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Research Article

In silico anti-Alzheimer potential of bioactive compounds in fungi from the African Natural Products Database

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Abstract

Research into treatments for Alzheimer's has led to several thousand bioactive compounds being tested in clinical trials, of which over 400 have failed. Given the exponential increase in the number of cases, it makes sense to intensify the search for new anti-Alzheimer's compounds. To this end, bioactive compounds from fungi are candidates for the treatment of Alzheimer's disease (AD). This work presents the anti-Alzheimer's potential *in silico* of fungi compounds contained in the African Natural Products Database (ANPDB) capable of crossing the blood-brain barrier (BBB). A virtual search for compounds of interest in ANPDB was carried out, during which 17 fungal families were identified and 91 bioactive compounds characterized. Among these molecules, those with have a log BB>0.3 in the pkCSM server were selected. Following this, a literature search using references on compelling compounds not studied in Alzheimer's disease was also selected. Conformational site analysis and docking parameters such as binding energy, and interaction profiles with AD target residues were determined. the selected compounds were: (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one, Diorcinol, Elgonene B and H, Ergosta-7-en-3beta-ol and Novae-zelandin. Ergosta-7-en-3beta-ol scored better than donepezil on BuchE. This interacts with His 438 of the catalytic triad, Trp 82 of the anionic site, and Tyr 332 of the peripheral site BuchE. Ergosta-7-en-3beta-ol, Elgonene B and H have higher docking scores on β-secretase. These and diorcinol also showed better score with GSK 3β. This study shows that ergosta-7-en-3beta-ol, Elgonene H and B and diorcinol have multi-targeted therapeutic potential for the treatment of AD.

Keywords: fungi compounds, ANPDB, *in silico*, AD and BBB

1. INTRODUCTION

Fungi have been appreciated for many decades for their culinary and nutritional value, but they are increasingly appreciated for their many medicinal properties, earning them the title of "superfood of the future" [1]. Although their use as a food supplement, nutraceutical, and mycotherapy product has been known since antiquity in Asian regions, they remain quite recent in Western regions [2]. In addition to these properties, fungi are well known for their bioactive compounds (lectins, polysaccharides (β-glucans), polysaccharide peptides, polysaccharide-protein complexes, terpenoids, alkaloids, sterols, and structured phenolic compounds), which are responsible for different biological and therapeutic activities ranging from antimicrobial, antioxidant, antidiabetic, anticancer, antiviral, anti-hypocholesterolemic, to neuroprotective properties [3] [4]. Due to these remarkable properties, several medicinal agents marketed today are derived from fungi including atorvastatin, aspirin, vincristine, taxol, vinblastine, cyclosporin A, lampterol, etc [5]. recently, bioactive compounds derived from fungi have shown promising effects against AD [6]. This makes them an ideal reservoir for research of therapeutic compounds to combat AD, one of the greatest ills of our time. AD is the most common dementia worldwide, and many urgent efforts are being made to find bioactive compounds with anti-Alzheimer properties that can reduce the incidence of the disease while improving the quality of life of patients [7]. These efforts have resulted in several thousand bioactive compounds entering clinical trials, of which more than 400 have failed [8]. Faced with this situation, intensifying research into new anti-Alzheimer compounds is proving to be a major asset for research. As the search processes for therapeutic agents against AD have so far demonstrated enormous complexity, the constant advances in computational approaches such as the prediction of pharmacokinetics and pharmacodynamics represent effective tools for filtering and selecting new candidate molecules for medication. However, the empirical methods of research and drug design being tedious and AD being of multifactorial origin, these methods are also highly recommended [9] because it makes it possible to provide a multi-targeted strategy by simultaneously targeting the multiple proteins involved in the progression of the disease, the main ones being

acetylcholinesterase, butyrylcholinesterase, Glycogen synthase kinase 3 β and β-secretase [10].

Africa is rich in diverse fungi families, and the recent creation of ANPDB containing bioactive fungal compounds facilitates the screening of natural products against Alzheimer's disease [11; 12]. This article presents the *in silico* anti-Alzheimer potential of compounds characterized from fungi contained in ANPDB capable of crossing the BBB based on their Log BB prediction values.

2. MATERIALS AND METHODS

2.1. Study design

A virtual search of ANPDB was carried out using relevant publications relayed in the database [11; 12]. As a result, 91 characterized bioactive compounds from 17 fungal species were identified. Among these compounds, those likely to respond positively to BBB crossing, as described by the BBB predictive log value using the *pkCSM* server, were selected. Next, a literature search using recent references on these probative compounds was performed to highlight those that have not yet been studied in AD.

