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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In silico screening of anticholinesterase alkaloids for cyclooxygenase-2 (COX-2) and matrix metalloproteinase 8 (MMP-8) inhibitory potentials as multi-target inhibitors of Alzheimer's disease

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Abstract
Alzheimer's disease (AD) is a progressive neurodegenerative disorder with yet no effective drug treatment; although several anticholinesterases are being used to offer relief from the symptoms of the disease. Recent studies have indicated that over-activation of cyclooxygenase-2 (COX-2) and matrix metalloproteinase-8 (MMP-8) may cause neuronal death in the brain of AD subjects, suggesting that inhibition of COX-2 and MMP-8 may be of therapeutic value in the management of AD. Therefore, it is important and rational to investigate new agents with anticholinesterase, COX-2 and MMP-8 inhibitory activities. In this study, molecular docking study was performed with earlier identified anticholinesterase alkaloids to search for compounds with high affinity for COX-2 and MMP-8. Molecular docking was done using Blind Docking Server while ligand-protein molecular interaction of compounds with remarkable inhibitory characteristics against COX-2 and MMP-8 were viewed with PyMOL. Alkaloids with high binding affinity and remarkable binding interaction with the target proteins were subjected to drug likeness investigation based on absorption-distribution-metabolism-excretion (ADME) properties using the Swiss online ADME web tool. Nine alkaloids (haloxysterol A, haloxysterol B, haloxysterol C, haloxysterol D, sarcodine, isosarcodine, axillaridine A, sarsalignenone and voacangine hydroxyindolenine) showed high affinities for both COX-2 and MMP-8. Thus, this in silico study identified 9 orally drugable, anticholinesterase alkaloids with COX-2 and MMP-8 multi-target activities that could be studied further as agents against AD.

Keywords Alkaloids · Anticholinesterase · Cyclooxygenase · Matrix metalloproteinase 8 · Molecular docking

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder named by Emil Kraepelin in honor of Alois Alzheimer, who first recognized the disease in 1906 (Möller and Graeber 1998). It is the most common neurodegenerative disorder that affects up to one-third of old individuals with the incidence doubling every 5 years among the elderly, where it is associated with memory impairment, excessive cholinesterase (ChE) activity, formation of neurotoxic amyloid plaque, and tau protein aggregation (Möller and Graeber 1998; Ahmed et al. 2013; Song et al. 2015; Rasool et al. 2018). Pathogenesis of Alzheimer's disease is characterized by alterations in the activity of several proteins including the 2 sister Cholinesterase (ChE) enzymes; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), cyclooxygenase-2 (COX-2) and matrix metalloproteinase-8 (MMP-8), also called neutrophil collagenase. The 2 sister ChE enzymes, AChE and BChE hydrolyze acetylcholine and butyrylcholine respectively; thus, their inhibitors are the major drugs currently used in the treatment of the symptoms of Alzheimer's disease (Orhan et al. 2011). Apart from the ChE enzymes, COX-2 is another important protein implicated in the pathology of AD (Rasool et al. 2018); it is an important target for chemoprevention and its pharmacological modulation is being exploited as target in many inflammation-associated conditions including AD and cancer (Plummer et al. 1999; Rasool et al. 2018). In the brain, the expression of COX-2, which is induced in the neurons in

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Table 1 Binding affinity of potential donepezil and selected alkaloids acetylcholinesterase, butyrylcholinesterase, cyclooxygenase-2 and matrix metalloproteinase 8

