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β-SECRETASE INHIBITORY ACTIVITY OF CLERODANE DITERPENOIDS AND APORPHINOID ALKALOIDS AND INSIGHT FROM *IN SILICO* ANALYSES

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ABSTRACT

The β-secretase, which is a β-site amyloid precursor protein cleaving enzyme 1 (BACE1), starts the synthesis of toxic amyloid β (Aβ), which is a key early factor in the pathophysiology of Alzheimer's disease (AD). No cure for AD so far which necessitates the search for new neurotherapeutic agents, especially from natural products (NPs). Inhibiting BACE1 using NP's inhibitors is considered a promising strategy. The study aims to evaluate the BACE 1 inhibitory potentials of clerodane diterpenoids and aporphinoid alkaloids from the stem of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms (Menispermaceae) and get Insight from in silico analyses using molecular docking and MM-GBSA. The enzyme assay was done in vitro using the UV-spectroscopic method. Molecular docking was carried out using Maestro software (Schrödinger). Ligands were docking with PDB ID: 3DV1 complex with NVP-ARV999. 8-hydroxycolumbin, a clerodane diterpenoid has the highest potential to inhibit the enzyme β-secretase (IC50 = 0.040±0.00 mg/mL, 58.58% inhibition) when compared to quercetin (IC_{50} = 0.055±0.00 mg/mL, 72.32% inhibition) at 0.1 mg/mL. Molecular docking demonstrated the presence of hydrogen bonding and π-π Interactions between ligands and enzyme active sites. N-formylanonaine has the best (ΔG_{bind}) (MM-GBSA) (-28.64 kcal/mol) followed by corydine (-21.64 kcal/mol). N-formylanonaine, corydine and 8-hydroxycolumbin could be a template in the discovery of new neurotherapeutic agent in the management of AD due to their abilities to effectively bind to β-secretase protein residues

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INTRODUCTION

The hallmark of Alzheimer's disease is the build-up of extracellular deposits in the brain known as amyloid plaques, which are made up of 38–43 amino acid amyloid β peptides. The brain's accumulation of amyloid β-peptide (Aβ) is one of the primary histopathological characteristics associated with Alzheimer's disease [1]. Amyloid precursor protein (APP) is converted to Aβ through the sequential action of β-secretase (BACE1). Currently, inhibitors of BACE1 are being investigated for their ability to reduce levels of Aβ in the brain and to both treat and prevent Alzheimer's disease [2]. When it comes to

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reducing cerebral Aβ concentrations in Alzheimer's patients, BACE1 is an excellent therapeutic target [3]. BACE1 inhibitor clinical development is being actively pursued, and intriguingly, many promising BACE1 inhibitors have recently entered human clinical studies [4].

Natural compounds have been exploited over the years as potential treatment or management for neurodegenerative diseases such as AD. Natural compounds such as catechins may aid in the decrease in the formation of amyloid plaques and enhance their cognitive ability [5]. According to research, isoflavones such as genistein are effective in managing AD [6]. It has been shown that substances including myricetin, quercetin, and 2,2',4'-trihydroxychalcone acid effectively block BACE1 activity at lower concentrations [7]. It was discovered that the substances salvianolic acid A, salvianolic acid C, and deoxyneocryptotanshinone had high BACE1 inhibitory potential [8].

Virtual screening techniques have been exploited in drug discovery processes. One well-known computational structure-based technique for drug development is molecular docking. It makes it possible to identify possible therapeutic targets and forecast atomic-level molecular ligand-target interactions. Therefore, it is possible to characterize both the metabolic processes and the behavior of the molecules at the binding site [9].

Given that amyloid β plaques are essential to the pathophysiology of Alzheimer's illness, searching for molecules that can inhibit the enzyme (BACE1) responsible for AD is considered a logical step. Therefore, the purpose of this study is to evaluate the BACE 1 inhibitory potentials of clerodane diterpenoids and aporphinoid alkaloids from the stem of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms (Menispermaceae) which is used as a memory enhancer and gets Insight from *in silico* analyses using molecular docking and Molecular mechanics with generalized Born and surface area solvation (MM-GBSA).

MATERIALS AND METHODS

Equipment

Electronic weighing balance (M411L, M-Metlar, Nigeria), Microplate reader (BioTek, EPOCH/2), rotary evaporator (Buchi, R-300)

Reagent

Quercetin, acetate buffer, BACE-1 (β-secretase enzyme), Nα-Benzoyl-D, and L-arginine 4-nitroanilide hydrochloride (DL-BAPNA) are from Sigma Aldrich.

