Neuroprotective Activities of New Monoterpenoid Indole Alkaloid from Nauclea officinalis

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Neuroprotective activities of new monoterpenoid indole alkaloid from *Nauclea officinalis*

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Abstract: Phytochemical investigation on the bark of *Nauclea officinalis* led to the isolation of a new monoterpenoid indole alkaloid, naucledіol. The structure of the compound was identified through extensive spectroscopic analysis. Naucledіol displayed cholinesterase inhibitory activities towards TcAChE and hBChE with IC_{50} values of 15.429 and 8.756 μM, respectively. The mode of inhibition of naucleadiol was mixed-type inhibition and the Ki value is correlated with the IC_{50} value in which naucledіol exhibited higher butyrylcholinesterase inhibition activity. Molecular docking revealed that naucledіol interacts with the choline-binding site and the catalytic triad of TcAChE and hBChE. This study also demonstrated the neuroprotective potential of naucledіol against amyloid beta-induced cytotoxicity and LPS-induced neuroinflammation activity in a dose-dependent manner.

Keywords: *Nauclea officinalis*, indole alkaloids, amyloid beta, neuroinflammation, cholinesterase

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1. Introduction

Alzheimer's disease (AD) is the most common type of dementia and it is estimated to affect 131 million people globally by the year 2050. Over the years, several hypotheses have been proposed to slow down the progression of AD including by improving type 2 diabetes mellitus [1] and the discovery of next-generation therapeutics for the management of AD. It includes cholinesterase, β-amyloid, tau, and neuroinflammation hypotheses. However, current drugs available to slow down the progression of AD are cholinesterase inhibitors (Donepezil, Rivastigmine, and Galantamine) and Memantine (NMDA antagonist), however, they are associated with limitations such as adverse effects, low bioavailability, and inconsistent efficacy [2]. These drugs are based on a one-drug-one target hypothesis whilst the etiology of AD is multifactorial [3]. Therefore, this necessitates the need for novel agents with multi-targeted potential. Natural products are of particular interest due to their ability to reduce oxidative stress, and anti-inflammation

and could potentially slow down the progression of AD [4,5]. This further reinforces the possibility of discovering new agents from natural derivatives.

Natural products also are known as secondary phytometabolites. These organic chemicals do not directly affect cell growth and proliferation, but they enhance survival mechanisms in living organisms [6]. Investigations in molecular mechanisms and docking analysis, have shed light on molecular activities of these secondary phytometabolites [7]. Most secondary phytometabolites are known to interfere with inflammatory mediators such as cytokines and peptides and inhibit macrophage activity [8] in which indole alkaloids; topsentins and dragmacidins [9] are some good examples. *Nauclea officinalis* (Pierre ex Pitard) Merr. & Chun, a traditional Chinese medicine from the Rubiaceae family, is widely used to treat exogenous fever, pink eye, pneumonia and acute jaundice in China. It has anti-inflammatory and anti-malarial properties [10-12]. In addition, *Nauclea officinalis* has been known to contain many indole alkaloids with biological activities. For example, 17-oxo-19-(*Z*)-naucline and naucleoffieine H displayed anti-inflammatory effects by decreasing the LPS-stimulated production of nitric oxide in RAW264.7 cell [13,14]. Hence, in continuous search for bioactive indole alkaloids from Rubiaceae family [15-17], a new monoterpenoid indole alkaloid, naucledio has been isolated from *Nauclea officinalis*. In the present study, the isolation and structural elucidation of the naucledio is reported together with the neuroprotective potential of the compound.

2. Materials and Methods

2.1. General Procedures

The general procedures were the same as previously described [18].

2.2. Plant Materials

The barks of *Nauclea officinalis* from Hutan Simpan Mersing, Johor were collected by a phytochemical group of the Department of Chemistry, Faculty of Science, Universiti Malaya. The botanical identification was conducted by the botanist, Mr. Teo Leong Eng. The voucher specimen (KL 4745) of the plant was deposited at the Herbarium of the Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, Malaysia.

2.3. Extraction, Isolation and Purification of Compound

The extraction procedure for 1.5 kg of dried and grounded bark of *Nauclea officinalis* was the same as previously described [18,19]. An amount of 6.2 g of CH_2Cl_2 extract was obtained. The CH_2Cl_2 extract was then subjected to column chromatography over silica gel using CH_2Cl_2 and MeOH solvent (100:0, 99:1, 98:2, 97:3, 96:4, 95:5, 94:6, 90:10, 83:17, and 75:25 v/v) and twenty fractions were finally obtained. Further purification from fraction 6 by PTLC yielded naucledio (8.2 mg, CH_2Cl_2:MeOH; 97:3 v/v saturated with NH_4OH). The structure of naucledio was elucidated using various spectroscopic methods such as NMR, UV, IR and LCMS-IT-TOF.

2.4. Cholinesterase Enzyme Inhibitory Activity

Naucledio l was evaluated for its enzyme inhibition potential against acetylcholinesterase from *Torpedo californica* (*Tc*AChE) and butyrylcholinesterase from *homo sapien (h*BChE). Cholinesterase inhibition was conducted using the Ellman's calorimetry method as described previously [20]. In the 96-well plate, buffer solution, DTNB (Ellman's reagent), ACTI/BUCL and naucledio l was added in ascending concentration across the row. 1% DMSO was added as the control for this experiment. Lastly, cholinesterase enzyme was added to the assigned enzymatic columns. The absorbance of the plate was then measured using a spectrophotometer at a wavelength of 412 nm for 30 minutes after the addition of enzymes. Each test was conducted in triplicate to ensure the consistency of the results.

2.5. Molecular Docking

Molecular docking was carried out following the method as described by Abdul Wahab et al [21]. Briefly, molecular docking of naucledio l was performed using Autodock 3.0.5 along with AutoDockTools (ADT) [22]. Hyperchem 8 was used to build the 3D-crystal structure of the naucledio l and the structure was subjected to energy minimization with a convergence criterion of 0.05 kcal/(molA). The three dimensional crystal structures of AChE from *Torpedo californica* (*Tc*AChE) (PDB ID: 1W6R) [23] and BChE from *Homo sapiens* (*h*BChE) (PDB ID: 2WIJ) [24] were retrieved from the Protein Data Bank. The proteins were edited using ADT to add hydrogen atoms and remove all the water molecules. Non-polar hydrogens and lone pairs were then merged and each atom was assigned with Gasteiger partial charges. A grid box was generated at the center of the active site gorge with 60×60×60 points and spacing of 0.375 Å. One hundred independent dockings were carried out for each docking experiment with a population size of 150 and 2,500,000 energy evaluations. The best conformation with the lowest docked energy in the most populated cluster was selected. Analysis and visualisation of the conformations from the docking experiments were conducted using Acceryls Discovery Studio 2.5 (*Accelrys* Inc., San Diego, CA, USA).