S/N	Compounds	Binding Affinity (Kcal/mol)				Reported CHE-inhibitory activity IC_{50} (μM)	Calculated pIC_{50}	References
		AChE	BChE	COX-2	MMP-8			
S	Donepezil (inhibitor)	−15.1	−12.5	−13.7	−16.0	0.058	7.2	(Mohsen 2012)
1	Assoanine	−9.8	−8.8	−8.0	−7.5	3.87	5.4	(López et al. 2002)
2	Buxamine B	−10.0	−11.2	−8.8	−8.1	79.6	4.1	(Ata et al. 2010)
3	Coronaridine	−8.8	−10.3	−8.2	−8.8	–	NA	(Andrade et al. 2005)
4	Epinorgalantamine	−10.6	−9.7	−9.7	−7.9	9.6	5.0	(López et al. 2002)
5	Galanthamine	−9.9	−9.2	−7.5	−7.1	1.07	6.0	(López et al. 2002)
6	Huperzine A	−9.4	−9.2	−7.3	−7.5	10^{-4}	10.0	(Orhan et al. 2004)
7	Oxoassoanine	−10.1	−9.2	−9.1	−7.7	47.2	4.3	(López et al. 2002)
8	Palmatine	−11.4	−9.6	−8.7	−8.7	5.8	5.2	(Kim et al. 2004)
9	Physostigmine	−9.5	−8.2	−7.0	−7.9	6×10^{-4}	9.2	(Karczmar 1998)
10	Protopine	−9.0	−10.3	−8.5	−9.7	16.1	4.8	(Kim et al. 2004)
11	Rivastigmine	−9.0	−7.7	−6.6	−6.6	501	3.3	(Krátký et al. 2016)
12	Rupicoline	−8.8	−11.4	−8.1	−9.3	–	–	(Andrade et al. 2005)
13	Sanguinine	−9.6	−9.4	−7.8	−8.1	0.10	7.0	López et al. 2002
14	Sarsalignone	−13.1	−10.9	−9.8	−9.6	7.02	5.2	(Choudhary 2001)
15	Vaganine A	−12.1	−11.2	−9.9	−9.8	8.59	5.1	(Choudhary 2001)
16	Vaganine D	−11.9	−11.4	−9.9	−9.8	46.89	4.3	(ul Haq and Uddin 2011)
17	Voacangine	−9.0	−10.8	−8.2	−8.1	–	NA	(Andrade et al. 2005)
18	1-O-Acetyllycorine	−12.6	−12.1	−12.6	−10.2	0.96	6.0	(Elgorashi et al. 2004)
19	Axillaridine A	**−17.7**	**−15.0**	**−16.2**	**−18.7**	5.21	5.3	(Atta-ur-Rahman et al. 2002)
20	Berberine	−12.4	−11.8	−12.5	−13.9	–	NA	(Howes and Houghton 2009)
21	Coptisine	−12.5	−12.4	−11.7	−13.7	–	NA	(Howes and Houghton 2009)
22	Dehydroevodiamine	−12.4	−12.4	−13.6	−13.3	37.8	4.4	(Park et al. 1996)
23	Crinine	−12.7	−12.3	−11.6	−12.9	461	3.3	(Elgorashi et al. 2004)
24	Haloxysterol A	**−18.7**	**−15.5**	**−15.9**	**−19.4**	8.3	5.1	(Ahmed et al. 2006)
25	Haloxysterol B	**−18.7**	**−15.4**	**−15.9**	**−19.4**	0.89	6.1	(Ahmed et al. 2006)
26	Haloxysterol C	**−18.4**	**−14.9**	**−15.6**	**−19.1**	1.0	6.0	(Ahmed et al. 2006)
27	Haloxysterol D	**−18.6**	**−15.3**	**−16.9**	**−20.2**	17.2	4.8	(Ahmed et al. 2006)
28	Sarcodine	**−16.1**	**−13.7**	**−14.6**	**−17.6**	18.3	4.7	(Khalid et al. 2004)
29	Isosarcodine	**−16.0**	**−13.4**	**−14.4**	**−17.6**	1.89	5.7	(Khalid et al. 2004)
30	Lycojapodine A	−12.4	−12.0	−13.1	−13.4	–	NA	(Dall'Acqua 2013)
31	Methyl isoplatydesmine	−11.4	−10.7	−10.5	−12.0	74.5	4.1	(Sultana and Sultana 2009)
32	Sarsalignenone	**−17.0**	**−14.2**	**−15.3**	**−18.3**	4.29	5.3	(ul Haq and Uddin 2011)
33	Voacangine hydroxyindolenine	−14.6	**−14.1**	**−13.8**	−14.2	–	NA	(Andrade et al. 2005)