Source of Compounds

The compounds in the classes of clerodane diterpenoids (columbin, 8-hydroxycolumbin) and aporphinoid alkaloids (N-formylanonaine, corydine) were isolated from the stem of *Tinospora cordifolia* and characterized using spectroscopic analyses such as ^{1}H-NMR,^{13}C-NMR, EI-MS, FT-IR, UV [10-11].

In vitro BACE1 Inhibitory Assay

The *in vitro* BACE 1 inhibitory assay was carried out according to the method described by Mancini *et al*. [12]. Acetate buffer (pH 4.5) was used to create 50 µL of test compounds (columbin, 8-hydroxycolumbin, N-formylanonaine, corydine) at 0.1–0.003125 mg/mL, which were then pre-incubated for 60 minutes at 37°C with 50 µL of BACE-1 solution (0.33 U/ml). After adding 50 µL of 3 mM DL-BAPNA, the mixture was incubated for 60 minutes. In tests, blank samples comprising all components except BACE-1 were employed to account for non-enzymatic reactions. Simultaneously, BACE-1 was pre-incubated without inhibitors under the same circumstances to measure the reference enzyme activity. After an hour at 37°C, the substrate was added, and the mixture was then incubated for a further hour at 37°C. Then, at 405 nm, the visible absorbance (DL-BAPNA) was measured. When DL-BAPNA was used, At 405 nm, BACE-1 activity was expressed as ΔA/h after the blank samples' contribution was subtracted. By contrasting the reaction rates with and without the inhibitors, the percentage of inhibition caused by the test compounds' presence was calculated. Quercetin served as a positive reference point. The following expression (equation 1) was used to compute the percentage of enzyme activity inhibition caused by the test compound's increasing concentrations:

$$100 - \left(\frac{vi}{vo} X100\right) - - - - - - equation\ 1$$

Where vo is the enzyme activity and vi is the rate computed with the inhibitor present.

Molecular Modeling

Virtual screening was conducted using the Maestro graphical user interface, which was developed by Schrödinger in New York, NY, USA [13]. The Protein Data Bank (PDB) of the Research Collaboratory for Structural Bioinformatics (RCSB) provided the crystal structures of the enzymes under examination, which matched the β-Secretase active site (PDB ID: 3DV1) in complex with NVP-ARV999 ((2S,5S,7R)-2,7-dimethyl-3,15-dioxo-1,4-diazacyclopentadecan-5-yl)-(2R,4S)-N-butyl-4-[4-hydroxy-2-methylbutanamide) derived from the *Homo sapiens* (Figure 1). The protein structure underwent a comprehensive optimization and minimization process using the OPLS3 force field. With the use of the Protein Preparation Wizard program from Schrodinger Maestro, polar hydrogens were added and unnecessary water molecules were eliminated from each of the crystal forms. The produced protein structures were used to create the receptor grids (20 Å), which were then centred on the unique binding pockets that Maestro's Glide module had found for each protein. Maestro Ligprep and Confgen tools were utilized for ligand preparation and conformers generation, respectively. The Ligand docking tool was utilized for the docking procedure employing the Extra Precision (XP) mode. The glide grides for the proteins and Confgen for the ligands were docked. Using XP GScore and

dG Bind (MM-GBSA), the docked position with the highest negative binding energy for each target was selected.

Statistical Analysis

Inhibition curves for each chemical were created and for every curve, the parameters for linear regression were identified, and the IC_{50} values were interpolated. GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA) is the computer tool that was utilized to analyze these data.

RESULTS

From the study, 8-hydroxycolumbin, a clerodane diterpenoid has the highest potential to inhibit the enzyme β-secretase *in vitro* (IC_{50} = 0.040±0.00 mg/mL, 58.58% inhibition), followed by corydine, an isoquinoline alkaloid (IC_{50} = 0.076±0.00 mg/mL, 53.88% inhibition) at 0.1 mg/mL when compared to quercetin (IC_{50} = 0.055±0.00 mg/mL, 72.32% inhibition) (Table 1).