2.6. Neuroprotective potential of naucledio l on Aβ-(1-42)-induced toxicity on SH-SY5Y cells

2.6.1. Cell culture and cell viability assay

Human neuroblastoma cells (SH-SY5Y) were cultured in a complete culture medium containing DMEM nutrient mixture F-12 (1:1) supplemented with 10 % fetal calf serum, glutamine, and 1 % antibiotics (penicillin-streptomycin), and kept at 37 °C under 5 % CO_2.

Cell viability assay was performed to determine the non-cytotoxic concentration of naucledio l in SH-SY5Y cells. Cells were seeded with a cell density of 25,000 cells/well in 96 well plates for 24 h. The cells were then treated with naucledio l at the concentration of 1, 2, 5, and 10 µM for 24 h. MTT assay was used to assess cell viability after drug treatment. In brief, 20 µl of the MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 hours. 100 µl of DMSO was added to lyse the cells after the removal of the

supernatant in each well. The amount of formazan generated was quantified using a microplate reader (SpectraMax ID3, USA) at an absorbance of 595 nm. All experiments were carried out 3 times.

2.6.2. Neuroprotective Assay Against Beta Amyloid Induced Toxicity

Aβ-(1-42) oligomers were prepared according to the protocol of Huang et al [25] with some modifications. Briefly, lyophilized Aβ-(1-42) powder was initially dissolved to 1.0 mM in hexafluoro-2-isopropanol (HFIP) and incubated at room temperature for 30 minutes. The solution was ensured to be clear and colourless. The HFIP was allowed to evaporate in the open tubes overnight in the fume hood. A thin clear film was formed at the bottom of the tube. The film was initially dissolved in DMSO and subsequently adjusted to 13 μM in DMEM/Hams F-12 media containing 1% Pen/strep and 1% FBS. The solution was incubated at 37 °C under 5 % CO_2.

The MTT assay was used to determine the protection of naucledіol toward SH-SY5Y cells against Aβ-(1-42) aggregate toxicity. In a 96-well plate, 25,000 cells/well were seeded and incubated overnight. Cells were treated with naucledіol at the concentration of 0.1, 1, 5, and 10 μM for 24 h. Aβ-(1-42) aggregates were added to the wells and incubated for another 24 h. 20 μl of the MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. 100 μl of DMSO was added to lyse the cells after removal of the supernatant in each well. The amount of formazan generated was quantified using a microplate reader (SpectraMax ID3, USA) at an absorbance of 595 nm. All experiments were carried out in triplicate.

2.7. Anti-Neuroinflammatory Effect of Naucledіol on LPS-Induced Neuro-Inflammation in BV2 Cells

2.7.1. Cell Culture and Cell Viability Assay

C57/BL6 microglial cells (BV2) were cultured in a complete culture medium containing DMEM supplemented with 10 % fetal calf serum, glutamine, and 1 % antibiotics (penicillin-streptomycin), and kept at 37 °C under 5 % CO_2.

Cell viability assay was performed to determine the non-cytotoxic concentration of naucledіol in BV2 cells. Cells were seeded with a cell density of 20,000 cells/well in 96 well plates for 24 h. The cells were then treated with naucledіol at the concentration of 0.625, 1.25, 2.5, 5, and 10 μM for 24 h. MTT assay was used to assess cell viability after drug treatment. In brief, 20 μl of the MTT solution (5 mg/mL) was added to each well and incubated at 37 °C for 4 h. 100 μl of DMSO was added to lyse the cells after removal of the supernatant in each well. The amount of formazan generated was quantified using a microplate reader (SpectraMax ID3, USA) at an absorbance of 595 nm. All experiments were carried out in triplicate.

2.7.2. Neuroprotective Assay Against LPS-Induced Inflammation

In a 96-well plate, 20,000 cells/well were seeded and incubated overnight. Cells were treated with naucledіol at the concentration of 0.0625, 0.125, 0.25, and 0.5 μM for 24 h. LPS (1 ug/ml) were added to the wells and incubated for another 24 h. A second 96-well plate was used for the measurement of nitride oxide. Sulfanilamide and NED solutions were equilibrated to room temperature for 30 min before use. 50 μl of the solution was transferred from the first plate to the second plate. Then, 50 μl of sulfanilamide solution was added to all wells in the second plate and incubated for 5 min. 50 μl of NED solution was subsequently added and incubated for another 5 min. The plate was then read at the absorbance of 540 nm using a microplate reader (SpectraMax ID3, USA). All experiments were carried out in triplicate. The absorbance was converted into a percentage of nitrite to determine the anti-inflammatory effects of naucledіol.

2.7.3. Statistical Analysis

Data is analysed using R programming. Data are expressed as mean ± standard deviation. Differences between groups are analysed using one-way ANOVA followed by Student's *t*-test for MTT assay and nitrite production. A *p*-value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Structural Elucidation of Naucledіol

Naucledіol (Figure 1) was isolated as a brownish amorphous solid. The LCMS-IT-TOF spectrum showed a pseudomolecular ion peak $[M+Na]^+$ at *m*/*z* 359.1275 (calc. 359.1372), corresponding to the molecular formula of $C_{20}H_{20}N_2O_3$. UV spectrum of naucledіol demonstrated strong absorptions at 374, 308, 277, 256 and 220 nm. In the IR spectrum, an absorption band at 1643 cm^{-1} was observed indicative of a corynanthe type of indole alkaloid with an N_4-C=O amide group [26,27]. In addition, a broad hydroxyl absorption band appeared at 3401 cm^{-1} suggesting the presence of hydrogen bonded OH group [27].

