https://doi.org/10.51483/AFJPS.3.1.2023.31-40

ISSN: 2789-5092

SvedbergOpen
DISSEMINATION OF KNOWLEDGE

African Journal of Pharmaceutical Sciences

Publisher's Home Page: https://www.svedbergopen.com/

Research Paper

Open Access

A Ceramide Isolated from *Tinospora cordifolia* (Menispermaceae) with Acetylcholinesterase Inhibitory Activity and Molecular Docking Study

O.J. Onoja[1,2*], T.O. Elufioye[3], J.I. Olawuni[4], Z.A. Sherwani[5], and Z. Ul-Haq[6]

[1]Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria. E-mail: joel.onoja@unn.edu.ng

[2]Institute of Drug-Herbal Medicine-Excipient Research and Development, University of Nigeria, Nsukka, Nigeria.

[3]Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria. E-mail: toonitaiwo@yahoo.com

[4]Department of Biochemistry and Molecular Biology, Faculty of Sciences, Obafemi Awolowo University Ile-Ife, Nigeria. E-mail: olawunij@gmail.com

[5]Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi-75270, Pakistan. E-mail: zaid_anis@live.com

[6]Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Karachi-75270, Pakistan. E-mail: zaheer_qasmi@hotmail.com

Article Info

Volume 3, Issue 1, March 2023

Received : 26 August 2022

Accepted : 11 February 2023

Published : 05 March 2023

doi: 10.51483/AFJPS.3.1.2023.31-41

Abstract

Alzheimer's Disease (AD) is a neurological disorder caused by acetylcholinesterase (AChE) via termination of the action of acetylcholine by catalytic hydrolysis. Inhibition of AChE is considered a useful approach to combat AD. The aim of the study is to evaluate the AChE inhibitory activity of compound isolated from the stem of *Tinospora cordifolia*. Chromatographic techniques were used to isolate and purify bioactive compounds. Their structures were determined by spectroscopic analysis. Ellman colorimetric assay method was used to determine the AChE inhibitory activity *in vitro*. The selected PDB was modeled with MOE 2019 using PDB ID: 4EY7. Chromatographic separation yielded one compound. Based on spectroscopic data, the molecule was identified as rel-(2S, 3S, 4R, 1 6E)- 2-[(2'R)-2'-hydroxynonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol reported for the first time in Menispermaceae. The molecule demonstrated good AChE inhibitory activity (IC_{50} = 0.055±0.00 mg/mL) at 0.1 mg/mL compared to eserine (IC_{50} = 0.009±0.00 mg/mL). The docked pose had an abundance of hydrophobic, hydrogen and Pi stacking interaction. The ceramide form potential lead for new AChEi drug for the management of AD.

Keywords: *Tinospora cordifolia, Menispermaceae, Ceramide, Acetylcholinesterase inhibition, Molecular docking*

1. Introduction

Alzheimer's disease is a type of brain disease caused by damage to nerve cells (neurons) in the brain. It is the most common cause of dementia, accounting for an estimated 60% to 80% of cases (Kapasi *et al*., 2017, Brenowitz

* *Corresponding author: O.J. Onoja, Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, Nigeria; Institute of Drug-Herbal Medicine-Excipient Research and Development, University of Nigeria, Nsukka, Nigeria. E-mail: joel.onoja@unn.edu.ng*

et al., 2017). The neurons that are damaged first are the parts of the brain responsible for memory, language, and thought. As a result, the first symptoms of Alzheimer's disease are problems with memory, speech, and thinking. Alzheimer's disease is a progressive disease that gets worse over time. The rate of progression and the skills affected vary from person to person (Jack *et al*., 2009, Braak *et al*., 2011). In the 20 years leading up to the Covid-19 pandemic, deaths from other major causes either declined significantly or remained roughly the same, but official records show that deaths from Alzheimer's disease have increased significantly. Between 2000 and 2019, the number of deaths recorded on death certificates from Alzheimer's disease more than doubled, an increase of 145.2%, while the number one cause of death (heart disease) decreased by 7.3% (WHO, 2004, Brunnstrom and Englund, 2009, Tejada-Vera, 2013, U.S. Department of Health and Human Services, 2021).

The enzyme acetylcholinesterase (AChE) is an important therapeutic target in AD (Silman and Sussman, 2005). Deterioration of cholinergic neurons in the brain and loss of neurotransmission are major causes of cognitive decline in AD patients (Bartus *et al*., 1982). According to the cholinergic hypothesis, the primary cause of AD is decreased acetylcholine (ACh) synthesis. Therefore, one possible therapeutic strategy is to increase cholinergic levels in the brain by inhibiting the biological activity of acetylcholinesterase (AChE). Therefore, AChE inhibitors are used to limit the degradation of ACh. AChE inhibitors can enhance neuronal function by increasing the concentration of ACh (Tabet, 2008).

Tinospora cordifolia belongs to the family Menispermaceae. It has been reported to be effective in enhancing memory in animals with memory impairment and to enhance cognitive function in patients with Alzheimer's disease (Lannert and Hoyer, 1998, Malve *et al*., 2014). Reported pharmacological activities also include anti-fertility, antioxidant and immunomodulating activities (Reddy and Rajasekhar, 2015). Molecules belonging to the class of clerodane diterpene such as Tinosponone and clerodane diterpene glucoside Tinocordioside were reported from the stem of *Tinospora cordifolia* (Rakesh *et al*., 1995, Atta-ur-Rahman *et al*., 1988), phenyl propene glycosides (Maurya *et al*., 1994) and aporphine alkaloids such as oxoglaucine, corydine (Onoja *et al*., 2020).