Virtual Screening Analyses of Compounds

The docking results between the ligands and β-Secretase active site showed that the compound columbin which is a bioactive diterpenoid furanolactone has interaction with protein residue Phe 108 and Thr 72. It was observed that the protein residue Phe 108 has hydrogen bonding with the hydroxy group at position 3 and another π-π Interaction between the protein residue Thr 72 and the furan ring (Figure 2, Table 2). The estimated free energy of binding (ΔG_{bind}), for XP G score is -3.963 kcal/mol and -5.97 kcal/mol for (ΔG_{bind}) (MM-GBSA). 8-hydroxycolumbin, a clerodane diterpenoid has hydrogen bonding interaction with Gln 73 and Thr 232 with the hydroxyl group at position 8 and position 4 of the moiety, respectively. The estimated free energy of binding (ΔG_{bind}), kcal/mol for XP G score to be (-7.317) and (-16.68) for (ΔG_{bind}) (MM-GBSA) (Figure 3, Table 2). Corydine a naturally occurring alkaloid has a hydrogen bond with protein residue Asp 32 was bound to the hydroxyl group at position 1 of the benzene ring and residue Asp 228 had hydrogen bonding with a methoxy group at position 2 of the benzene ring and another interaction with Thr 231 at position 5 of the nitrogen-containing ring. The estimated free energy of binding (ΔG_{bind}), kcal/mol for XP G score to be (-4.063) and (-21.64) for (ΔG_{bind}) (MM-GBSA) (Figure 4, Table 2). N-Formylanonaine a naturally occurring alkaloid has a hydrogen bond with Thr 72, Asp 32 and a π-π Interaction with Gln 73. One of the hydrogen bond interactions was between the residue Thr 72 and the carbonyl group attached to Nitrogen at position 12 while the other residue Gln 73 bonded with one of the aromatic rings not attached to methylenedioxy ring (–OCH_2O-) and Asp 32 bonded with the nitrogen-containing ring at position 4. The estimated free energy of binding (ΔG_{bind}), kcal/mol for XP G score to be (-5.026) and (-28.64) for (ΔG_{bind}) (MM-GBSA) (Figure 5, Table 2). The co-crystallize ligand (NVP-ARV999) has several hydrogen bonding interactions with the following protein residues Gln 73, Thr 72, Gly 34, Asp 228 and Gly 230. Hydrogen bond was observed with the residue Asp 228 attached to the hydroxyl group of the molecule and another two-hydrogen bonding attached to two (2) carbonyl groups with the residues Thr 72 and Gln 73. We also observed two other hydrogen bonding interactions with the protein residue Gly 34 and Gly 230 attached to the amine groups. The estimated free energy of binding (ΔG_{bind}), kcal/mol for XP G score to be (-9.164) and (-57.75) for (ΔG_{bind}) (MM-GBSA) (Figure 6, Table 2).

DISCUSSION

Many scientific groups worldwide have been working hard to find and create inhibitors that can enter the brain and efficiently block BACE1. To further corroborate our findings, several diterpenoids has been reported for their anti-Alzheimer's potentials. Onoja *et al*. [10-11] reported the acetylcholinesterase inhibitory potentials of 8-hydroxycolumbin, Tinosporide and columbin belonging to the class of clerodane diterpenoids. 7-Oxo-ent-pimara-8(14),15-diene-19-oic acid, 16α-Hydroxy-17-isovaleroyloxy-ent-kauran-19-oic acid, 17-Hydroxy-ent-kaur-15-en-19-oic acid are diterpenes reportedly isolated from *Aralia cordata* as BACE1 inhibitors [14]. Also, several plant-derived alkaloids in the class of aporphines have served as drug leads for Alzheimer's disease. Magnoflorine an aporphine alkaloid from *Coptidis rhizome* was found to inhibit BACE1 [15]. Oxoisoaporphine alkaloids such as 1-azabenzanthrone have been shown to possess various biological properties, such as cholinesterase and β-amyloid inhibition [16].