AChE acetylcholinesterase, *BChE* butyrylcholinesterase, *COX-2* cyclooxygenase-2, *MMP-8* matrix metalloproteinase 8, *NA* not available

response to excitatory synaptic activity and glial cells in response to inflammation, is upregulated in AD subjects (Aisen 2002; Woodling et al. 2016). Studies have shown that the administration of selective COX-2 inhibitors attenuate inflammation and cholinergic hypofunction (Giovannini et al. 2003). Another important target protein implicated in the development of AD is MMP-8, which has been linked to the development of many diseases, including cancer metastasis, chronic inflammation and neurological disorders (Rasool et al. 2018). Matrix metalloproteinases (MMPs) are said to contribute to the formation and degradation of amyloid proteins in AD and cause death of dopaminergic neurons in Parkinson's disease (Rosenberg 2009). In normal physiological conditions, tissue inhibitors of matrix metalloproteinases (TIMPs) control the activity of MMPs; overexpression of MMP activity, or inadequate control by TIMPs, have been reported in a number of disease conditions, including Alzheimer's disease (Peress et al. 1995; Pochetti et al. 2009). Although the major therapeutic strategies for AD is the use of anticholinesterase, such as donepezil, tacrine, galantamine, and ensaculin (Sugimoto et al. 2000; Hoerr and Noeldner 2002; Camps et al. 2010; Kavanagh et al. 2011); the employment of multi-target-directed molecules is now been considered to be a promising strategy for AD treatment, because of the multifactorial mechanisms implicated in the pathology of the disease, hence the global interest in finding new multi-target-directed ligands for the treatment of AD especially from phytochemicals since prolonged usage of the current synthetic drugs used to manage the symptoms of AD have been associated with some side effect (Lin et al. 2017; Hu et al. 2018). Interestingly, some studies and have considered the use of multi-target-directed ligands for AD; however, to the best of our knowledge, very few of such studies relate to the co-inhibition of important target proteins