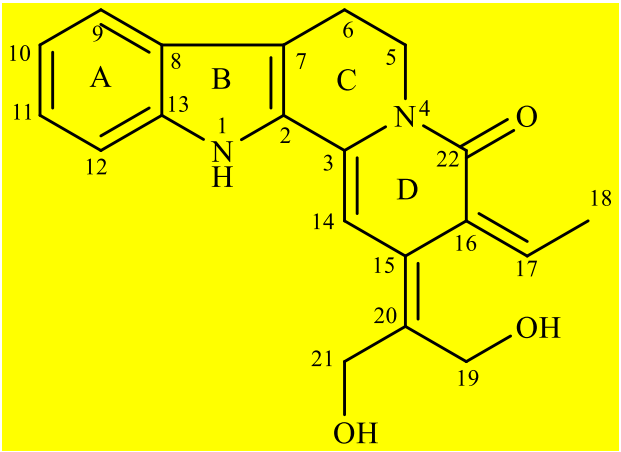

Figure 1. Structure of naucledіol.

In the ^{1}H-NMR spectrum (Table 1), the presence of four aromatic protons signals at the downfield region and a -CH_2-CH_2-N- group were observed, indicating the existence of a tetrahydro-*β*-carboline skeleton [10,27]. Two of the four aromatic protons in ring A appeared as doublets at δ_H 7.53 and 7.37, corresponding to H-9 (1H, *d*, *J* = 7.8 Hz) and H-12 (1H, *d*, *J* = 7.8 Hz), respectively. The other two protons, H-10 (δ_H 7.04, 1H, *t*, *J* = 7.8 Hz) and H-11 (δ_H 7.19, 1H, *t*, *J* = 7.8 Hz), appeared as triplets. Besides, the COSY spectrum also showed the correlations between the four aromatic protons; H-9 with H-10, H-10 with H-11 and H-11 with H-12 (Figure 2). In addition, two sets of triplet signals which appeared at δ_H 4.33 (2H, *t*, *J* = 6.9 Hz) and δ_H 3.06 (2H, *t*, *J* = 6.9 Hz) are attributable to methylene protons, H_2-5 and H_2-6 respectively. H-14 of ring D appeared as a singlet at δ_H 7.00 indicating the presence of neighbouring quaternary carbons, C-3 (δ_C 135.6) and C-15 (δ_C 141.2). Furthermore, the presence of downfield quartet at δ_H 6.46 (1H, *q*, *J* = 6.9 Hz, H-17) coupled with an upfield doublet at δ_H 1.86 (3H, *d*, *J* = 6.9 Hz, H_3-18) implied the presence of a trisubstituted olefin group. Two downfield singlets were observed at δ_H 4.45 (2H, *s*, H_2-19) and 4.55 (2H, *s*, H_2-21) which correlated with the carbon signals at δ_C 64.1 (C-19) and δ_C 64.3 (C-21), respectively in HSQC spectrum, suggesting the existence of two hydroxyl groups (a diol).

Table 1. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) Spectral Data of naucledioI in MeOD.

Position	^{1}H, δ_H (multiplicity, *J* in Hz)	^{13}C (δ_C)
2	-	127.5
3	-	135.6
5	4.33 (*t*, 6.9)	40.5
6	3.06 (*t*, 6.9)	19.0
7	-	113.0
8	-	125.7
9	7.53 (*d*, 7.8)	118.9
10	7.04 (*t*, 7.8)	119.6
11	7.19 (*t*, 7.8)	124.0
12	7.37 (*d*, 7.8)	111.3
13	-	138.6
14	7.00 (*s*)	95.5
15	-	141.2
16	-	120.0
17	6.46 (*q*, 6.9)	123.8
18	1.86 (*d*, 6.9)	12.2
19	4.45 (*s*)	64.1
20	-	129.2
21	4.55 (*s*)	64.3
22	-	160.9

As indicated in the ^{13}C-NMR and DEPT-135 spectra, naucledio has a total of twenty carbon signals comprising one methyl, one carbonyl, four methylenes, six methines and eight quaternary carbons. The signal of a carbonyl carbon was observed at δ_C 160.9 (C-22). HMBC spectrum showed correlation of H_2-19 and H_2-21 with C-20 (δ_C 129.2) indicating the connectivity of the two methylenes with quaternary carbon C-20 as shown in Figure 2. Besides, HMBC correlations between H_3-18 and C-16 (δ_C 120.0), H-19 and C-15, H-21 and C-15 were observed, thus supporting the connectivity of trisubstituted olefin group and propane-1,3-diol group to ring D through C-16 and C-15, respectively. The COSY spectrum revealed the correlation peaks between H_2-5 and H_2-6 and H_3-18 and H-17 indicating the respective protons are coupled with each other (Figure 2).

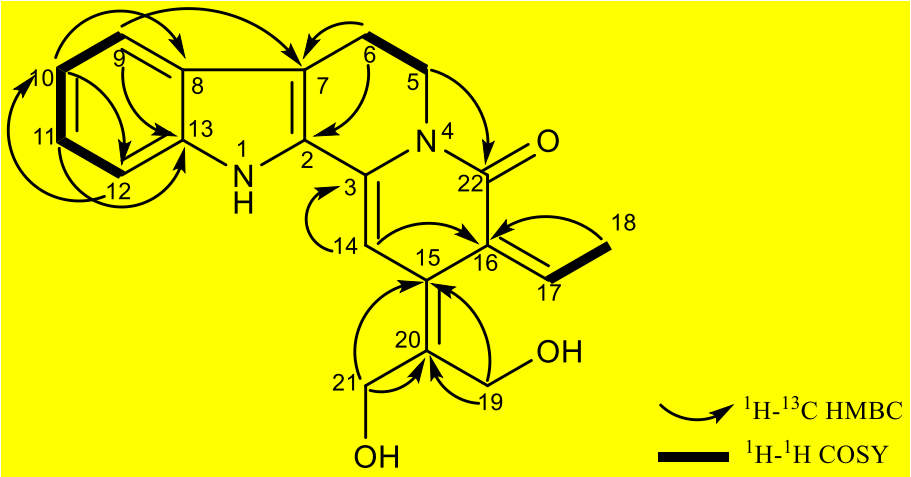

Figure 2. ^{1}H-^{1}H COSY and selected ^{1}H-^{13}C HMBC correlations of naucledio.

Based on the aforementioned and extensive analysis of all spectral data, the structure of naucledio was established and it is identified as a new corynanthe type of monoterpenoid indole alkaloid. The biogenetic precursor of naucledio would be strictosidine. Strictosidine could undergo cleavage of glycoside to strictosidine aglycone followed by cleavage of ring D to form dialdehyde intermediate. Subsequently, a series of rearrangement could lead to the formation of 21-oxogeissoschizine followed by dehydration to form naucledio as shown in Figure 3.