Molecular docking studies were carried out to understand the binding pattern (referred together as the "pose") between the ligands isolated from *Tinospora cordifolia* and β-Secretase active site (PDB ID: 3DV1) in complex with NVP-ARV999 **(**(2R,4S)-N-butyl-4-[(2S,5S,7R)-2,7-dimethyl-3,15-dioxo-1,4-diazacyclopentadecan-5-yl]-4-hydroxy-2-methylbutanamide) from the organism *Homo sapiens*. Ligand-protein docking facilitates the prediction of the binding mechanism between a ligand and a protein with established three-dimensional structures. A larger negative docking score from XP G Score and Molecular mechanics with generalised Born and surface area solvation (MM-GBSA) suggests a stronger binding affinity between the ligand and the target protein. This negative value signifies the anticipated binding free energy, with diminished values denoting more advantageous interactions [17]. From the virtual screening, it was observed that the natural compounds N-formylanonaine, corydine and 8-hydroxycolumbin can be said to be a potential β-Secretase inhibitor due to their abilities to bind favourably to protein residues of the β-Secretase active site. N-formylanonaine and 8-hydroxycolumbin has hydrogen bonding interaction with same protein residues Thr 72 and Gln 73 as the co-crystallized ligand and also based on their docking and MM-GBSA scores which is an indication of their stability within the active pocket of the protein. Hydrogen bond and π-stacking interaction as displayed by the compounds play a crucial role in determining

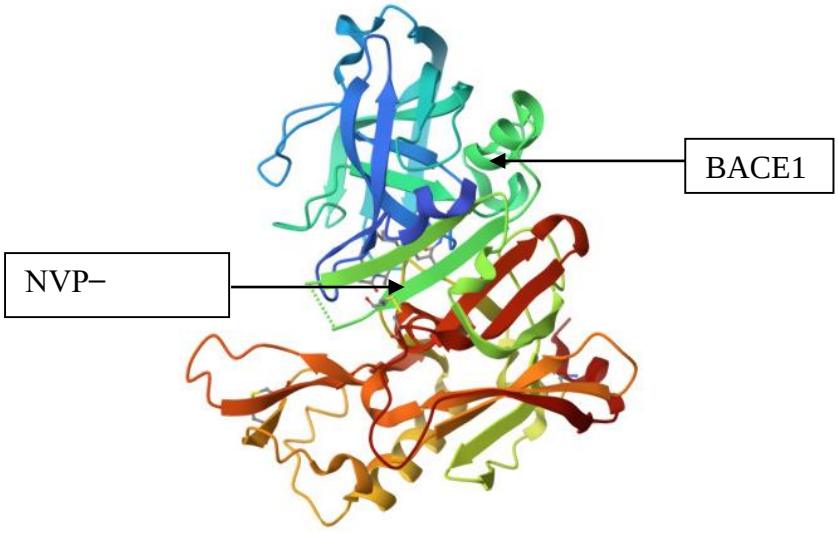

Figure 1: Crystal structure of human β-secretase in complex with NVP-ARV999

Table 1: β-Secretase inhibitory activity of compounds isolated from *Tinospora cordifolia* at 0.1 mg/mL

Test compound(s)	**IC_{50} (mean±SD)**
Columbin	0.109±0.00
8-hydroxycolumbin	0.040±0.00
Corydine	0.076±0.00
N-Formylanonaine	0.117±0.00
Quercetin	0.055±0.00