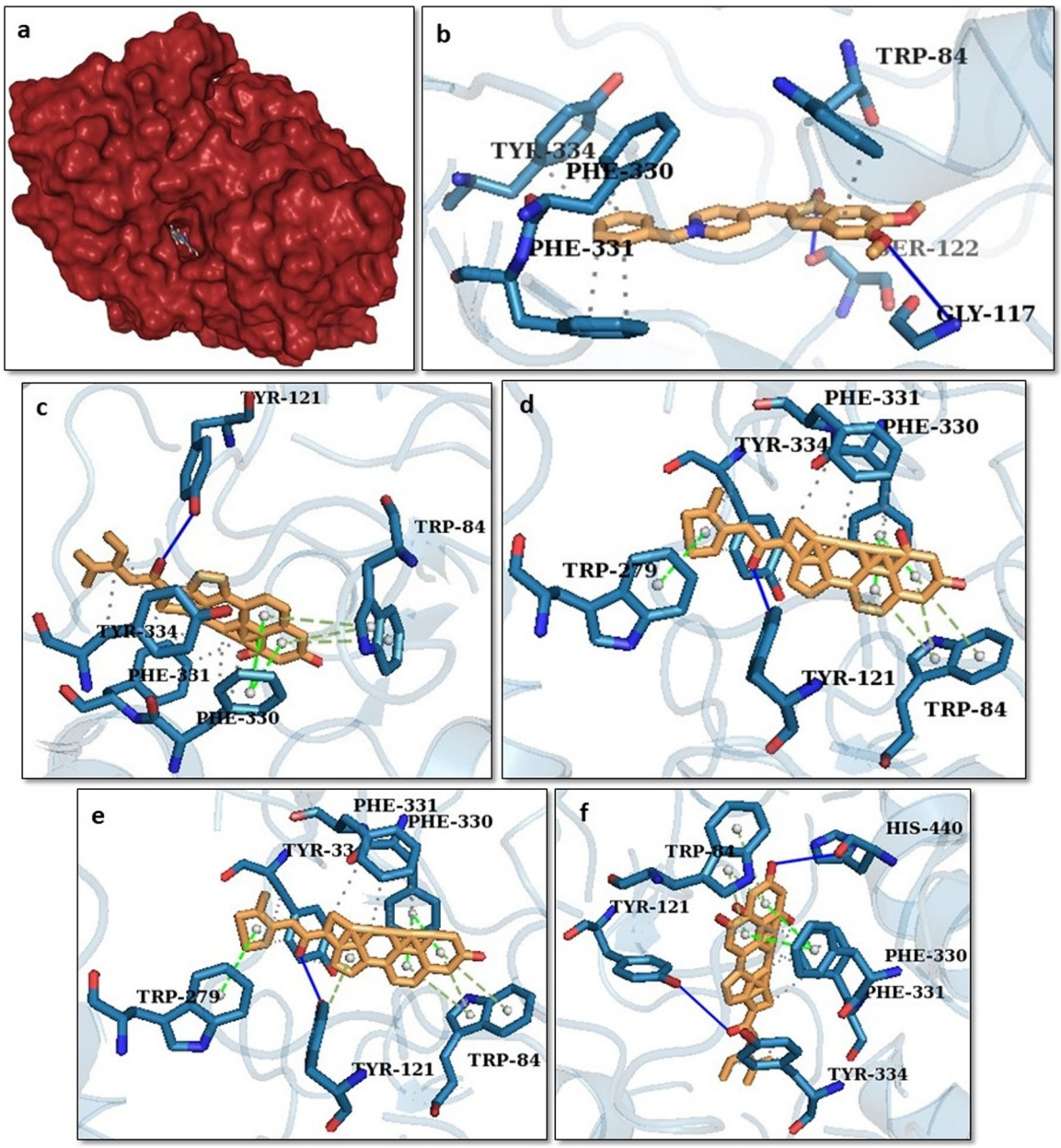

Fig. 1 a–f Interaction between acetylcholinesterase and potential inhibitors and **a** binding pocket occupied by donepezil and other ligands. Interaction between amino acids in the binding site of acetylcholinesterase: **b** donepezil, **c** Haloxysterol A, **d** Haloxysterol B, **e** Haloxysterol C, **f** Haloxysterol D

(AChE, BChE, COX-2 and MMP-8) by alkaloids, a popular group of phytochemicals identified as the major constituents responsible for a variety of the bioactivities of most plant extracts, including anticholinesterase, antioxidant as well as inhibitory activity against many proteins (Ahmed et al. 2013; Hussain et al. 2018). Therefore, in an effort to identify potential drug agents against AD, we have decided to carry out a study into AChE, BChE, COX-2 and MMP-8 targeted alkaloids against AD, using In silico methods, since phytochemicals are not only less toxic relative to the synthetic drugs, they also have other beneficial effects like antioxidant and antiinflammatory activities (Kumar and Khanum 2012; Kim et al. 2014; Venkatesan et al. 2015). To achieve this aim, 33 alkaloids were selected for molecular docking studies, based on literature search, to explore their binding modes with AChE, BChE, COX-2 and MMP-8, using donepezil, a standard neurological drug as the bench mark. Molecular docking was done using Blind Docking Server (Sánchez-Linares et al. 2012) while ligand-protein molecular interaction of compounds with remarkable inhibitory characteristics against the selected target proteins were viewed with PyMOL (PyMOL Molecular Graphics System, Version 2.0 Schrodinger LLC)

