6-naphthyridine core from the Vietnamese marine sponge *Aaptos aaptos*. Tetrahedron Lett 50: 2580–2582. https://doi.org/10.1016/j.tetlet.2009.03.096

van Beek TA, Tetala KKR, Koleva II, Dapkevicius A, Exarchou V, Jeurissen SMF, Claassen FW, van der Klift EJC (2009) Recent developments in the rapid analysis of plants and tracking their bioactive constituents. Phytochem Rev 8: 387–399. https://doi.org/10.1007/s11101-009-9125-9

Van Soest RWM, Boury-Esnault N, Vacelet J, Dohrmann M, Erpenbeck D, De Voogd NJ, Santodomingo N, Vanhoorne B, Kelly M, Hooper JNA (2012) Global diversity of sponges (Porifera). PLoS One 7: e35105. https://doi.org/10.1371/journal.pone.0035105

Varijakzhan D, Loh JY, Yap WS, Yusoff K, Seboussi R, Lim SHE, Lai KS, Chong CM (2021) Bioactive compounds from marine sponges: Fundamentals and applications. Mar Drugs 19: 246. https://doi.org/10.3390/md19050246

Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD (2002) Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem *45*(12): 2615-2623. https://doi.org/10.1021/jm020017n

Wajid S, Khatoon A, Khan MA, Zafar H, Kanwal S, Choudhary MI, Basha FZ (2019) Microwave-assisted organic synthesis, structure–activity relationship, kinetics and molecular docking studies of non-cytotoxic benzamide derivatives as selective butyrylcholinesterase inhibitors. Bioorg Med Chem *27*(18): 4030-4040. https://doi.org/10.1016/j.bmc.2019.07.015

Wang P, Huang J, Kurtán T, Mándi A, Jia H, Cheng W, Lin W (2020) Aaptodines A–D, *spiro* naphthyridine–furooxazoloquinoline hybrid alkaloids from the sponge *Aaptos suberitoides*. Org Lett 22: 8215–8218. https://doi.org/10.1021/acs.orglett.0c02645

Widayanti TF, Djafar EM, Hakim MZ, Rivanie SS, Ashri M (2022) Legal protection concerning marine environment and coastal areas in Indonesia based on the convention on biological diversity (CBD). IOP Conf Ser Earth Environ Sci 1119: 012006. https://doi.org/10.1088/1755-1315/1119/1/012006

Zhong HA (2017) ADMET properties: overview and current topics. In: Grover A (eds), Drug Design: Principles and Applications. Singapore: Springer, pp. 113-133. https://doi.org/10.1007/978-981-10-5187-6_8

Zilbeyaz K, Stellenboom N, Guney M, Oztekin A, Senturk M (2018) Effects of aryl methanesulfonate derivatives on acetylcholinesterase and butyrylcholinesterase. J Biochem Mol Toxicol 32: e22210. https://doi.org/10.1002/jbt.22210

AUTHOR CONTRIBUTION:

Contribution	Suciati S	Putri HR	Palaniveloo K	Hung OK	Rizman-M	Widyawaruyanti A	Nuengchamnong N	Suphrom N	Kristiana R	Mudianta IW	George M	Setiawan E
Concepts or ideas	x											
Design	x		x		x	x						
Definition of intellectual content	x		x		x	x						
Literature search	x	x	x	x	x	x	x	x	x	x	x	x
Experimental studies	x	x		x			x	x	x	x		x
Data acquisition		x		x			x		x	x		
Data analysis	x		x		x	x	x	x			x	x
Statistical analysis	x	x										
Manuscript preparation	x	x	x	x							x	
Manuscript editing	x		x									
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x

Citation Format: Suciati S, Putri HR, Palaniveloo K, Hung OK, Rizman-Idid MR, Widyawaruyanti A, Nuengchamnong N, Suphrom N, Kristiana R, Mudianta IW, George M, Setiawan E (2025) *In vitro* and *in silico* cholinesterase inhibitory activities of aaptamine and derivatives from *Aaptos suberitoides*. J Pharm Pharmacogn Res 13(3): 757–773. https://doi.org/10.56499/jppres24.2064_13.3.757

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/ licenses/by/4.0/), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.