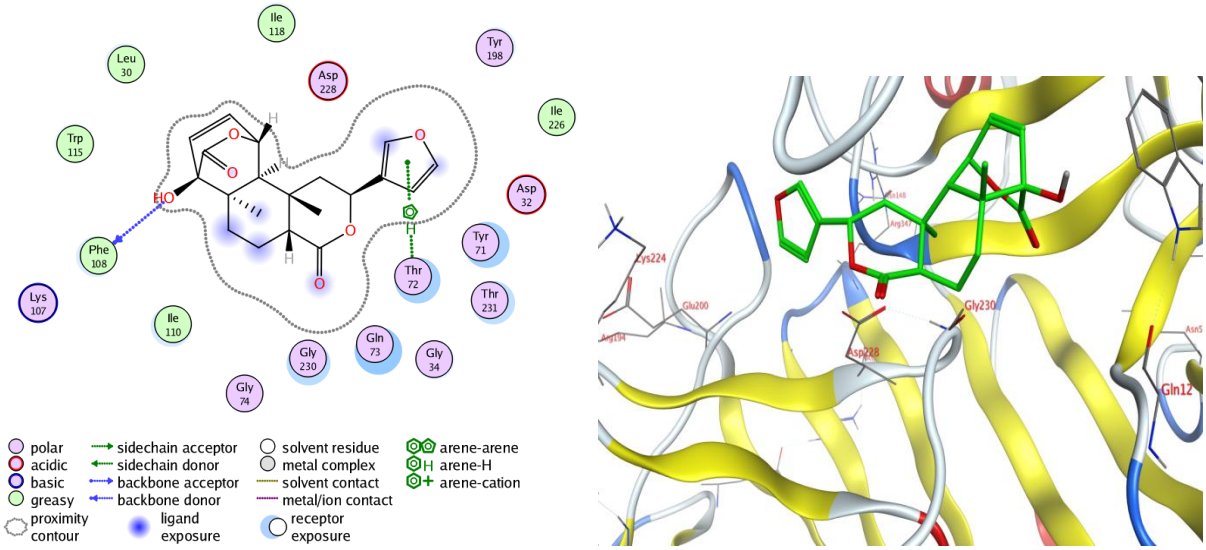

Figure 2: 2D and 3D binding interactions of columbin with β-Secretase active site (PDB ID: 3DV1)

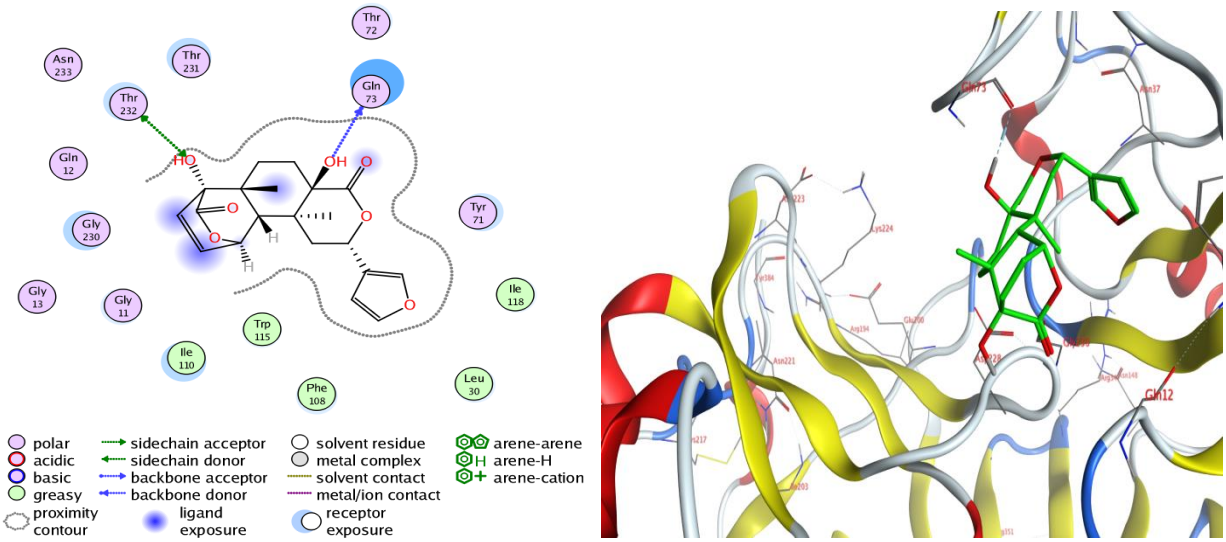

Figure 3: 2D and 3D binding interactions of 8-hydroxycolumbin with β-Secretase active site (PDB ID: 3DV1)