2.2. Molecular docking

2.2.1. Software used

Python 2.5, Python 2.7, *Molecular Graphics Lab Tools* (MGL), *AutoDockTools-1.5.7*, *Discovery Studio Visualizer 2.5.5*, and *ChemDraw* were used. Python 2.7 was downloaded from www.python.com, Cygwin (a data store) c:\program; and Python 2.5 were downloaded simultaneously from www.cygwin.com. Molecular Graphics Lab Tools (MGL) and *AutoDockTools-1.5.7* were downloaded from www.scripps.edu; *Discovery Studio Visualizer 2.5.5* was downloaded from www.accelerys.com; and *ChemDraw* was downloaded from www.clubic.com. Online smile scoring was performed using cactus.nci.nih.gov/translate/ [13].

2.2.2. Preparation of the target enzyme

The crystal structures of AchE, BuchE, β-secretase, and GSK-3β proteins were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database. Preparation of the target proteins with *AutoDock* tools involved the addition of all hydrogen atoms to the macromolecule, a necessary step for the correct calculation of partial atomic charges. Three-dimensional affinity grids (which close the amino acids of the active site of each protein [14]) sized with a spacing of 0.375 Å were centered on the geometric center of the proteins and were calculated for each of the following atom types (HD, C, A, N, OA and SA), representing all possible atom types in a protein [15].

2.2.3. Preparation of the ligand

The two-dimensional (2D) structures of the ligands of interest and reference drugs (donepezil, valiltamiprosate, and hydromethylthionine) were drawn using ChemDraw software (Figure 1). Three-dimensional (3D) structures were obtained using ChemDraw 3D software and energy minimization was performed using the molecular mechanics force field (MM2) and saved in PDB format using the same software.

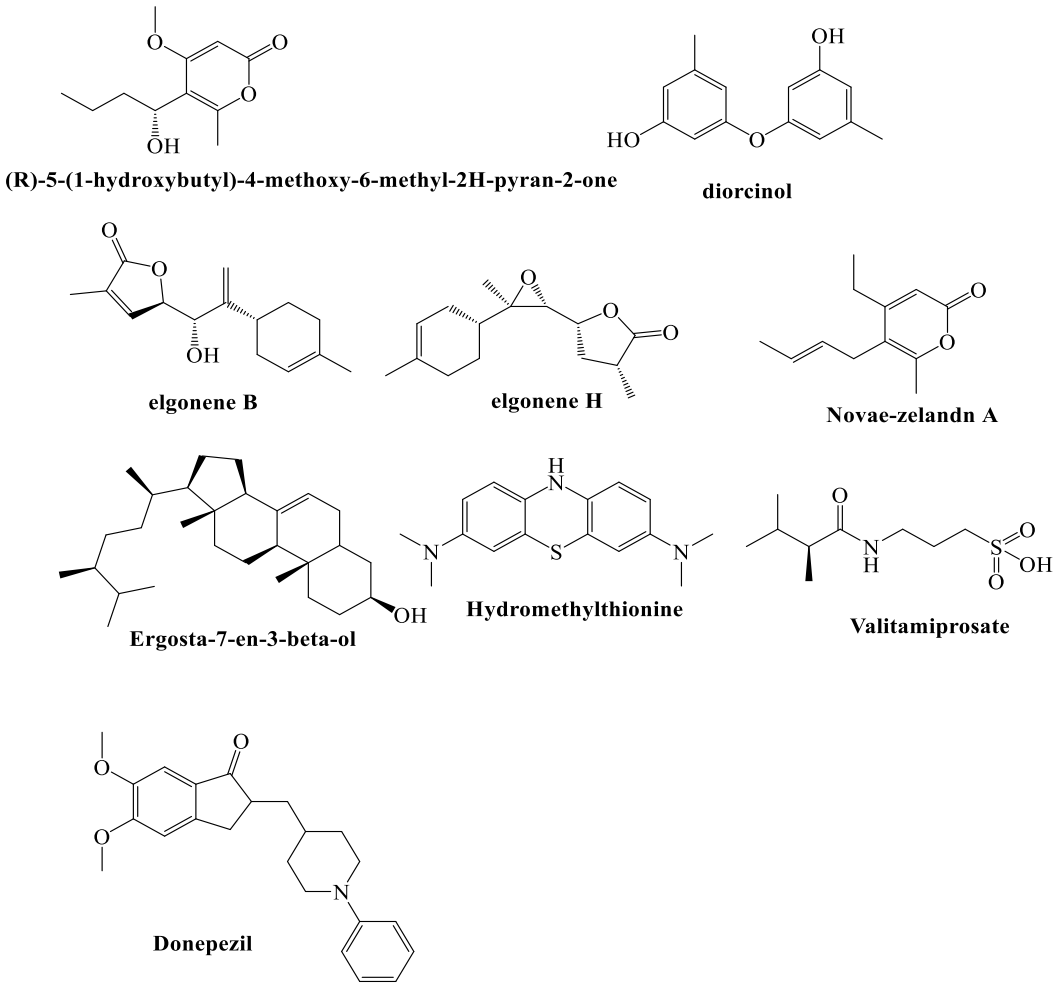


Figure 1: structure of interest ligands and reference drugs

2.2.4. In silico inhibition

The molecular docking used the *Lamarckian* genetic algorithm method, which performed a total of 2,500,000 energy calculations for every run and 10 total generations. The algorithm method works using traditional force fields, providing empirical free energy functions and a binding energy constant (μM) with "master equation" as follows:

$$\mathbf{\Delta G} = \Delta Gvdw + \Delta Ghbond + \Delta Gelec + \Delta Gconform + \Delta Gtor + \Delta Gsol$$