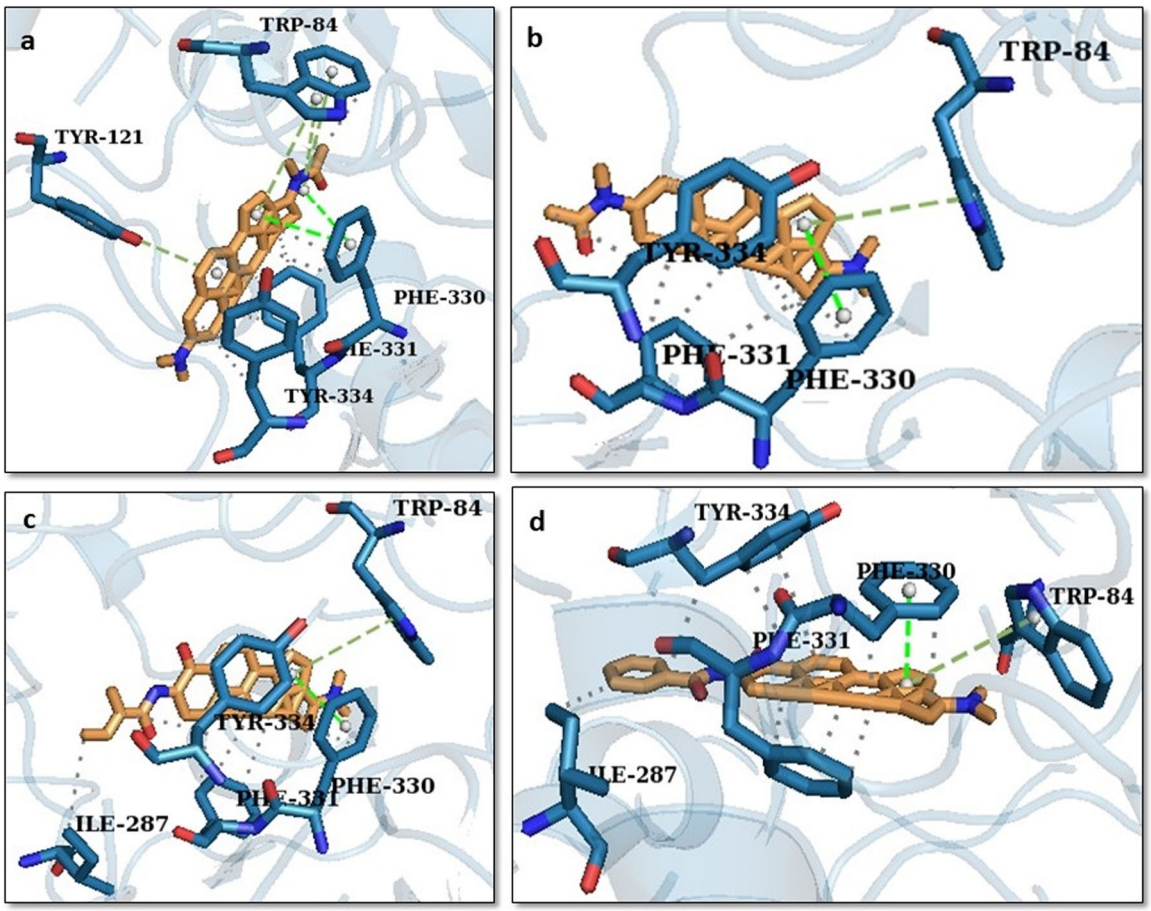

Fig. 2 **a**–**d** Interaction between amino acids in the binding site of acetylcholinesterase and **a** sarcodine, **b** isosarcodine, **c** sarsalignenone, **d** axillaridine A

to gain structural insight into the binding interaction, including the types of bonding interaction and the amino acids involved in such interactions, compared to the selected standard anticholinesterase inhibitor. Furthermore, alkaloids with high binding affinity and remarkable binding interaction with the target proteins were subjected to drug likeness investigation based on absorption-distribution-metabolism-excretion (ADME) properties using the Swiss online ADME web tool. This is in order identify orally drugable AChE, BChE, COX-2 and MMP-8 inhibitors that qualify for further drug development studies.