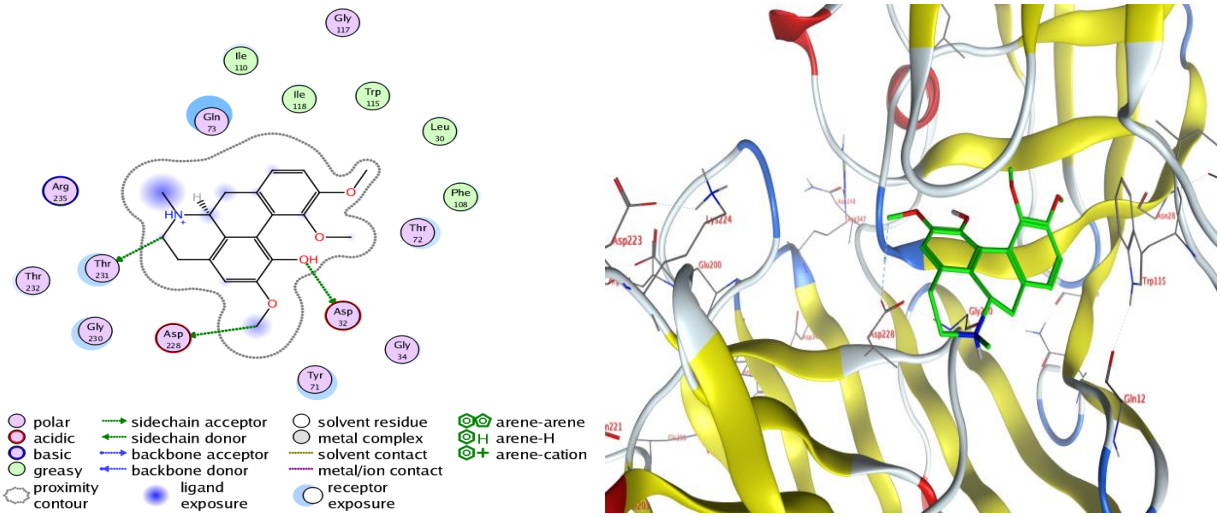

Figure 4: 2D and 3D binding interactions of corydine with β-Secretase active site (PDB ID: 3DV1)

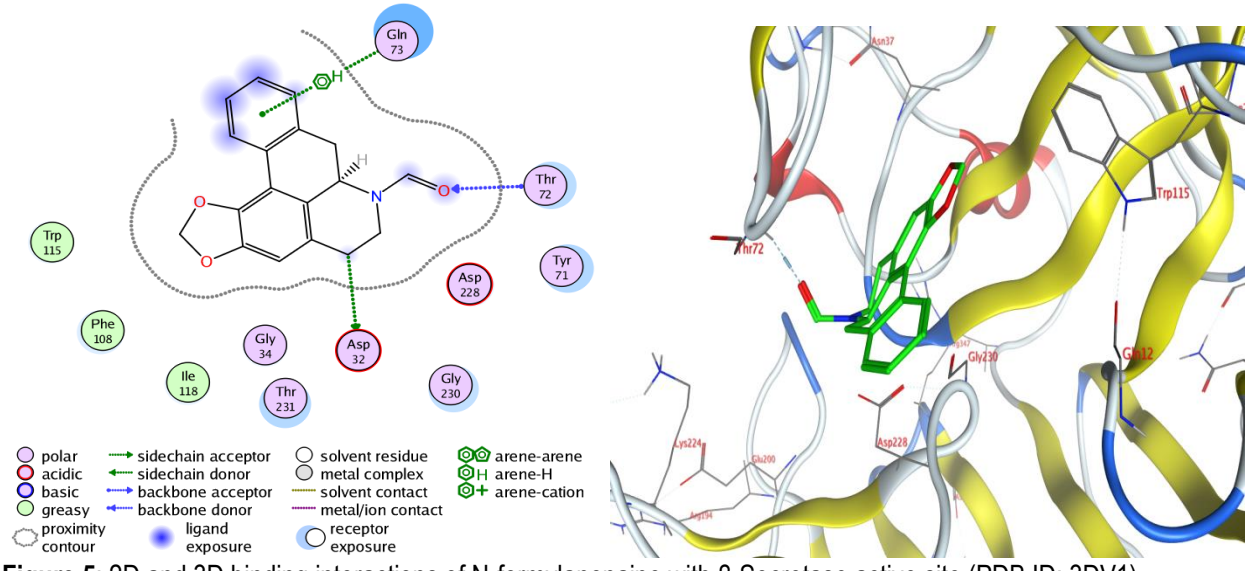

Figure 5: 2D and 3D binding interactions of N-formylanonaine with β-Secretase active site (PDB ID: 3DV1)