The typical molecular mechanics terms are the van der Waals interaction (ΔGvdw + desolv), electrostatic interaction (ΔGelec), torsional energy (ΔGtor), desolvation upon binding, and the hydrophobic effect (+ΔGsol). The other docking simulations were carried out with *AutoDock* default parameters. Finally, 10 different conformations were studied for a single compound. For each ligand, 25 best docking simulations were obtained against the target molecule [16]. The results were visualized using *the Discovery Studio visualizer*.

3. RESULTS

3.1. Prediction of BBB permeability and Lipinski Violations

Table 1 shows the prediction of BBB permeability and Lipinski violations.

Table 1: Prediction of BBB permeability and Lipinski violations of potential anti-Alzheimer compounds

	BBB permeability (log BB)	Lipinski Violations
(R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one	0.458	0
Diorcinol	0.347	0
Elgonene B	0.598	0
Elgonene H	0.401	0
Ergosta-7-en-3beta-ol	0.77	1
Novae-zelandin A	0.369	0

The compounds that responded positively to the BBB cross and drug- descriptors (Lipinski violations) are represented in Table 1. Among the 91 compounds screened in the 'African Natural Product Database', only the 6 compounds having responded positively to the BBB crossing parameter and not having been subject of any recent work on AD were taken into account (Table 1). Those presenting low logBB predictive values, and have been subject of previous studies on AD were omitted. The selected compounds presented permeability scores (logBB) varying from acceptable (Diorcinol: 0.347) to very acceptable (Ergosta-7-en-3beta-ol: 0.77). Although having shown a significant capacity for its action, research and development processes of drugs are also accelerated thanks to the study of their characteristics. Thus, Lipinski violation parameters are used as criteria to locate possible drug candidates. The compounds investigated except for ergosta-7-en-3beta-ol did not present any Lipinki violation.

3.2. Molecular docking scores of interest ligands on therapeutic targets in Alzheimer's disease

3.2.1. Docking scores of interest compound on Alzheimer's disease therapeutic targets

Table 2 shows the docking scores of compounds of interest on Alzheimer's disease therapeutic targets.

The docking scores of the target compounds and various conventional compounds from the pre-clinical studies are shown in Table 2. According to the AutoDock software, the compounds most likely to establish bonds with negative energy (ΔG) represent those possessing a better existential affinity between the compound and its target. From this perspective, a value is all the more negative as its affinity score is better.

The compounds investigated respectively presented docking scores between -5.70 and -9.38 kcal/mol for AchE, -5.15 and -10.38 kcal/mol for BuChE, -5.35 and -9.80kcal/mol for β -secretase and finally -5.43 and -8.92 for GSK3β. Likewise, the compounds used as standards presented docking scores of -10.91kcal/mol, -9.02kcal/mol, -6.80kcal/mol, and -6.79kcal/mol corresponding respectively to the affinity energies established with AChE, BuChE, β -secretase and GSK3β. Compared to the energies presented with the lead compounds, ergosta-7-en-3beta-ol presented an affinity close to that of donepezil (Table 2) for acetylcholinesterase and better than that of all the other standards for their respective targets (Table 2). Furthermore, elgonene H and B presented respective affinity energies of -7.53 and -7.68kcal/mol better than that of valiltamiprosate in β -secretase. Moreover, in the case of GSK3β, the latter as well as diorcinol also presented affinity energies better than that of hydromethylthionine used as a standard.

Table 2: Molecular docking scores (Kcal/mol) of interest compounds with pipeline drugs against four different target proteins associated with AD

Compounds / Standards	Selected target			
	AChE	**BuChE**	**BACE1**	**GSK3β**
(R)-5-(1-hydroxy butyl)-4-methoxy-6-methyl-2H-pyran-2-one	-5.70	-5.15	-5.35	-5.43
Diorcinol	-7.45	-7.62	-6.48	-7.28
elgonene B	7.85	-7.84	-7.53	-7.42
elgonene H	-7.77	-7.79	-7.68	-7.12
ergosta-7-en-3beta-ol	-9.38	-10.38	-9.80	-8.92
novae-zelandin A	5.98	-5.81	-6.15	-5.66
Donepezil	-10.91	- 9.02	/	/
Valiltamiprosate	/	/	-6.80	/
hydromethylthionine	/	/	/	-6.79

AchE: *Acetylcholinesterase,* ***BuchE:*** *Butyrylcholinesterase,* ***GSK-3β:*** *Glycogen synthetase kinase 3 beta*