A number of cholinesterase inhibitors have been developed to treat AD and are currently available on the market. However, the efficacy of these drugs is limited, and these drugs have shown various dose-dependent side effects. This necessitated the continuous search for new neurotherapeutic agents from nature with good cholinesterase inhibitory potentials and no side effects. The aim of the study was to evaluate the acetylcholinesterase inhibitory activity of compound isolated from the stem of *Tinospora cordifolia*.

2. Materials and Methods

2.1. Reagents and Solvents

Acetylcholinesterase (AChE), Acetylthiocholine iodide (ATChI), 5:5-dithiobis-2-nitrobenzoic acid (DTNB), eserine and sodium phosphate buffer salts, column silica gel, n-hexane, dichloromethane, ethyl acetate, methanol were of analytical grade purchased from Sigma Aldrich, USA.

2.2. Spectroscopic Instrument

^{1}H and ^{13}C-NMR (Bruker AVANCE-III AV-400 MHz), Electron Impact Mass Spectrometry (EI-MS) performed on JEOL MS route, Fourier Transform Infrared spectroscopy (FT-IR) was recorded on Bruker Vector 22, Melting point range was also determined to ascertain purity using BUCHI (M-560).

2.3. Collection and Authentication of Plant

Tinospora cordifolia (Willd.) Miers ex Hook. F. and Thoms (Menispermaceae) stem was obtained from Obollo-afor of Enugu State in the month of November / December 2016. Plant was authenticated at Forestry Herbarium Ibadan (FHI) by Mr. Adeyemo, A. and Chukwuma C. Emmanuel where voucher specimen was deposited as *Tinospora cordifolia* (FHI 112287).

2.4. Preparation of Plant Extract

9 Kg of powder of *Tinospora cordifolia* stem was macerated with 100% methanol for 72 h. The filterate was concentrated *in vacuo*. The dried concentrated extract was stored in a refrigerator at 4 °C until required. The

methanolic crude extract was suspended in water and separating funnel was used to partition the extract successively with n-hexane, dichloromethane, ethyl acetate and aqueous methanol.

2.5. Isolation of Bioactive Molecules from Ethyl Acetate Fraction

Firstly, ethyl acetate fraction yielded 91.8 g out of which 30 g was chromatographed using column chromatography on 600 g column silica gel (60-200 mesh size) on a 6 cm diameter and 75 cm long column using gradient elution of n-hexane (100%), n-hexane: dichloromethane (9:1 to 1:9), dichloromethane (100%) and dichloromethane: methanol (99:1 to 93:7, 3:1 to 1:3) to yield 252 fractions. Secondly, a slurry of two (2) grams of sub fraction labeled EA-26 (pooled fractions 174-185) eluted with solvent dichloromethane: methanol (97:3) was chromatographed using column chromatography on 60 g column silica gel (60-200 mesh size) on a 3 cm diameter and 80 cm long column using the solvent systems n-hexane (100%), n-hexane: dichloromethane (1:1 to 1:3), ethyl acetate (100%) and ethyl acetate: methanol (97:3 to 3:1). The chromatography yielded 78 sub-fractions which were pooled together to 7 sub fractions (labeled as EA-26-1- EA-26-7). The eluent 100% ethyl acetate eluted light yellow eluates EA-26-4 (pooled sub-fractions 32-43) which contain a white armourphous solid known as compound 1 (60 mg) soluble in pyridine. The TLC plates were visualized using UV lamp (254 nm and 366 nm) and spray with 20% sulphuric acid, then heated with a spray gun to visualize spots.

2.6. Acetylcholinesterase Inhibitory Assay

Acetylcholinesterase inhibition was measured spectrophotometrically using acetyl thiocholine iodide (ATChI) according to method of Ellman *et al*. (1961). Add 240 μL of buffer (50 mM Tris-HCl, pH 8.0) and 20 μL of various concentrations of compound (0.1-0.00313 mg / mlL, 20 μL of enzymepreparation (0.28 U / mL) to a 96-well plate. The reaction mixture was then incubated for 30 min at 37 ^{O}C, after which 20 μL of 10 mM DTNB was added. The reaction was then initiated by the addition of 20 μL of 25 mM ATChI. The hydrolysis rate of ATChI was then measured by measuring the change in absorbance per minute (ΔA / min) due to the formation of the yellow 5-thio-2-nitrobenzoate anion at 412 nm over a period of 4 min at 30 s interval. Buffer was used as negative control. All assays were carried out in triplicate. Eserine ((-) physostigmine) was used as positive control.