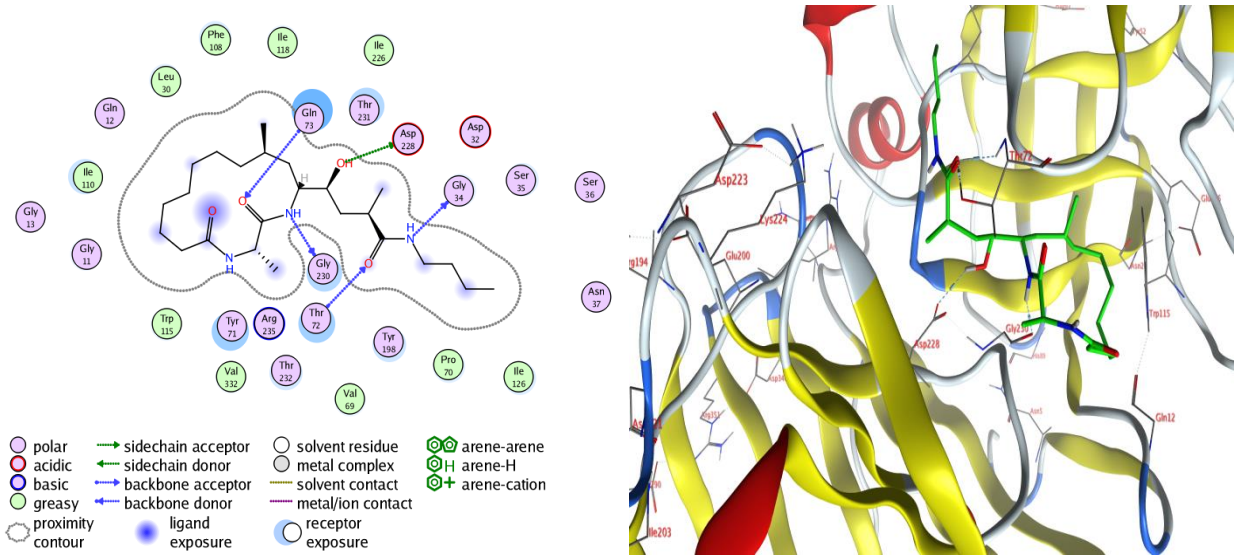

Figure 6: 2D and 3D binding interactions of co-crystallize ligand (NVP-ARV999) with β-Secretase active site (PDB ID: 3DV1)

Table 2: Estimated free energy binding score and ligand interaction with β-Secretase active site (PDB ID: 3DV1)

Ligand(s)	XP G Score (kcal/mol)	(ΔG_{bind}) (MM-GBSA) (kcal/mol)	Ligand interactions
Columbin	-3.963	-5.97	Hydrogen bond with Phe 108 and π-π Interaction with Thr 72
8-hydroxycolumbin	-7.317	-16.68	Hydrogen bond with Gln 73 and Thr 232
Corydine	-4.063	-21.64	Hydrogen bond with Asp 32, Asp 228, and Thr 231
N-formylanonaine	-5.026	-28.64	Hydrogen bond with Thr 72, π-π Interactions with Gln 73 and Asp 32
NVP-ARV999	-9.164	-57.75	Hydrogen bond with Gln 73, Thr 72, Gly 34, Asp 228 and Gly 230

the stability and specificity of the complex and also can increase the binding affinity of the inhibitor for its target [18]. To corroborate our research finding, compounds in the class of terpenoids such as lupeol and lupenone isolated from *Pueraria lobata* inhibit BACE1 and also demonstrated interaction with the enzyme active site [19] and also several alkaloids have been reported to be a promising dual inhibitor against β and γ-Secretase proteins *in silico* [20].

CONCLUSION

N-formylanonaine, corydine and 8-hydroxycolumbin could be a template for structural modifications in the discovery of new neurotherapeutic agent in the management of Alzheimer's disease due to their ability to effectively bind to BACE 1 as revealed by MM-GBSA. Hence the compounds might be able to prevent the accumulation of β-amyloid plaques.

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AUTHORS' CONTRIBUTION

Onoja O. Joel designed the experiment, collected and extracted the plant, isolated and characterized the compounds, wrote the first draft of the manuscript, and supervised and performed the *in silico* studies. Julius I. Olawuni performed the bio-assay *in vitro*. Both authors read and approved the manuscript.

CONFLICT OF INTEREST

There are no conflicts of interest.

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REFERENCES

1. Duran-Aniotz C, Moreno-Gonzalez I, Gamez N, Perez-Urrutia N, Vegas-Gomez L, Soto C, Morales R. Amyloid pathology arrangements in Alzheimer's disease brains modulate in vivo seeding capability.