Materials and methods

Protein preparation

The crystal structures of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), cyclooxygenase-2 (COX-2) and neutrophil collagenase (MMP-8) with PDB IDs 1QTI, 1P0P, 5W58 and 3TT4 respectively were retrieved from the protein databank (www.rcsb.org). The crystal structures were prepared individually by removing existing ligands and water molecules while missing hydrogen atoms were added using Autodock v4.2 program, Scripps Research Institute. Thereafter, non-polar hydrogens were merged while polar hydrogen where added to each protein. The process was repeated for each protein and subsequently saved into dockable pdbqt format in preparation for molecular docking.

Ligand preparation

SDF structures of the standard drug donepezil and 33 potential inhibitors of cholinesterase were retrieved from the PubChem database (www.pubchem.ncbi.nlm.nih.gov). The compounds were converted to mol2 chemical format using Open babel (O'Boyle et al. 2011). Polar hydrogen charges of the Gasteiger-type were assigned and the nonpolar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set to zero. The compounds were further converted to the dockable pdbqt format using Autodock tools.

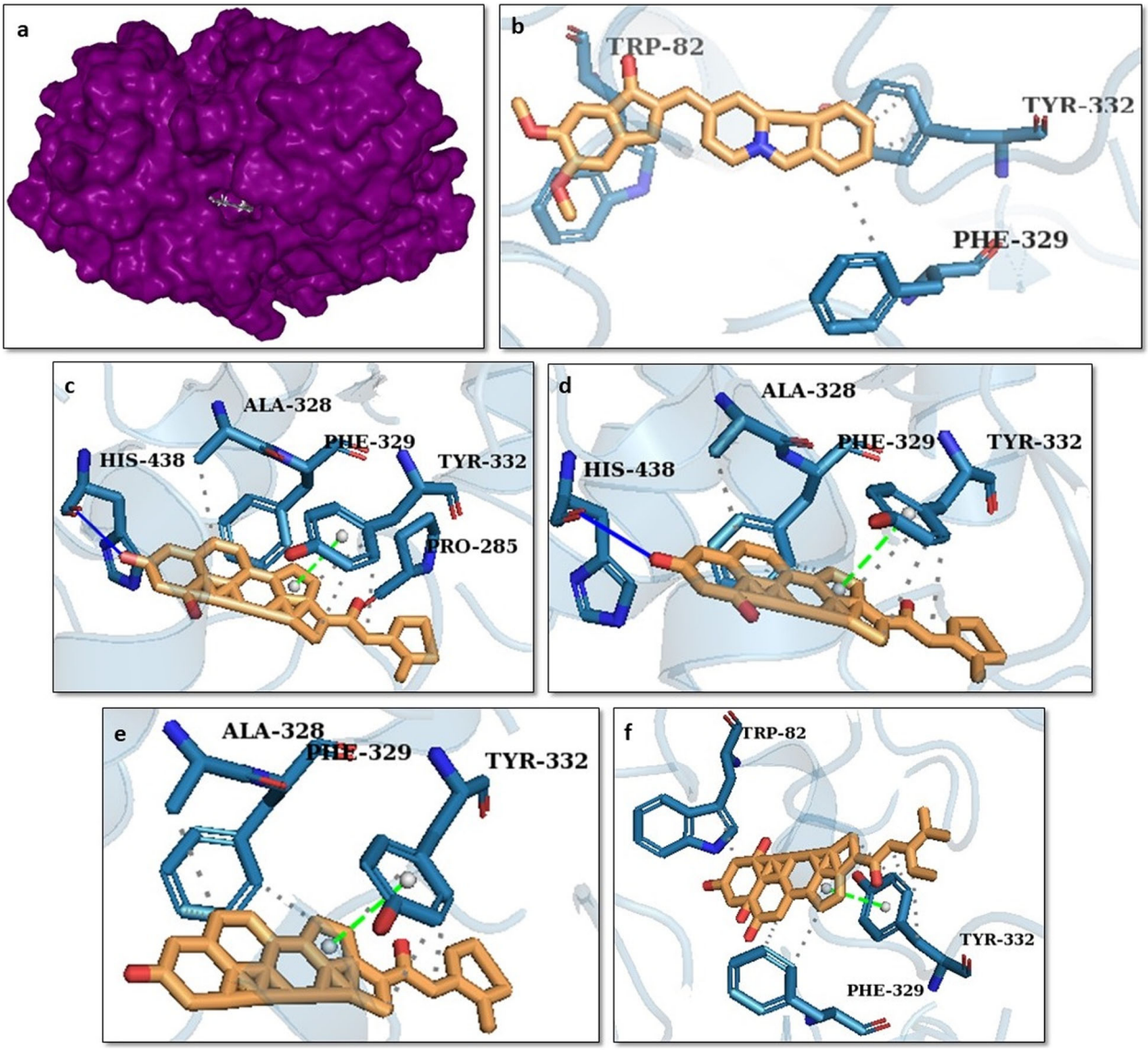

Fig. 3 a–f Interaction between butyrylcholinesterase and potential inhibitors and **a** binding pocket occupied by donepezil and other ligands. Interaction between amino acids in the binding site of acetylcholinesterase: **b** donepezil, **c** haloxysterol A, **d** haloxysterol B, **e** haloxysterol C, **f** haloxysterol D

Molecular docking

Docking of the ligands to AChE, BChE, COX-2 and MMP-8, as well as determination of binding affinities was carried out using Blind Docking Server (Sánchez-Linares et al. 2012). The web base tool utilizes a modified version of Autodock Vina (Trott and Olson 2010) to sample across the whole protein surface to determine the best pose. Pdbqt form of individual protein and ligands were uploaded into their respective columns and the online tool was run. Blind Docking Server performs exhaustive docking simulations on each alpha carbon of the protein and uses its pose clustering algorithm to detect new binding modes and calculate binding affinities. After the affinities were calculated, the tool clusters the results according to spatial overlapping of the resulting poses. For each cluster, the pose with the best affinity was taken as the representation of this cluster. The binding affinities of compounds for three proteins were calculated. The compounds were then ranked by their affinity scores. Thereafter, molecular interaction between AChE, BChE, COX-2 and MMP-8 and compounds with binding affinity higher than that of donepezil (standard inhibitor) were viewed with PyMOL (PyMOL Molecular Graphics System, Version 2.0 Schrodinger LLC).