3.2.2. Interaction profiles for compounds of interest and reference drugs with AD therapeutic targets

Figures 2, 3, 4, and 5 present the interactions between various active site residues of the different targets and the target compounds, as well as the reference compounds. Analysis of the 2D and 3D diagrams of the interactions established with AchE showed that the compounds penetrate the active site of acetylcholinesterase by binding to residues involved in the catalytic mechanism (Table 3 and Figure 2). Concerning BuchE, the compounds investigated established convincing interactions with the amino acid residues of these same sites for butyrylcholinesterase (Table 4 and Figure 3). In addition to this, interaction with the His438 residue of the butyrylcholinesterase catalytic triad was noted on the profiles of diorcinol, elgonene B, and ergosta-7-en-3beta-ol. On the other hand, so far donepezil has not shown any favorable interaction with the amino acid residues of the catalytic triad. On the other hand, with the case of β-secretase, these target compounds showed no interaction with the amino acid residues Asp32 and Asp228 of the catalytic dyad. However, a favorable interaction was noted between an important peripheral residue (Tyr71) and the compounds elgonene H and ergosta-7-en-3beta-ol (Figure 4). Ultimately, the analysis of the interactions established between the compounds investigated and GSK 3β showed that the compounds investigated establish favorable interactions with important amino acid residues involved in the catalytic mechanism of the enzyme (Figure 5).

Table 3: Profiles of important amino acid residues for AchE that interact with target compounds

	Catalytic triad	Oxyanion hole	Acyl pocket	Anionic site	Peripheral site
1	/	/	Phe295,		Tyr341, Tyr124, Trp286, Tyr72
2	/	Gly121	/	Trp86	/
3	/	/	Phe295,	/	Trp286, Tyr124, Tyr72
4	/	/	Phe295, Phe297	/	Tyr124, Tyr341, Trp286
5	/	/	/	/	Tyr124, Tyr341, Trp286, Tyr72
6	/	/	Phe295, Phe297, Phe338	/	Tyr124, Tyr341, Trp286, Tyr72
7	/	/	Phe295, Phe297, Phe338	Trp86	Tyr124, Tyr341, Trp286

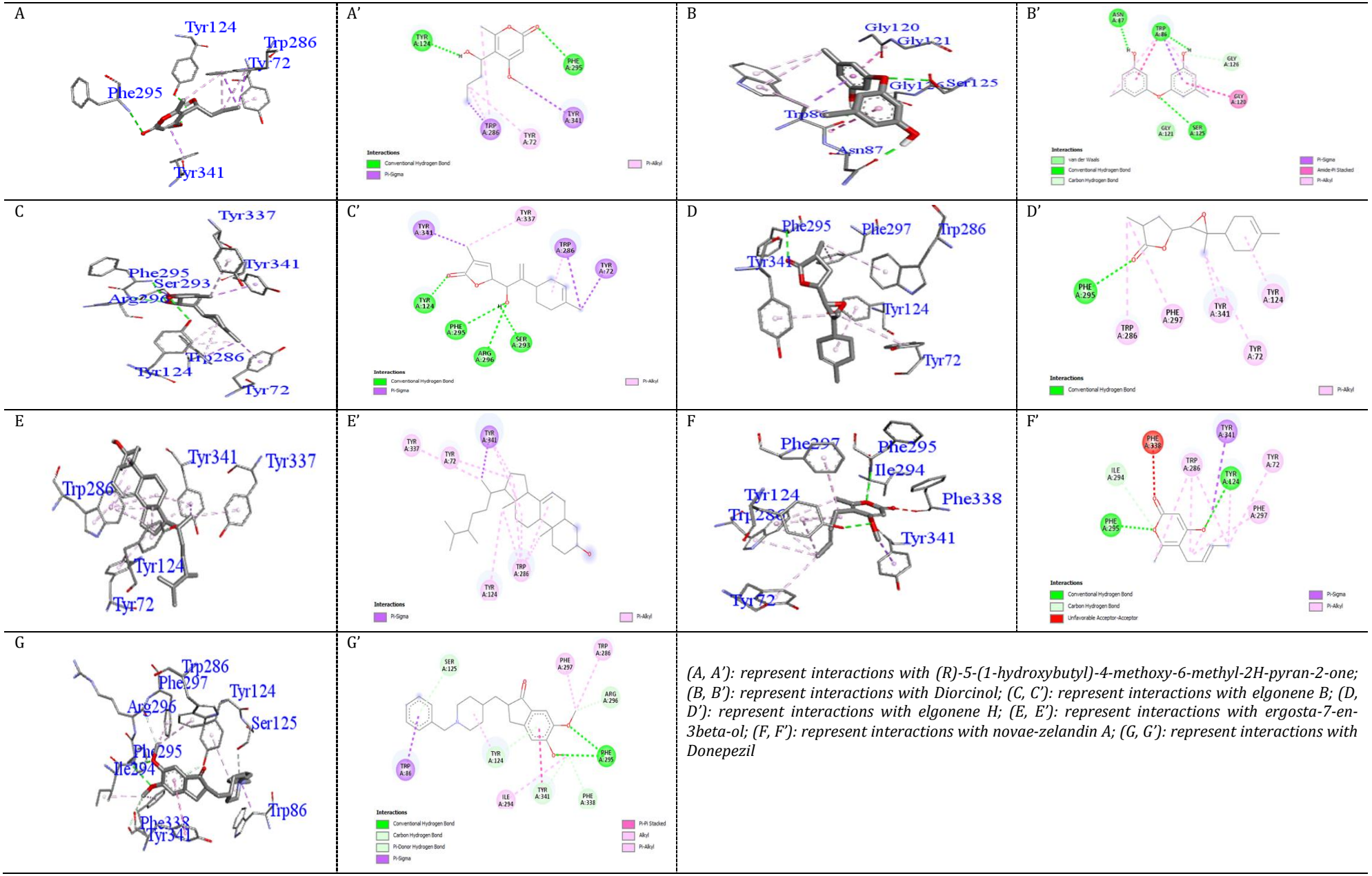
1: (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; 2: Diorcinol; 3: elgonene B; 4: elgonene H; 5: ergosta-7-en-3beta-ol; 6: novae-zelandin A; 7: Donepezil