Acta Neuropathologica Communications, 9, 2021:1-3.
2. Coimbra JR, Marques DF, Baptista SJ, Pereira CM, Moreira PI, Dinis TC, Santos AE, Salvador JA. Highlights in BACE1 inhibitors for Alzheimer's disease treatment. Frontiers in Chemistry. 6, 2018:178.
3. Hampel H, Vassar R, De Strooper B, Hardy J, Willem M, Singh N, Zhou J, Yan R, Vanmechelen E, De Vos A, Nisticò R. The β-secretase BACE1 in Alzheimer's disease. Biological psychiatry, 89(8), 2021:745-56.
4. Yan R, Vassar R. Targeting the β secretase BACE1 for Alzheimer's disease therapy. The Lancet Neurology, 13(3), 2014:319-29.
5. Fernando W, Somaratne G, Goozee KG, Williams S, Singh H, Martins RN. Diabetes and Alzheimer's disease: can tea phytochemicals play a role in prevention?. Journal of Alzheimer's Disease, 59(2), 2017:481-501.
6. Youn K, Park JH, Lee S, Lee S, Lee J, Yun EY, Jeong WS, Jun M. BACE1 inhibition by genistein: biological evaluation, kinetic analysis, and molecular docking simulation. Journal of Medicinal Food, 21(4), 2018:416-20.
7. Naushad M, Durairajan SS, Bera AK, Senapati S, Li M. Natural compounds with anti-BACE1 activity as promising therapeutic drugs for treating Alzheimer's disease. Planta Medica, 85(17), 2019:1316-25.
8. Yu T, Paudel P, Seong SH, Kim JA, Jung HA, Choi JS. Computational insights into β-site amyloid precursor protein enzyme 1 (BACE1) inhibition by tanshinones and salvianolic acids from Salvia miltiorrhiza via molecular docking simulations. Computational Biology and Chemistry, 74, 2018:273-85.
9. Yang C, Chen EA, Zhang Y. Protein–ligand docking in the machine-learning era. Molecules, 27(14), 2022:4568.
10. Onoja OJ, Elufioye TO, Sherwani ZA, Ul-Haq Z. Molecular docking studies and anti-Alzheimer's potential of isolated compounds from *Tinospora cordifolia*. Journal of Biologically Active Products from Nature, 10(2), 2020:100-21.
11. Onoja JO, Elufioye TO, Sherwani ZA, Ul-Haq Z. Molecular docking study on columbin isolated from *Tinospora cordifolia* as a cholinesterase inhibitor. Tropical Journal of Pharmaceutical Research, 20(2), 2021:337-43.
12. Mancini F, Naldi M, Cavrini V, Andrisano V. Multiwell fluorometric and colorimetric microassays for the evaluation of beta-secretase (BACE-1) inhibitors. Analytical and Bioanalytical Chemistry, 388, 2007:1175-83.
13. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, Repasky MP, Knoll EH, Shelley M, Perry JK, Shaw DE. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. Journal of Medicinal Chemistry, 47(7), 2004:1739-49.
14. Jung HA, Lee EJ, Kim JS, Kang SS, Lee JH, Min BS, Choi JS. Cholinesterase and BACE1 inhibitory diterpenoids from *Aralia cordata*. Archives of Pharmacal Research, 32, 2009:1399-408.
15. Jung HA, Min BS, Yokozawa T, Lee JH, Kim YS, Choi JS. Anti-Alzheimer and antioxidant activities of *Coptidis rhizoma* alkaloids. Biological and Pharmaceutical Bulletin, 32(8), 2009:1433-8.
16. Zhang J, Chen L, Sun J. Oxoisoaporphine Alkaloids: Prospective Anti-Alzheimer's Disease, Anticancer, and Antidepressant Agents. ChemMedChem, 13(13), 2018:1262-74.
17. Agu PC, Afiukwa CA, Orji OU, Ezeh EM, Ofoke IH, Ogbu CO, Ugwuja EI, Aja PM. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific Reports, 13(1), 2023:13398.
18. Salentin S, Haupt VJ, Daminelli S, Schroeder M. Polypharmacology rescored: Protein–ligand interaction profiles for remote binding site similarity assessment. Progress in Biophysics and Molecular Biology, 116(2-3), 2014:174-86.
19. Koirala P, Seong SH, Jung HA, Choi JS. Comparative molecular docking studies of lupeol and lupenone isolated from *Pueraria lobata* that inhibits BACE1: Probable remedies for Alzheimer's disease. Asian Pacific journal of tropical medicine, 10(12), 2017:1117-22.
20. Onifade Jr I, Umar H, Aborode A, Awaji A, Jegede I, Adeleye B, Fatoba D. In silico study of selected alkaloids as dual inhibitors of β and γ-Secretases for Alzheimer's disease. bioRxiv, 2024:2024-09.