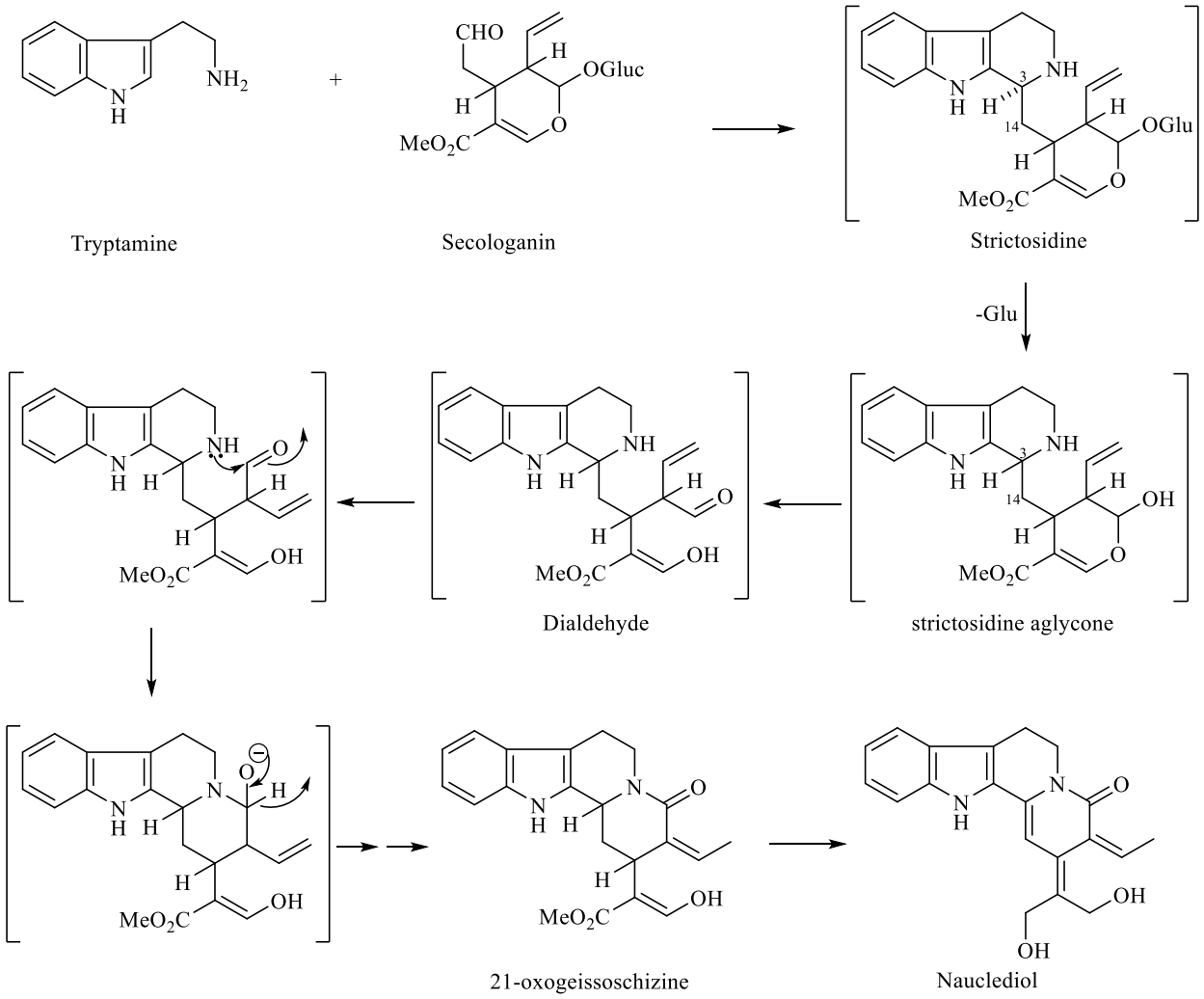

Figure 3. A proposed biogenesis process of naucledio.

3.2. Cholinesterase Inhibition of Naucledio

Cholinesterase inhibitors (ChEIs) are the first and most commonly recognized treatment strategy for AD. It works by reducing the metabolism of cholinergic neurotransmitter, acetylcholine released from the cholinergic neurons hence increasing synaptic levels of ACh and BCh [28]. In this study, naucledio was tested for its inhibitory potential against *Tc*AChE and *h*BChE, respectively. Naucledio showed cholinesterase inhibition activity in both cholinesterase enzymes with an IC_{50} values of 15.429 and 8.756 μM with two-fold more selective toward hBChE inhibition (Figure 4). Furthermore, naucledio was three times more potent as an inhibitor of BChE compared to galanthamine (standard or drug) with reported IC_{50} value of 28.29 μM [19].

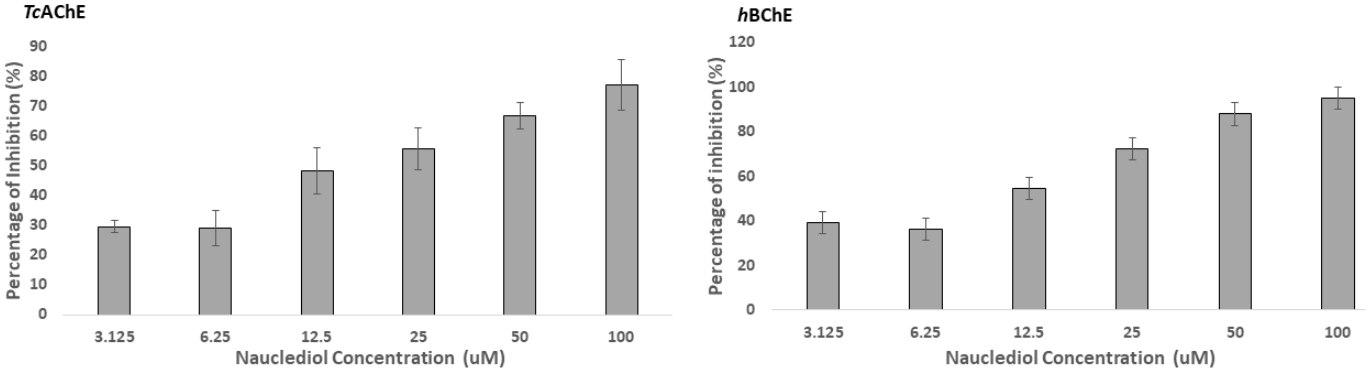

Figure 4. Cholinesterase inhibition of naucledial.