The percentage inhibition (%I) of test sample was obtained using the formula:

$$I\,(\%) = [(V_o - V_i) / V_o] * 100$$

where: I (%) = Percentage inhibition

V_i = Enzyme activity in the presence of test sample

V_o = Enzyme activity in the absence of test sample

2.7. Molecular Docking Studies

Human Acetylcholinesterase structure complexed with clinically approved inhibitor drug Donepezil (PDB ID: 4EY7) was downloaded from RCSB PDB website and was subsequently prepared by correction, Hydrogenation, minimization and partial charges application under AMBER : EHT10 forcefield. MOE 2019 was used throughout for docking. Redocking *in-silico* experiment was carried out and showed a good value of substantially less than 1 Å. The ligand was built in molecular builder module of MOE 2019 and prepared by Quick prep application available in the MOE Suite. The Reference compound donepezil and the purported inhibitor compound were subsequently docked with settings validated in redocking (Triangle matcher as placement, London DG as scoring method, GBVI as rescoring method and the induced fir docking was performed.

3. Statistical Analyses

The statistical analysis was carried out using Graphpad prism 7. All data was expressed as mean ± S.D. and of triplicate parallel measurements. Statistical analyses were performed using One-way ANOVA followed by Dunnett's Multiple Comparisons test at α 0.05. Differences between means at 5% level ($p \leq 0.05$) were considered significant. Standard curves were generated and calculation of the 50% inhibitory concentration (IC_{50}) values was done using Microsoft Excel.

4. Results and Discussion

4.1. Characterization of Compound 1

It has retardation factor (R_f) value of 0.8 in solvent system methanol: ethyl acetate (3:4). Melting point (M.p): 144.1 °C-145.2 °C, Mass (m/z): 653.2 g/mol [M+]. IR (γ_{max}^{KBr} cm^{-1}): 3816.0, 3424.0, 2920.0, 2850.0, 1618.7, 1551.9, 1468.0, 1072.5, 721.1 and NMR data (Table 1). Molecular formula ($C_{40}H_{78}NO_5$) and structure has been assigned to the substance named re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2 ' - hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol (Figure 1) based on the physical data and spectroscopic analysis (UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR). Its low resolution electron impact (EI) mass spectrum on JEOL MS Route instrument showed major fragmentation at m/z (%): 774.4 (1.8), 760.5 (4.0), 732.5 (8.4), 718.5 (6.0), 693.3 (12.4), 679.4 (23.9), 665.3 (43.7), 647.3 (26.1), 620.3 (9.1), 592.3 (7.2), 524.3 (2.0), 467.1 (10.5), 453.2 (21.4), 439.2 (60.4), 422.2 (30.4), 408.2 (72.3), 394.2 (44.1), 384.1 (71.2), 370.1 (39.7), 357.0 (100.0), 339.1 (75.6), 308.1 (16.0), 298.1 (4.7), 280.0 (13.5), 265.1 (25.1), 226.1 (11.6), 125.0 (11.9), 97.0 (29.3), 83.0 (45.6), 69.0 (44.9), 59.9 (81.0), 43.0 (67.4). Its IR spectrum ranges from 3816.0 to 3424.0 cm^{-1} with several broad peaks characteristic of bond N—H or O—H stretching, amide carbonyl at 1618.7 cm^{-1} , followed by the N-H bending at 1551.9 cm^{-1}. A long aliphatic chain was characterized by a band at 721.1 cm^{-1}. The ^{1}H-NMR spectrum shows characteristic signals for the amide proton at δ 8.59 (d, 1H, J = 9.2 Hz), δ 7.63, 6.70 (d, 2H, J = 6.4 Hz) and 6.22 (d, 1H, J=6.4 Hz), all displayed as broad singlets. Signal at δ 5.12 (m, H-2) for nitrogen-bound methine. Hydroxymethylene signals at δ 4.53 (dd, J=6.4 Hz, H-1a) and 4.44 (dd, 1H, J=4.8 Hz, H-1b) and δ 4.62 (m, 1H, H-2´), 4.37 (dd, 1H, J=6Hz, H-3) and 4.29 (d, 1H, J=6.4Hz, H-4) correspond to three oxymethines. Additional signals for double bonds at δ5.64 (td, J=15.4 and 5.4 Hz, H-16) and 5.52 (td, J=15.4 and 5.8 Hz, H-17), two additional signals at δ 0.85 (dd, 5H) A terminal methyl, J = 4.8 Hz, 5.2 Hz, 3H-19´/3H-21) and several methylene hydrogens at δ2.26-1.24, two corresponding to aliphatic chains, are also observed. The COSY spectrum showed that the nitrogen-bonded methine (H-2) bound to proton oxymethylene (2H-1) and oxymethine (H-3), the latter to oxymethine H-4. As expected, the ^{13}C-NMR spectra exhibited three low-field carbon signals at δ175.23 (C-1') 131.30 (C-16) and 131.11 (C-17). These correspond to a carbonyl amide and a double bond, respectively. Nitrogen methine (C-2) at ä 53.00, oxymethylene (C-1) at δ 62.06, three oxymethine signals at d 76.81 (C-3), 73.03 (C-4), 72.48 (C-2`). In addition, several carbon signals in the range δ 35.73-22.94 assigned to methylene groups and a carbon signal at 14.28 corresponding to two terminal methyl groups were also obtained from the ^{13}C-NMR spectrum, confirming that the compound is a ceramide was suggested. The distinct positions of hydroxyl groups were deduced based on the HMBC spectrum. Here, the proton signal at δ 8.59 (NH) showed correlation with carbonyl (C-1') and nitrogen-containing methine (C-2), whereas the proton signal at δ5.12 (H-2) Signals showed correlation with carbon signals at δ 62.06 (C-1), 76.81 (C-3), and 73.03 (C-4). Furthermore, the HMBC correlation between the proton signal at δ 4.62 (H-2') and the carbonyl (C-1') confirmed the presence of α-hydroxy fatty acid side chains. The spectra and physical data were in complete concurrence with the literature (Maia *et al*., 2010).