Table 4: Profiles of important amino acid residues for BuchE that interact with target compounds

	Catalytic triad	Oxyanion hole	Acyl pocket	Anionic site	Peripheral site
1	/	Gly116	/	Trp82	/
2	His438	/	/	Trp82	Tyr332
3	His438	/	/	Trp82	Tyr332
4	/	/	/	Trp82	Tyr332
5	His438	/	Phe329	Trp82	Tyr332
6	/	/	Phe329	Trp82	Tyr332
7	/	Gly116	Phe329	Trp82	Tyr332, Asp70

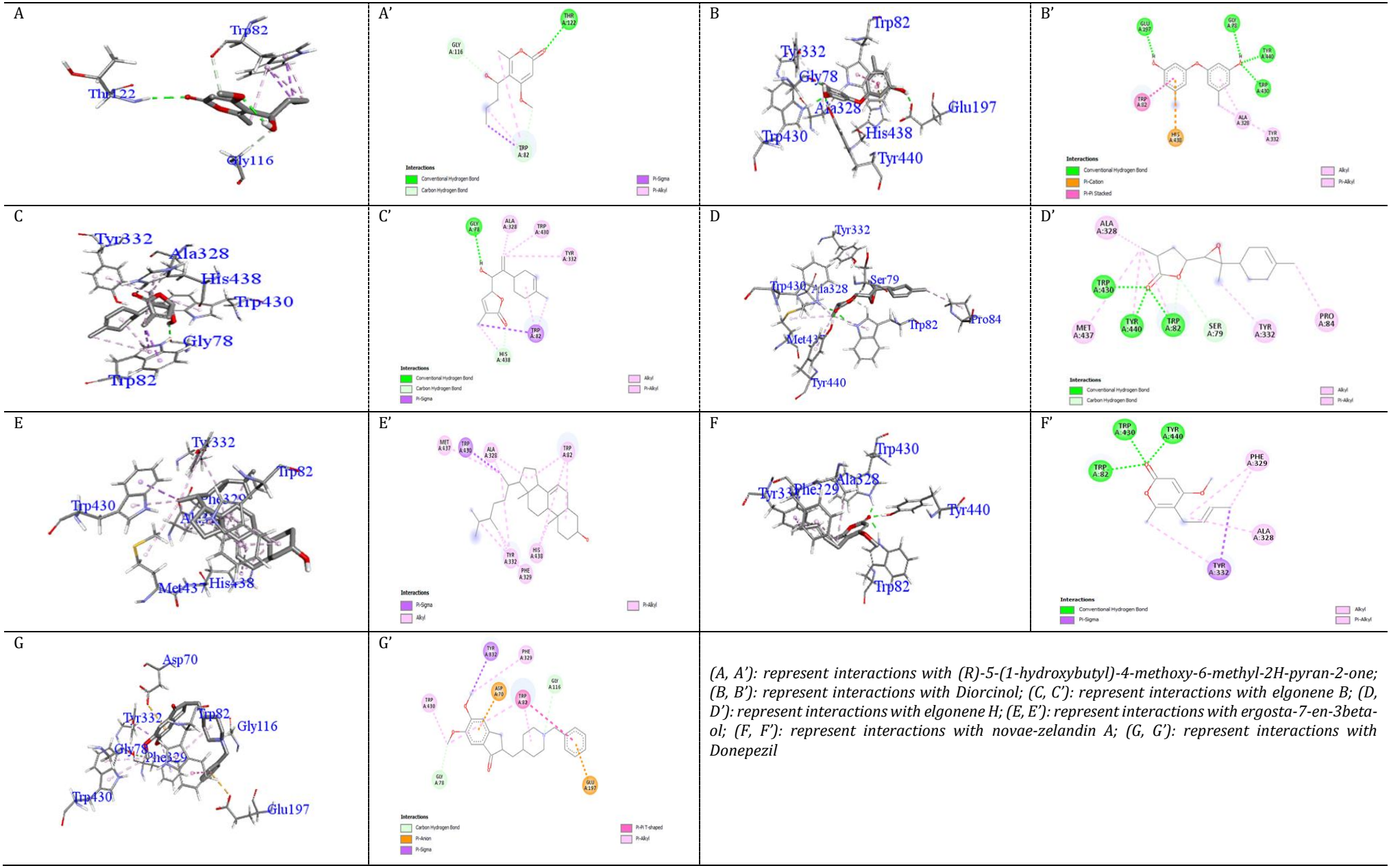
1: (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; 2: Diorcinol; 3: elgonene B; 4: elgonene H; 5: ergosta-7-en-3beta-ol; 6: novae-zelandin A; 7: Donepezil