The mode of inhibition was determined by means of Lineweaver-burk plot. Figure 5 shows the kinetic plots of naucledial and the mode of inhibition of naucledial for both enzymes were identified to be mixed types inhibition. The Ki values of naucledial were 25.287 and 20.401 for both *Tc*AChE and *h*BChE, respectively (Appendix S1), which is well correlated with IC_{50} value obtained. Secondary plots (Kis) for naucledial were presented as mixed type inhibitors that could bind in the free enzyme (Ki) and inhibit substrate bound-enzymes. By comparing the potency of the nauclesdiol in inhibiting cholinesterase enzymes, the Ki and Kis of the naucledial for hBChE (20.40 and 45 μM) was stronger than TcAChE (25.287 and 36.51 μM) as well as lower Ki value indicating naucledial bind more firmly to the free cholinesterase enzyme than the enzyme-substrate complex.

It is worth noting that all drugs available in the market are AChE inhibitors and compounds acting on BChE might be an interesting target for anti-cholinesterase discovery. Naucledial appeared to be a mixed mode inhibitor of both AChE and BChE.

By comparing with the cholinesterase inhibitory activities of other indole alkaloids that isolated from *N. officinalis* [19], a brief structure-activity relationship studies can be conducted. Angustidine, angustine, angustoline and naucline are also corynanthe type of indole alkaloids [19] with the same precursors as naucledial. According to the proposed biogenesis pathway, the difference between naucledial and the four known indole alkaloids is starts after the formation of strictosidine. Rotation at C-14 of strictosidine is needed to form strictosidine lactam followed by the formation of the four known indole alkaloids. On the other hand, formation of naucledial does not require the rotation at C-14 of strictosidine but involve a ring opening in strictosidine aglycone. Hence, the four known indole alkaloids possess ring E (pyridine or pyran ring) but naucledial does not. Based on the previously reported data, angustidine, angustine, angustoline and naucline exhibited moderate to weak AChE inhibition with angustidine having the lowest IC_{50} values (21.72 μM) [19], In comparison with the four known indole alkaloids, nauledial possess better inhibitory effect towards AChE. It was suggested that the potency of naucledial on AChE inhibition may due to the presence of ring-opened system in ring E and the two hydroxyl

groups. For BChE, angustidine and angustine (IC_{50} = 1.03 and 4.98, respectively) [19] showed better inhibitory effect compared to naucledio l. However, naucledio l was 2.5 and 4 times more potent than angustoline and naucline, respectively in which angustoline has only one hydroxyl group in its structure while naucline has a pyran ring instead of pyridine ring (ring E). Based on structure-relationship studies, the potency in inhibiting BChE may be contributed by the presence of pyridine ring and the potency could be increased if having two hydroxyl groups.

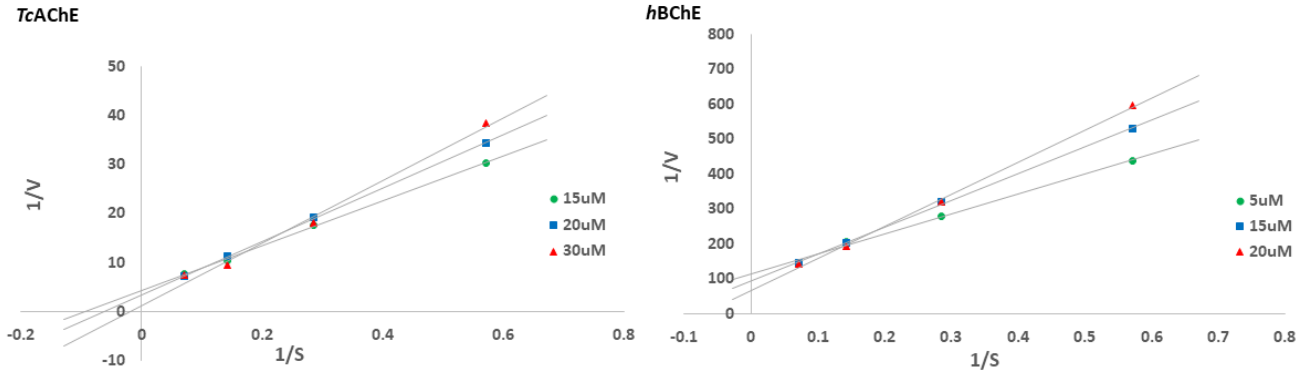

Figure 5. Lineweaver-Burk plots of naucledio l.

3.3. Molecular Docking of Naucledio l

Since naucledio l is potent in inhibiting both enzymes, molecular docking analysis was carried out in order to understand its binding interaction with the enzymes. The results revealed that naucledio l docked well in the catalytic triad and choline binding site of *Tc*AChE (Table 2). The hydroxyl group of C-19 formed a hydrogen bond with His 440 at a distance of 2.60 Å in the catalytic triad (Table 2). Catalytic triad serves as the active site of the enzyme where acetylcholine, the substrate, is hydrolysed into choline and acetate [21]. Besides, hydrogen bond and hydrophobic interaction were observed between the compound and the amino acid residues in the choline binding site which anchored this compound to the *Tc*AChE active site gorge (Figure 6). Oxygen atom of the carbonyl group C-22 interacted with Tyr 130 through hydrogen bond at a distance of 2.26 Å while the ring B and D of the compound formed π-π stacking interaction with Trp 84. For *h*BChE, naucledio l also bound with amino acid residues of the catalytic triad and choline binding site with hydrophobic interaction (Table 2). A π-π stacking interaction was observed between the indole ring of naucledio l with His 438 from the active site of *h*BChE as shown in Figure 7. In addition, a π-σ interaction was observed between Trp 82 with C-17 of naucledio l. This finding was in agreement with the mode of inhibition of naucledio l; mixed type of inhibition, which indicated that the compound was able to bind to the active site and allosteric site (choline binding site) of both enzymes.

Table 2. Binding interaction data for naucledio with amino acid residues of *Tc*AChE and *h*BChE.