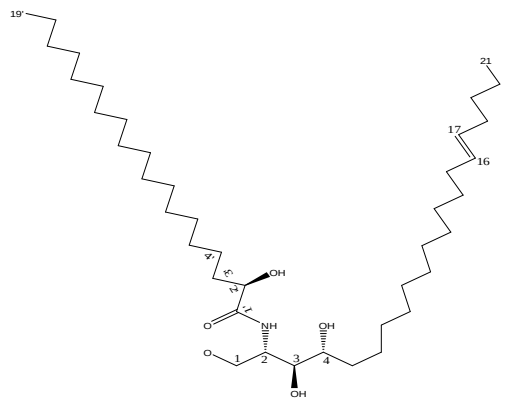

Figure 1: re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2' - hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol

Table 1: ^{1}H and ^{13}C-NMR Chemical Shift Values for re l (2 S , 3 S , 4R, 1 6E)-2[(2'R) 2 ' hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol recorded in C5D5N (AVANCE-III AV-400 MHz)

Position	^{1}H-NMR	^{13}C-NMR
1	4.53 (dd, 1H, J=6.4Hz), 4.44 (dd, 1H, J=4.8Hz)	62.06
2	5.12 (m, 1H)	53.00
3	4.37 (dd,1H, J=6Hz)	76.81
4	4.29 (d,1H,J=6.4Hz)	73.03
5	2.26 (m,1H), 1.96 (m,1H)	34.17
6	1.75 (m, 3H)	26.66
7-14	1.24 -1.42	30.01-30.34
15	2.03 (m, 1H)	32.13[a]
16	5.64 (td, J=15.4Hz; 5.4Hz)	131.30[b]
17	5.52 (td, J=15.4Hz; 5.8Hz)	131.11[b]
18	2.03 (m)	34.17
19	1.24 -1.42	32.13
20	1.24 -1.42	22.94
21	0.85 (dd, 5H, J=4.8Hz, 5.2Hz)	14.24
1'		175.23
2'	4.62 (m, 1H)	72.48
3'	2.22 (m), 2.03 (m)	35.73
4'	1.24 -1.42	26.66
5-16'	1.24 -1.42	30.01-30.34
17'	1.24 -1.42(m)	32.13
18'	1.24 -1.42(m)	22.94
19'	0.85 (dd, 5H, J=4.8Hz, 5.2Hz)	14.24
N-H	8.59 (d, 1H, J=9.2Hz)	150.24
HO-1	6.70 (d, 2H, J=6.4Hz)	-
HO-3	6.70 (d, 2H, J=6.4Hz)	-
HO-4	6.22 (d, 1H, J=6.4Hz)	-
HO-2'	7.63 (d, 1H, J=5.2Hz)	135.80

Note: Chemical shift values are in δ (ppm); Coupling constants are in Hz.

Table 2: Result of Powder Microscopy of *J. secunda* Leaf

CERA (Compound 1)		Eserine	
Conc. (mg/mL)	**% Inhibition of AChE**	**Conc. (mg/mL)**	**% Inhibition of AChE**
0.1	66.30±0.87	0.1	81.98±2.92
0.05	52.88±3.53	0.05	77.74±0.14
0.025	38.13±2.41	0.025	66.28±4.50
0.0125	30.75±0.91	0.0125	55.44±5.59
0.00625	23.78±5.13	0.00625	45.31±5.98
0.00313	9.91±1.89	0.00313	37.57±7.20
IC_{50} (mg/mL)	0.055±0.00	IC_{50} (mg/mL)	0.009±0.00

Note: Values are presented as mean ± standard deviation (n=3).

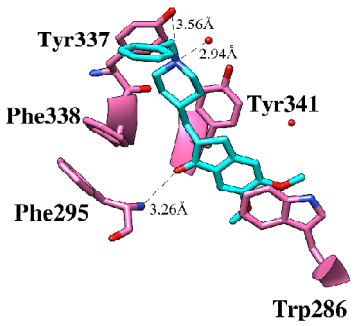

Figure 2: The Best Ranking Docked Pose of the Reference Compound Donepezil on Human Acetylcholinesterase PDB ID: 4EY7

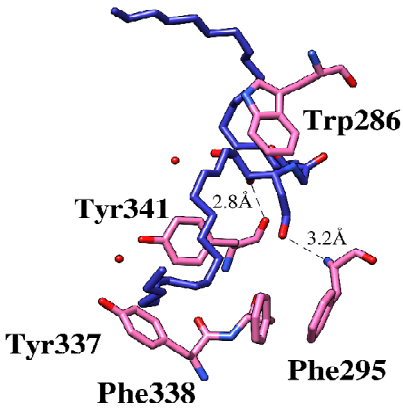

Figure 3: The Best Ranking Docked Pose of re l-(2S , 3S , 4R, 1 6E)-2-[(2'R)-2'-hydroxy Nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol on Human Acetylcholinesterase PDB ID: 4EY7