Figure 2: 3D and 2D views of molecular interactions of interest compounds and standard in development on AchE



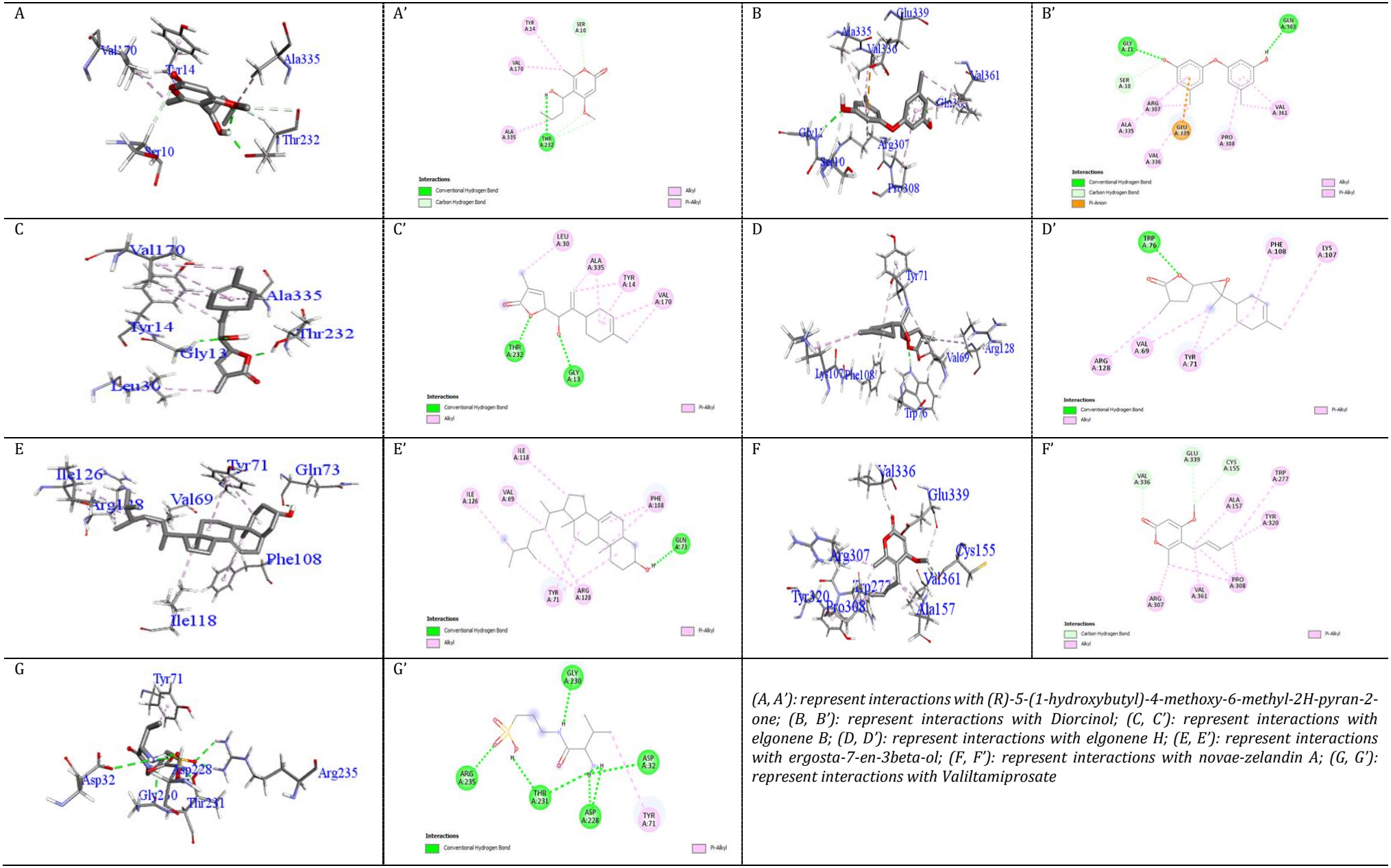
(A, A'): represent interactions with (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; (B, B'): represent interactions with Diorcinol; (C, C'): represent interactions with elgonene B; (D, D'): represent interactions with elgonene H; (E, E'): represent interactions with ergosta-7-en-3beta-ol; (F, F'): represent interactions with novae-zelandin A; (G, G'): represent interactions with Donepezil