Ligand/ Compound	Enzyme	Binding Energy (kcal/mol)	Number of conformations in the cluster	Interacting site	Residue	Type of Interaction	Distance (Å)	Ligand Interacting
Naucledio	*Tc*AChE	-13.01	64	Catalytic triad	His 440	Hydrogen	2.60	Hydroxyl group at C-19
				Choline binding site	Trp 84	Hydrophobic	-	Ring B and D
					Tyr 130	Hydrogen	2.26	Oxygen atom of the carbonyl group C-22
	*h*BChE	-12.19	60	Catalytic triad	His 438	Hydrophobic	-	Ring A and B
				Choline binding site	Trp 82	Hydrophobic	-	C-17

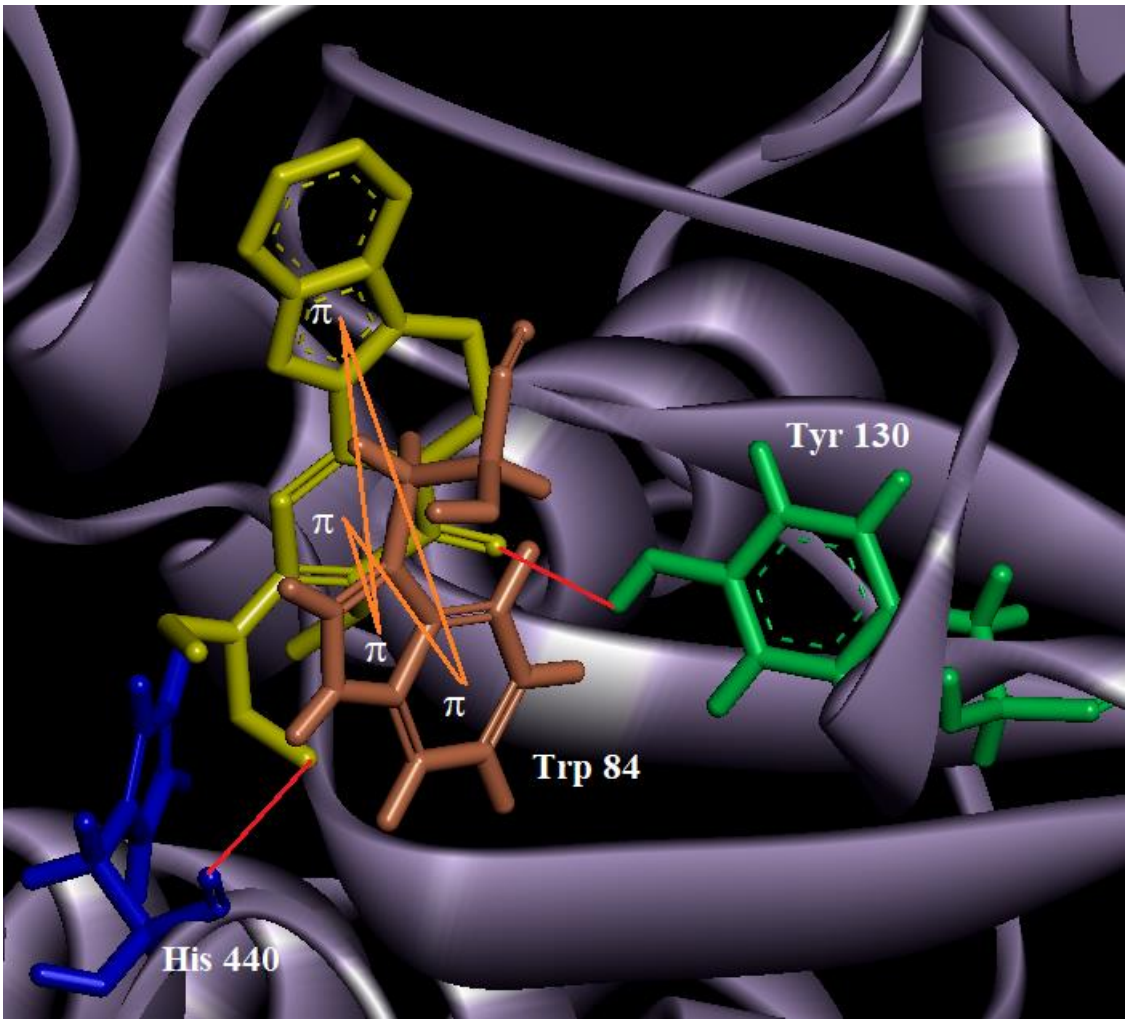

Figure 6. Binding interaction of naucledio1 (in yellow) with amino acid residues of *TcAChE*. The hydrogen bond between the ligand and amino acid residues are shown in red line.