Ceramides are naturally present in many food sources, including grains, tubers, and legumes such as wheat (Ohnishi *et al*., 1985; Fujino and Ohnishi, 1983), rice (Sugawara *et al*., 2010), corn (Aida *et al*., 2005), potato and sweet potato (Bartke *et al*., 2006), soybean (Gutierrez *et al*., 2004), and konjac (Usuki *et al*., 2016). Also series of ceramides have been isolated from African medicinal plants, e.g., Tithoniamide from *Tithonia diversifolia* (Guan and Wenk, 2006), Paullinomide A from *Paullinia pinnata* (Miemanang *et al*., 2006), Citropremide from *Citropsis gabunensis* (Tsassi *et al*., 2011), Glumoamide from *Ficus glumosa* (Nana *et al*., 2012), Laportomide A from *Laportea ovalifolia* (Tazoo *et al*., 2007), (2S,3S,4R,8E)-2N-[(20R)-20-Hydroxytetracosanoyl]-8(E)-octadecene-1,3,4-triol from *Cordia* (Tapondjou *et al*., 2005). Ceramides remain interesting as many reports have presented some of them as potent anti-inflammatory agents (Cheng *et al*., 2009). Ceramides are among the most important ingredients in cosmetic products. As a result, many scientific documents have revealed their role in the water-retention capacity of the skin (Lee *et al*., 2003). Phytoceramide-based skin care products also help rehydrate the skin and strengthen its protective barrier. Both oral and topical administration of phytoceramide can enhance the endogenous epidermal lipid-synthetic capability (Shimoda *et al*., 2012, Tokudome *et al*., 2014) or replace depleted skin ceramides in some skin conditions and ageing (Ueda *et al*., 2009). The most widely studied biological activity of ceramides is the ability to induce apoptosis, a programmed cell death essential to maintain normal cellular homeostasis and an important physiological response to many forms of cellular stress (Haimovitz-Friedman *et al*., 1994, Dbaibo *et al*., 1998, Dbaibo *et al*., 2001). New

ceramide, lutaoside, and benjaminamide, isolated from the twigs of *Ficus benjamina* (Poumale *et al*., 2011) and from the wood of *Ficus lutea* harvested in Cameroon, inhibited the growth of several microorganisms, including the bacteria *Scenedesmus subspicatus*, *Chlorella sorokiniana*, *Mucor miehei*, *Bacillus subtilis*, and the fungus *Candida albicans* (Simo *et al*., 2008).

Currently, in diseases such as Alzheimer's disease, the search for new drugs that can improve memory and learning or slow neurodegenerative processes is important. A biologically important enzyme known as acetylcholinesterase has been implicated in the pathology of Alzheimer's disease because it is involved in the hydrolysis of acetylcholine, a key neurotransmitter in the brain involved in learning and memory (Herbert *et al*., 1995). The use of acetylcholinesterase inhibitors is considered a promising tactic to elevate acetylcholine levels in the brain (Enz *et al*., 1993). Medicinal plants such as Huperzine A, an alkaloid isolated from *Huperzia serrata*, Galanthamine, an alkaloid isolated from *Galanthus nivalis* has been reported to have neuroprotective potentials in conditions such as AD (Marston *et al*., 2002, Jones *et al*., 2006), Cladamide a new ceramide from the marine-derived endophytic fungus *Cladosporium cladosporioides* isolated from the leaves of the mangrove, *Avicennia marina* (Forssk.) Vierh. has been reported to inhibit acetylcholinesterase (Amal *et al*., 2021) and ceramides (MEC-1) from the Red Sea Marine Sponge *Mycale euplectellioides* has been reported to show a moderate anti-choline esterase activity *in vitro* and tight binding to AChE as confirmed by docking study by interacting with aromatic residue of peripheral anionic site and penetrated deeply till catalytic triad residue of the active site (Reda *et al*., 2016). In this study, the acetylcholinesterase inhibitory potential of re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol isolated from stem of *Tinospora cordifolia* was evaluated using ATChI and DTNB *in vitro.* The principle involves the measurement of the rate of production of thio-choline as acetylthiocholine is hydrolyzed. This is accomplished by the continuous reaction of the thiol group with DTNB to produce the yellow anion of 5-thio-2-nitro-benzoic acid (TNB). The molecule demonstrated good AChE inhibitory activity (IC_{50}= 0.055±0.00 mg / mL) at 0.1 mg / mL compared to eserine (IC_{50} = 0.009±0.00 mg / mL) (Table 2). In order to elucidate the mechanism of inhibition by re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol on Human acetylcholinesterase evidenced by single protein assay molecular docking studies were carried out. The docking results showed that the purported inhibitor compound re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol showed a better docking score than the reference compound Donepezil but since re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol was much larger than donepezil ligand efficiency metrics were applied as true measure of binding affinity. The ligand efficiency was better for donepezil than re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol in the study. The docked pose of Donepezil shows good distance Hydrogen bonding with crucial residues Phe295 and Tyr337. There was plethora of hydrophobic interactions with a number of important residues. A water bridge was also formed as shown in figure 2 with a bond length of 2.94Å and estimated free energy of binding (ΔG), kcal / mol to be (-9.8853) while the docked pose of re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol had an abundance of hydrophobic interaction explaining its higher dock score. It also formed hydrogen bonds with Phe295 and Tyr341. Water Bridges were also formed with residue Tyr72, Ser 293 and Arg296. Two Pi stacking interactions were noted with estimated free energy of binding (ΔG), kcal / mol to be (-11.1739) (Figure 3). Detailed post-docking analysis showed that the purported inhibitor was showing a very good docking profile as the reference compound is a well-established inhibitor and the compound was showing a very similar interaction profile and thus likely to be a true inhibitor. The insinuation of the findings is that the molecule has the potential to prevent the catalytic hydrolysis of acetylcholine by acetylcholinesterase thereby increasing its half-life in the brain and in turn improve learning and memory.