Figure 3: 3D and 2D views of molecular interactions of interest compounds and standard in development on BuchE



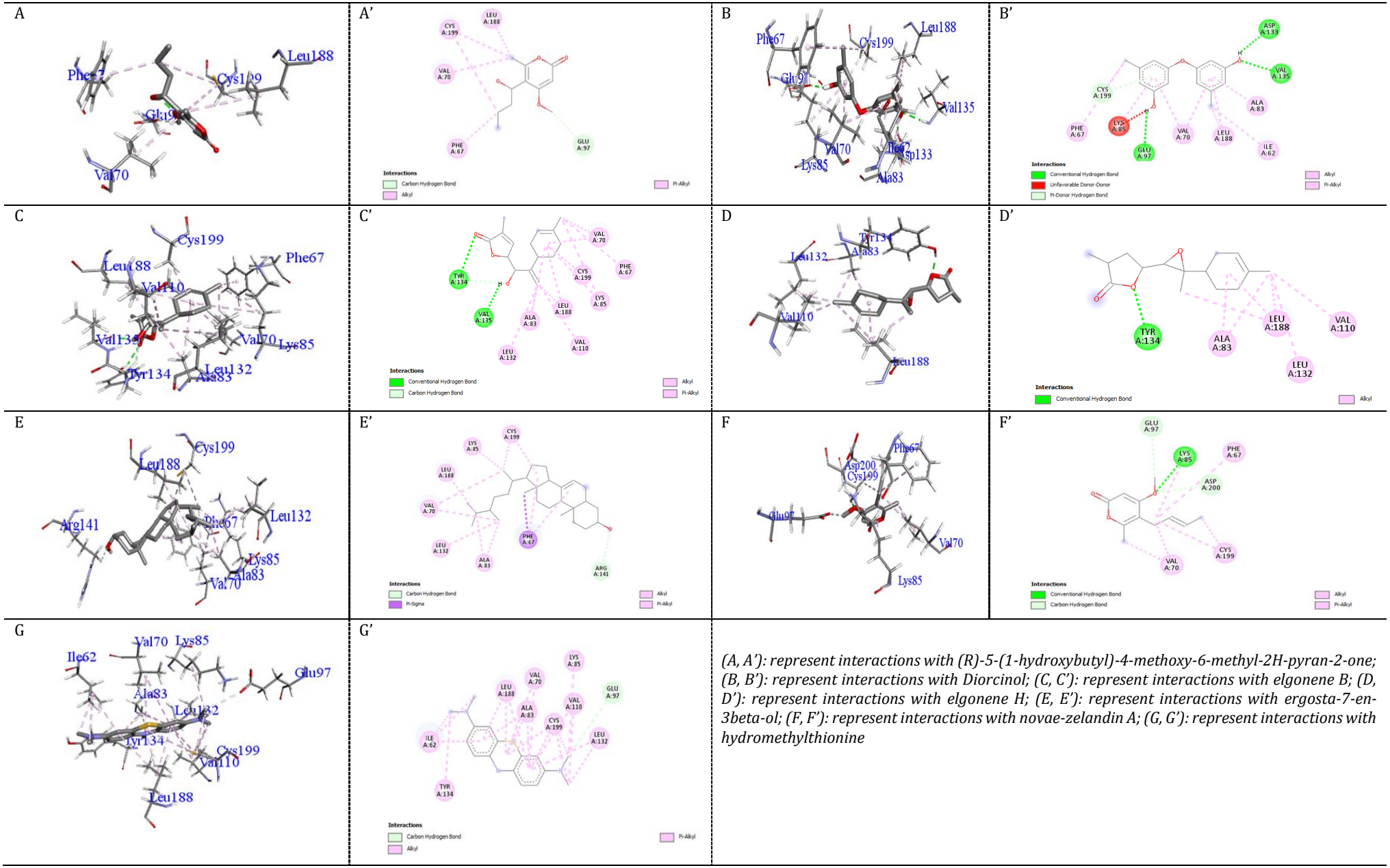
(A, A'): represent interactions with (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; (B, B'): represent interactions with Diorcinol; (C, C'): represent interactions with elgonene B; (D, D'): represent interactions with elgonene H; (E, E'): represent interactions with ergosta-7-en-3beta-ol; (F, F'): represent interactions with novae-zelandin A; (G, G'): represent interactions with Donepezil

Figure 4: 3D and 2D views of molecular interactions of interest compounds and standard in development on β-secretase