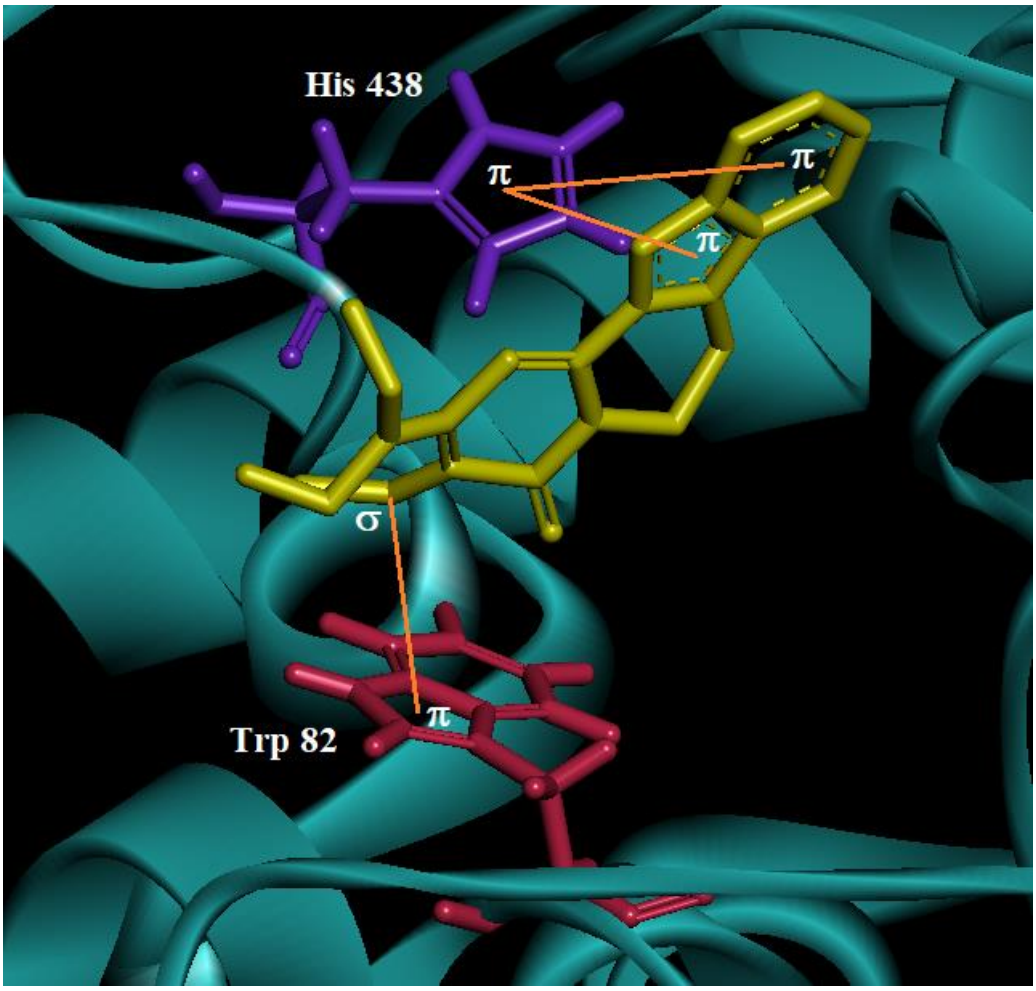

Figure 7. Binding interaction of naucledio1 (in yellow) with amino acid residues of *hBChE*.

3.4. Naucledio1 Reduces Aβ-(1-42)-Induced Toxicity on SH-SY5Y Cells

As illustrated in Figure 8A, naucledio1 had no cytotoxic effect on SH-SY5Y cells from 1 to 10 μM. Further, the cells were pretreated with 1 to 10 μM of naucledio1 followed by Aβ-(1-42) aggregates. Figure 8B shows that 13 μM Aβ-(1-42) aggregates significantly reduced cell viability to 54.5 ± 6.52% compared to the untreated cells. The cell viability of neuroblastoma SH-SY5Y cells was increased by 33.28 to 64.87% at the tested concentrations (0.1 to 10 μM) compared to cells treated with Aβ-(1-42) aggregates alone. Overall, our study showed that naucledio1 protected neuroblastoma SH-SY5Y cells against Aβ-(1-42) induced cytotoxicity.

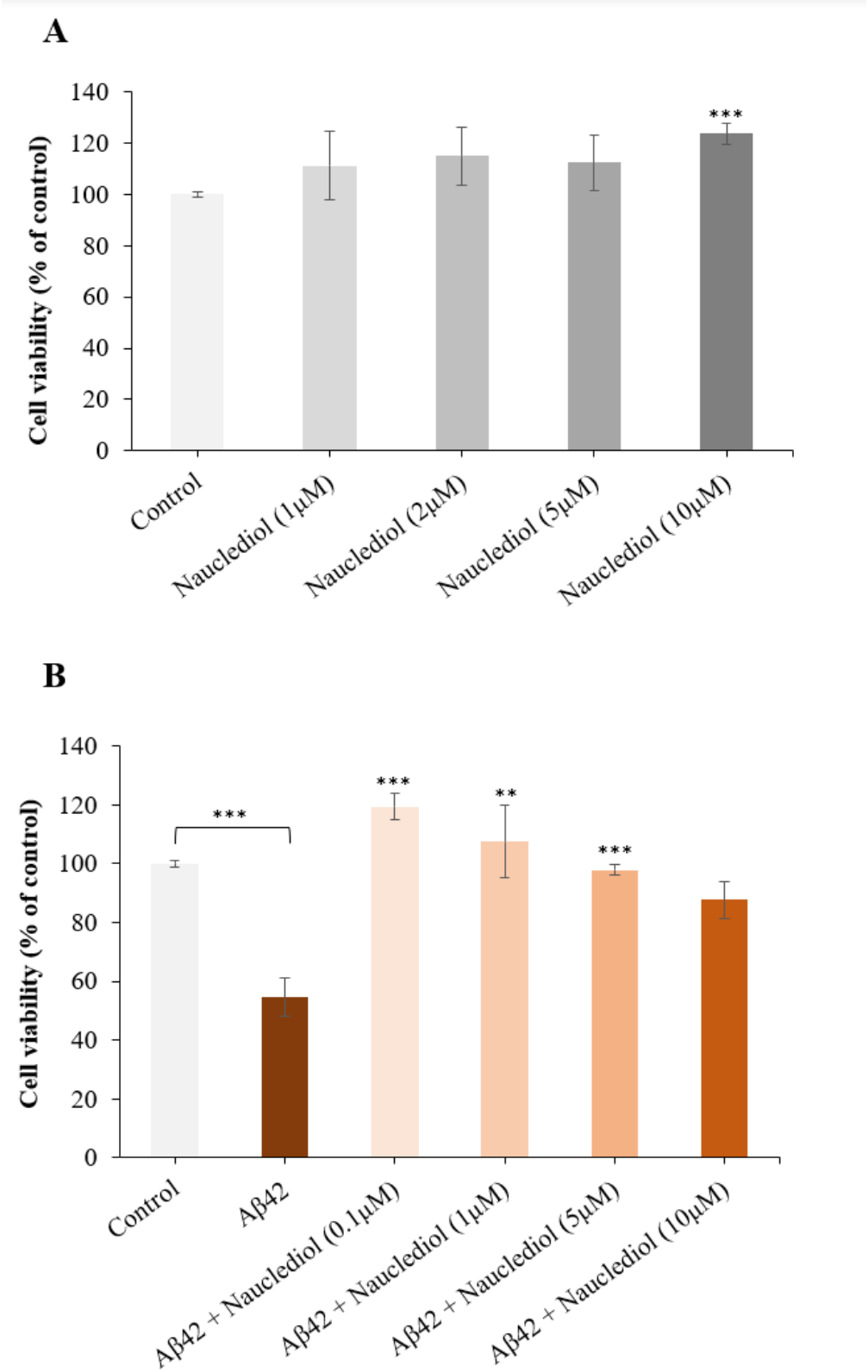

Figure 8. Neuroprotective activity of naucledial on SH-SY5Y cells. (A) Cells were treated with various doses of naucledial for 24 h. (B) Cells were pre-treated with non-detrimental concentrations of naucledial for 24 h before exposure to 13 µM Aβ-(1-42) aggregates for 24 h. Data are expressed as mean ± standard deviation of three independent experiments, statistical significance was performed by one-way ANOVA followed by Student's t-test; *, **, ***, and **** indicate significant statistical differences between treated and untreated control cells or versus Aβ-(1-42) aggregates ($p < 0.05$).