5. Conclusion

re l - (2S , 3S , 4R, 1 6E) - 2 - [(2'R) - 2'- hydroxy nonadecanoylamino]-heneicosadec-16-ene-1,3,4-triol a compound belonging to a class of ceramides has been reported for the first time in Menispermaceae. The ceramide isolated will contribute to knowledge in natural product chemistry as well as form possible potential lead for new AChEi drug for the management of Alzheimer's disease. Further validation studies and ADME profile for clinical applicability is a logical next step.

Conflicts of Interest

There are no conflicts of interest.

Acknowledgment

Authors would like to acknowledge Third World Academy of Science (TWAS) and International Center for Chemical and Biological Sciences (ICCBS), University of Karachi for funding part of this research.

References

Aida, K., Kinoshita, M., Tanji, M., Sugawara, T., Tamura, M., Ono, J., Ueno, N. and Ohnishi, M. (2005). Prevention of Aberrant Crypt Foci Formation By Dietary Maize And Yeast Cerebrosides in 1,2-dimethylhydrazine-treated mice. *J Oleo Sci.* 54 (1), 45-49.

Amal, S., Mohamed, E., Mohamed, A. and Sabry , M.E. (2021). Cladamide: A New Ceramide From The Endophytic Fungus *Cladosporium cladosporioides, Nat Prod Res*., 8, 1-10. doi: 10.1080/14786419.2021.1986709

Atta-ur-Rahman, Ahraad, S., Rycroft, D.S., Parkanyl, L., Choudhary, M.I. and Clardy, J. (1988). *Tetrahedron Letters,* 29(34), 4241-4244.

Bartke, N., Fischbeck, A. and Humpf, H.U. (2006). Analysis of Sphingolipids in Potatoes (*Solanum tuberosum* L.) and Sweet Potatoes (*Ipomoea batatas* (L.) lam.) by Reversed Phase High-performance Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (HPLC-ESI-MS/MS). *Mol Nutr Food Res.,* 50(12), 1201-1211.

Bartus, R.T., Dean, R.L. III., Beer, B. and Lippa, A.S. (1982). The Cholinergic Hypothesis of Geriatric Memory Dysfunction. *Science,* 217, 408417.

Braak, H., Thal, D.R., Ghebremedhin, E. and Del Tredici, K. (2011). Stages of the Pathologic Process in Alzheimer Disease: Age Categories from 1 to 100 years. *J Neuropathol Exp Neurol*., 70(11), 960-9.

Brenowitz, W.D., Hubbard, R.A., Keene, C.D., Hawes, S.E., Longstreth, W.T., Woltjer, R.L. and Kukull, W.A. (2017). Mixed Neuropathologies and Estimated Rates of Clinical Progression in a Large Autopsy Sample. *Alzheimers Dement*., 13(6), 654-62.

Brunnstrom, H.R. and Englund, E.M. (2009). Cause of Death In Patients With Dementia Disorders. *Eur J Neurol*., 16(4), 488-92.

Cheng, S.Y., Wen, Z.H., Chiou, S.F., Tsai, C.W., Wang, S.K., Hsu, C.H., Dai, C.F., Chiang, M.Y., Wang,W.H. and Duh, C.Y. (2009). Ceramide and Cerebrosides from the Octocoral *Sarcophyton ehrenbergi*. *J Nat Prod.,* 72(3), 465-8.

Dbaibo, G.S., El-Assad, W., Krikorian, A., Liu, B., Diab, K., Idriss, N.Z., El-Sabban, M., Driscoll, T.A., Perry, D.K. and Hannun, Y.A. (2001). Ceramide Generation By Two Distinct Pathways In Tumor Necrosis Factor Alpha-induced Cell Death. *FEBS Lett.,* 503(1), 7-12.

Dbaibo, G.S., Pushkareva, M.Y., Rachid, R.A., Alter, N., Smyth, M.J., Obeid, L.M. and Hannun, Y.A. (1998). Dependent Ceramide Response To Genotoxic Stress., *J Clin Invest*., 102(2), 329-39.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M. (1961). A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity. *Biochemical Pharmacology,* 7(2), 88-95.

Enz, A., Amstuz, R., Boddeke, H., Gmelin, G. and Malanowski, J. (1993). Brain Selective Inhibition of Acetylcholinesterase: A Novel Approach To Therapy for Alzheimer's disease. *Progress in Brain Research,* 98, 431-438. doi: 10.1016/s0079-6123(08)62429-2.

Fujino, Y. and Ohnishi, M. (1983). Sphingolipids in Wheatgrain. *J Cereal Sci*., 1(2), 159-168.