(A, A'): represent interactions with (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; (B, B'): represent interactions with Diorcinol; (C, C'): represent interactions with elgonene B; (D, D'): represent interactions with elgonene H; (E, E'): represent interactions with ergosta-7-en-3beta-ol; (F, F'): represent interactions with novae-zelandin A; (G, G'): represent interactions with Valiltamiprosate

Figure 5: 3D and 2D views of molecular interactions of interest compounds and standard in development on GSK 3β



(A, A'): represent interactions with (R)-5-(1-hydroxybutyl)-4-methoxy-6-methyl-2H-pyran-2-one; (B, B'): represent interactions with Diorcinol; (C, C'): represent interactions with elgonene B; (D, D'): represent interactions with elgonene H; (E, E'): represent interactions with ergosta-7-en-3beta-ol; (F, F'): represent interactions with novae-zelandin A; (G, G'): represent interactions with hydromethylthionine

4. DISCUSSION

The progressive and debilitating nature of AD and the lack of effective treatments for it contribute to a significant economic and societal burden on the global healthcare system [17]. This has led to increased efforts in the search for treatments capable of preventing AD, delaying its onset, slowing its progression, and alleviating its symptoms [8]. Because of the remarkable therapeutic properties of fungi, many drugs on the market today are derived from them, including atorvastatin, aspirin, vincristine, taxol, vinblastine, cyclosporine A, lampterol, and many others. Many researchers have turned to them in their search for anti-Alzheimer's candidates [6]. This work aimed to present the anti-Alzheimer's potential *in silico* of compounds characterized from fungi contained in ANPDB capable of crossing the BBB based on their Log BB prediction values. A virtual search of ANPDB identified 91 characterized bioactive compounds from 17 fungal species. Of these compounds, only six responded positively to BBB crossing, as described by the BBB predictive log value using the *pkCSM* server and not yet explored in AD. These are (R)-5-(1-hydroxy butyl)-4-methoxy-6-methyl-2H-pyran-2-one, Diorcinol, Elgonene B, Elgonene H, Ergosta-7-en-3beta-ol and Novae-zelandin A. Except for Ergosta-7-en-3beta-ol, which has a lipinski violation score of 1, the other compounds do not violate this rule. In the drug discovery setting, the *Rule of 5* predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500, and the calculated Log P (CLog P) is greater than 5 [18]. Bioactive compounds that cross the BBB can act as scaffolding to regulate several biological processes in the brain. Due to severely damaged cholinergic neurons, the amount of AChE decreases by 90% in advanced AD. Meanwhile, BChE levels and function reach 105-165% of normal, making it the main metabolic enzyme for AD [19]. As a result, licensed selective AChE inhibitors, such as galantamine and donepezil, have a very limited effect on severe AD, which is linked to a significant drop in AChE levels in people with severe AD. In recent years, a growing number of researchers have focused their efforts on designing BChE inhibitors for the treatment of advanced AD [20]. The compounds examined recorded relatively high docking scores compared with AchE and BuchE. However, Ergosta-7-en-3beta-ol scored better than donepezil on BuchE. This interacts with His 438 of the catalytic triad, Trp 82 of the anionic site, and Tyr 332 of the peripheral site on BuchE. It could be a good candidate against memory loss in the advanced stages of Alzheimer's disease. Accumulation of senile plaques is central to AD, occurring as a result of fibrillation of amyloid β-peptide, which is produced by the action of β-secretase on the β-amyloid precursor protein. β-secretase is a key target in AD. The results show that, in addition to ergosta-7-en-3beta-ol, elgonene H and B score higher than valiltamiprosate (a drug in phase 3 clinical trials [21]) in docking β-secretase. In contrast, the interaction profile with β-secretase showed no interaction with Asp32 and Asp228 amino acid residues of the catalytic dyad, but a favorable interaction was noted between an important peripheral residue (Tyr71) (Figure 4) which controls the accessibility of the substrate to the active site of β-secretase [22]. Hyperphosphorylation of the tau protein is one of the main alterations leading to AD. Tau protein is mainly phosphorylated by GSK 3β [23]. The result unveils that ergosta-7-en-3beta-ol, elgonene H and B, as well as diorcinol show better affinity energies than hydromethylthionine, used as a standard [24] with regards to GSK 3β. Analysis of the interactions established between the compounds studied and GSK 3β showed that the study compounds establish favorable interactions with important amino acid residues involved in the enzyme's catalytic mechanism (Figure 5).

5. CONCLUSION

The *in-silico* study of the potential of bioactive compounds from ANPDB fungi revealed that ergosta-7-en-3beta-ol, elgonene H and B, and diorcinol are promising candidates for the treatment of Alzheimer's disease. *In vitro* and *in vivo* studies are needed to confirm their anti-Alzheimer's potential.

Competing Interests

The authors have declared that no competing interests exist.

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Consent for Publication

All authors read and approved the manuscript for publication.

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