3.5. Naucledіol Reduces LPS-Induced Neuroinflammation in BV2 Cells

Naucledіol was tested for its cytotoxic effect on BV2 cells at 1.25 to 10 μM. Figure 9 shows BV2 cell viabilities upon exposure to naucledіol. Treated doses at 1.25 to 10 μM were cytotoxic towards BV2 cells from 9.28 to 22.7% when compared to untreated cells. Non-toxic doses at 0.0625 to 0.5 μM were used for further experiments. Naucledіol demonstrated a statistically significant reduction in nitrite at the doses of 0.25 to 0.5 μM.

In this test, we sought to evaluate whether naucledіol could reduce the elevated levels of the nitric oxide production of the LPS-induced inflammation in BV2 cells. Several studies have shown the relationship between nitric oxide and the pathogenesis of AD such as neuronal death and S-nitrosylation of cellular components [29]. In addition, in postmortem brain tissues from AD patients, Aβ plaques are strongly associated with reactive microglia and nitric oxide production from activated microglia [30].

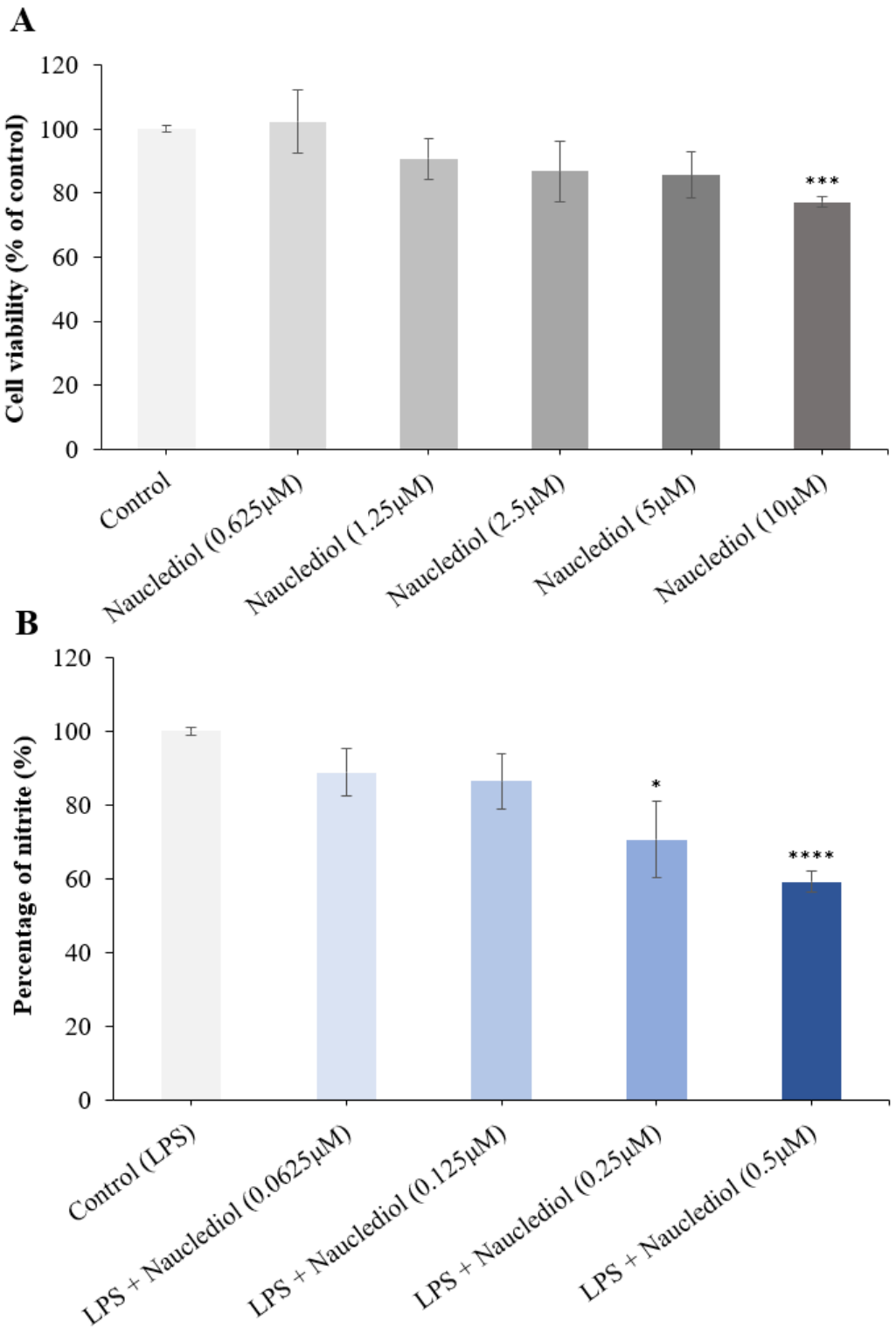

Figure 9. Anti-neuroinflammation activity of naucledíol. (**A**) BV2 cells were treated with various doses of naucledíol for 24 h. (**B**) BV2 cells were pre-treated with non-detrimental concentrations of naucledíol for 24 h before exposure to LPS (1 μg/ml) for 24 h. Data are expressed as mean ± standard deviation of three independent experiments, statistical significance was performed by one-way ANOVA followed by Student's t-test; *, **, ***, and **** indicate significant statistical differences between treated and untreated control cells or versus LPS ($p < 0.05$).

5. Conclusions

In conclusion, the present study has yielded a new monoterpenoid indole alkaloid, naucledіol from *Nauclea officinalis* which is a dual cholinesterase inhibitor with two-fold more selective against butyrylcholinesterase enzyme. Upon a molar basis comparison with galantamine; drug or medication used in treatment of Alzheimer disease, naucledіol was three times more potent in inhibiting BChE. Besides, lineweaver burk plot and molecular docking showed that naucledіol is a mixed inhibitor. In addition, naucledіol was significantly protected cells against beta amyloid induced toxicity and neuroinflammation at submicromolar levels. Overall, the findings in this study are worth further investigation for its potential in other neurodegenerative diseases related assay.

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