Guan, X.L. and Wenk, M.R. (2006). Mass Spectrometry-based Profiling of Phospholipids and Sphingolipids in Extracts from *Saccharomyces cerevisiae*. *Yeast,* 23(6), 465-77.

Gutierrez, E., Wang, T. and Fehr, W.R. (2004). Quantification of Sphingolipids in Soybeans. *J Am Oil Chem Soc.*, 81(8), 737-742.

Haimovitz-Friedman, A., Kan, C.C., Ehleiter, D., Persaud, R.S., McLoughlin, M., Fuks, Z. and Kolesnick, R.N. (1994). Ionizing Radiation Acts On Cellular Membranes To Generate Ceramide And Initiate Apoptosis. *J Exp Med.,* 180(2), 525-35.

Herbert, L.E., Scherr, P.A. and Beckeff, L.A. (1995). Age Specific Incidence of Alzheimer's Disease in a Community Population. *JAMA*, 273, 1354-1359.

Jack, C.R., Lowe, V.J., Weigand, S.D., Wiste, H.J., Senjem, M.L., Knopman, D.S., Shiung, M.M., Gunter, J.L., Boeve, B.F., Kemp, B.J., Weiner, M. and Petersen, R.C. (2009). Serial PiB and MRI in Normal, Mild Cognitive Impairment and Alzheimer's Disease: Implications for Sequence of Pathological Events in Alzheimer's Disease. *Brain*, 132(5),1355-1365.

Jones, W.P., Chin, Y.W. and Kinghorn, A.D. (2006). The Role of Pharmacognosy in Modern Medicine and Pharmacy. *Current Drug Targets*, 7(3), 247-264.

Kapasi, A., DeCarli, C. and Schneider, J.A. (2017). Impact of Multiple Pathologies on the Threshold for Clinically Overt Dementia. *Acta Neuropathol*., 134(2), 171-86.

Lannert, H. and Hoyer, S. (1998). Intracerebroventricular aDministration Of Streptozotocin Causes Long-term Diminutions in Learning and Memory Abilities and in Cerebral Energy Metabolism in Adult Rats. Behav Neurosci 112:1199-1208.

Lee, M.L., Lee, G.H., Yoo, J.S. (2003). Analysis of Ceramides in Cosmetics by Reversed-phase Liquid Chromatography / Electrospray Ionization Mass Spectrometry With Collision Induces Dissociation. *Rapid Commun Mass Spectrom,* 17(1), 34-75.

Maia, Ana Isabel V., Maria, L.V., Raimundo, B., Norberto, P., Lopes, Edilberto R., Silveiraa. and Otilia Deusdênia L. Pessoa. (2010). New Ceramides from *Acnistus arborescens*. *J. Braz. Chem. Soc*., 21(5), 867-871.

Malve, H., Raut, S., Marathe, P. and Rege, N. (2014). Effect of Combination of *Phyllanthus emblica*, *Tinospora cordifolia* and *Ocimum sanctum* on Spatial Learning and Memory in Rats. *J Ayurveda Integr Med.,* 5(4), 209-215.

Marston, A., Kissling, J. and Hostettmann, K. (2002). A Rapid TLC Bioautographic Method For The Detection of Acetylcholinesterase and Butyrylcholinesterase Inhibitors in Plants. *Phytochemical Analysis,* 13(1), 51-54.

Maurya, R., Wazir, V., Kapil, A. and Kapil, R. S. (1994). Clerodane Diterpenoids from *Tinospora cordifolia. Tetrahedron Letters in Press,* 705-11.

Miemanang, R.S., Krohn, K., Hussain, H. and Dongo, E. (2006). Paullinoside A and Paullinomide A: A New Cerebroside and a New Ceramide from Leaves of *Paullinia pinnata*. *Z Naturforsch*., 61b(1), 1123-7.

Nana, F., Sandjo, L.P., Keumedjio, F., Ambassa, P., Malik, R., Kuete, V., Rincheval, V., Choudhary, M. I. and Ngadjui, B.T. (2012). Ceramides and Cytotoxic Constituents from *Ficus glumosa Del.* (Moraceae). *J Braz Chem Soc*., 23(3), 482-7.

Ohnishi, M., Ito, S. and Fujino, Y. (1985). Sphingolipid Classes and their Molecular Species In Wheatflour. *Agric Biol Chem Tokyo*, 49(12), 3609-3611.

Onoja, O.J., Elufioye, T.O., Zaid, A. S. and Zaheer, Ul-Haq. (2020). Molecular Docking Studies and Anti-Alzheimer's Potential of Isolated Compounds from *Tinospora cordifolia*. *Journal of Biologically Active Products from Nature*, 10(2), 100-121.

Poumale, H.M.P., Songfack, Djoumessi A.V.B., Ngameni, B., Sandjo, L.P., Ngadjui, B.T. and Shiono, Y.A. (2011). New Ceramide Isolated from *Ficus lutea Vahl* (Moraceae). *Acta Chim Slov*., 58(1), 81-6.

Rakesh, M., Versha, W., Anjulika, T., Randhir, S. and Kapil. (1995). Clerodane Diterpenoids from *Tinospora cordifolia. Phytocheralstry*, 38(3), 659-61.

Reda, Abdelhameed., Mohamed, Saleh Elgawish., Amira Mira., Amany, K. Ibrahim., Safwat, A., Ahmed., Kuniyoshi, Shimizu. and Koji, Y. (2016). Anti-choline Esterase Activity of Ceramides from the Red Sea Marine Sponge *Mycale euplectellioides. RSC Advances*, 10(20), 46.

Reddy, N. and Rajasekhar, R. (2015). *Tinospora cordifolia* Chemical Constituents and Medicinal Properties: A Review. *Sch Acad J Pharm.,* 4(8), 364-369.

Shimoda, H., Terazawa, S., Hitoe, S., Tanaka, J., Nakamura, S., Matsuda, H. and Yoshikawa, M. (2012). Changes in Ceramides and Glucosylceramides in Mouse Skin and Human Epidermal Equivalents by Rice-derived Glucosylceramide. *J Med Food*, 15(12), 1064-1072.

Silman, I. andSussman, J.L. (2005). Acetylcholinesterase: Classical and Nonclassical Functions and Pharmacology. *Curr Opin Pharmacol*., 5(3), 293?302.

Simo, C.C.F., Kouam, S.F., Poumale, H.M.P., Simo, I.K., Ngadjui, B.T., Green, I.R. and Krhon, K. (2008). Benjaminamide: A New Ceramide and Other Compounds from the Twigs of *Ficus benjamina* (Moraceae). *Biochem Syst Ecol.,* 36(3), 238-43.

Sugawara, T., Aida, K., Duan, J. and Hirata, T. (2010). Analysis of Glucosylceramides From Various Sources By Liquid Chromatography-ion Trap Mass Spectrometry. *J Oleo Sci*., 59(7), 387-394.

Tabet, N. (2008). Acetylcholinesterase Inhibitors for Alzheimer's Disease: Antiinflammatories in Acetylcholine Clothing. *Age Ageing,* 35(4), 336-338.

Tapondjou, L.A., Mitaine-Offer, A.C., Sautour, M., Miyamoto, T. and Lacaille-Dubois, M.A. (2005). Sphingolipids and Other Constituents from *Cordia platythyrsa*. *Biochem Syst Ecol.,* 33(12), 1293-7.

Tazoo, D., Krohn, K., Hussain, H., Kouam, S.F. and Dongo, E. (2007). Laportoside A and Laportomide A: A New Cerebroside and a New Ceramide from Leaves of *Laportea ovalifolia. Z Naturforsch*., 62b(1), 1208-12.

Tejada-Vera, B. (2013). Mortality from Alzheimer's Disease in the United States: Data for 2000 and 2010. National Center for Health Statistics Data Brief, No. 116. National Center for Health Statistics, Hyattsville, MD.

Tokudome, Y., Endo, M. and Hashimoto, F. (2014). Application of Glucosylceramide-based Liposomes Increased the Ceramide Content in a Three-dimensional Cultured Skin Epidermis. *Skin Pharmacol Physiol*., 27(1), 18-24.

Tsassi, B.V., Hussain, H., Geagni, A., Dongo, E., Ahmed, I., Riaz, M. and Krohn, K. (2011). Citropremide and Citropridone: A New Ceramide and a New Acridone Alkaloid from the Stem Bark of *Citropsis gabunensis*. *Helv Chim Acta.,* 94(6), 1035-40.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics. CDC WONDER online database: About Underlying Cause of Death, 1999-2019, available at: https: / / wonder.cdc.gov / ucd-icd10.html. Accessed on December 28, 2021.

Ueda, O., Hasegawa, M., Kitamura, S. (2009). Distribution in Skin of Ceramide After Oral Administration To Rats. *Drug Metab Pharmacokinet*, 24(2), 180-184.

Usuki, S., Tamura, N., Sakai, S., Tamura, T., Mukai, K. and Igarashi, Y. (2016). Chemoenzymatically Prepared Konjac Ceramide Inhibits Ngf-induced Neurite Outgrowth by a Semaphoring 3A-like Action. *Biochem Biophys Rep*., 5, 160-167. doi: 10.1016 / j.bbrep.2015.11.016.

World Health Organization. (2004). *International Statistical Classification of Diseases and Related Health Problems.* 10th Revision. 2nd Edition. WHO Press, Geneva, Switzerland.

Appendix

Abbreviations

AD: Alzheimer's disease

AChE: Acetylcholinesterase; PDB: Protein Data Base

ACh: Acetylcholine

NMR: Nuclear Magnetic Resonance

IR: Infrared spectroscopy

FT-IR: Fourier-Transform Infrared Spectroscopy

UV: Ultraviolet-visible spectroscopy

MOE: Molecular Operating Environment

RMSD: Root Mean Square Deviation

RCSB: Research Collaboratory for Structural Bioinformatics

ATChI: Acetylthiocholine iodide

DTNB: 5:5-dithiobis-2-nitrobenzoic acid

Cite this article as: O.J. Onoja, T.O. Elufioye, J.I. Olawuni, Z.A. Sherwani. and Z. Ul-Haq. (2023). A Ceramide Isolated from *Tinospora cordifolia* (Menispermaceae) with Acetylcholinesterase Inhibitory Activity and Molecular Docking Study. *African Journal of Pharmaceutical Sciences*, 3(1), 31-41. doi: 10.51483/AFJPS.3.1.2023.31-41.