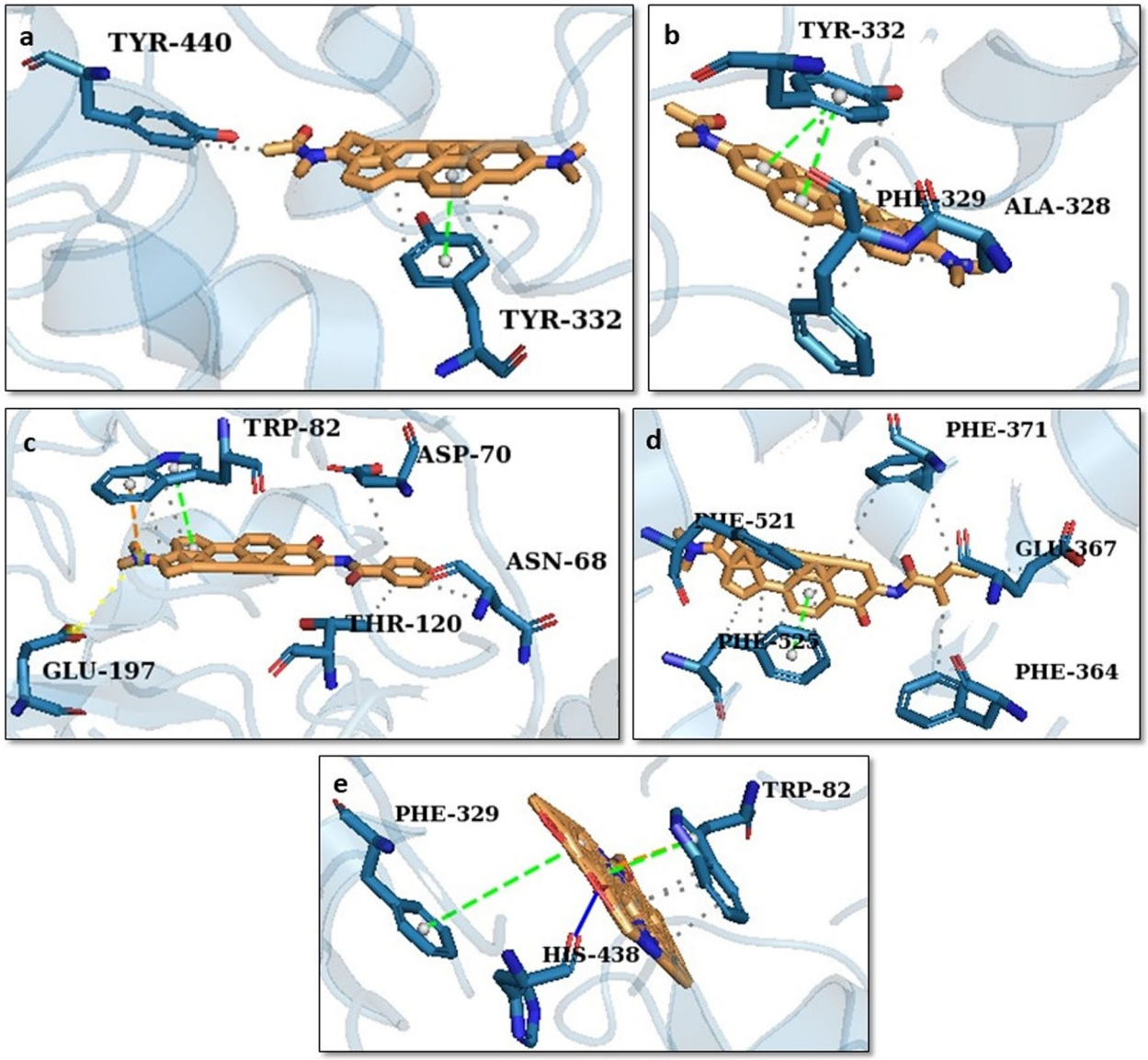

Fig. 4 **a**–**e** Interaction between amino acids in the binding site of butyrylcholinesterase and **a** Sarcodine, **b** isosarcodine, **c** axillaridine A, **d** sarsalignenone, **e** voacangine hydroxyindolenine

Drug likeness and prediction of ADME

In-silico methods for the determination of Absorption-Distribution-Metabolism-Excretion (ADME) parameters rely on theoretically derived statistical models, which have been generated by relating the structural characteristics of compounds that have been measured in a given assay to their biological responses and is now widely used due to its low resource requirement (Gleeson et al. 2011). Therefore, nine of the compounds that showed reasonable binding modes with AChE, BChE, COX-2 and MMP-8 based on similar binding pattern to donepezil and higher negative binding affinity were subjected to physico-chemical studies. ADME studies were carried out using the Swiss online ADME web tool (Daina et al. 2014; Daina and Zoete 2016; Daina et al. 2017) to evaluate the drug-likeness of the selected compounds. A graph of WLOGP against TPSA was plotted using GraphPad Prism 6 software (GraphPad Software, California, USA) to determine the blood brain barrier (BBB) properties of the compounds.

Determination of pIC_{50} value

IC_{50} (μM) value of the donepezil and the 33 anticholinesterase alkaloids used in this study were retrieved from literature. pIC_{50} values were calculated as $-\log(IC_{50})$ (Selvaraj et al. 2011).

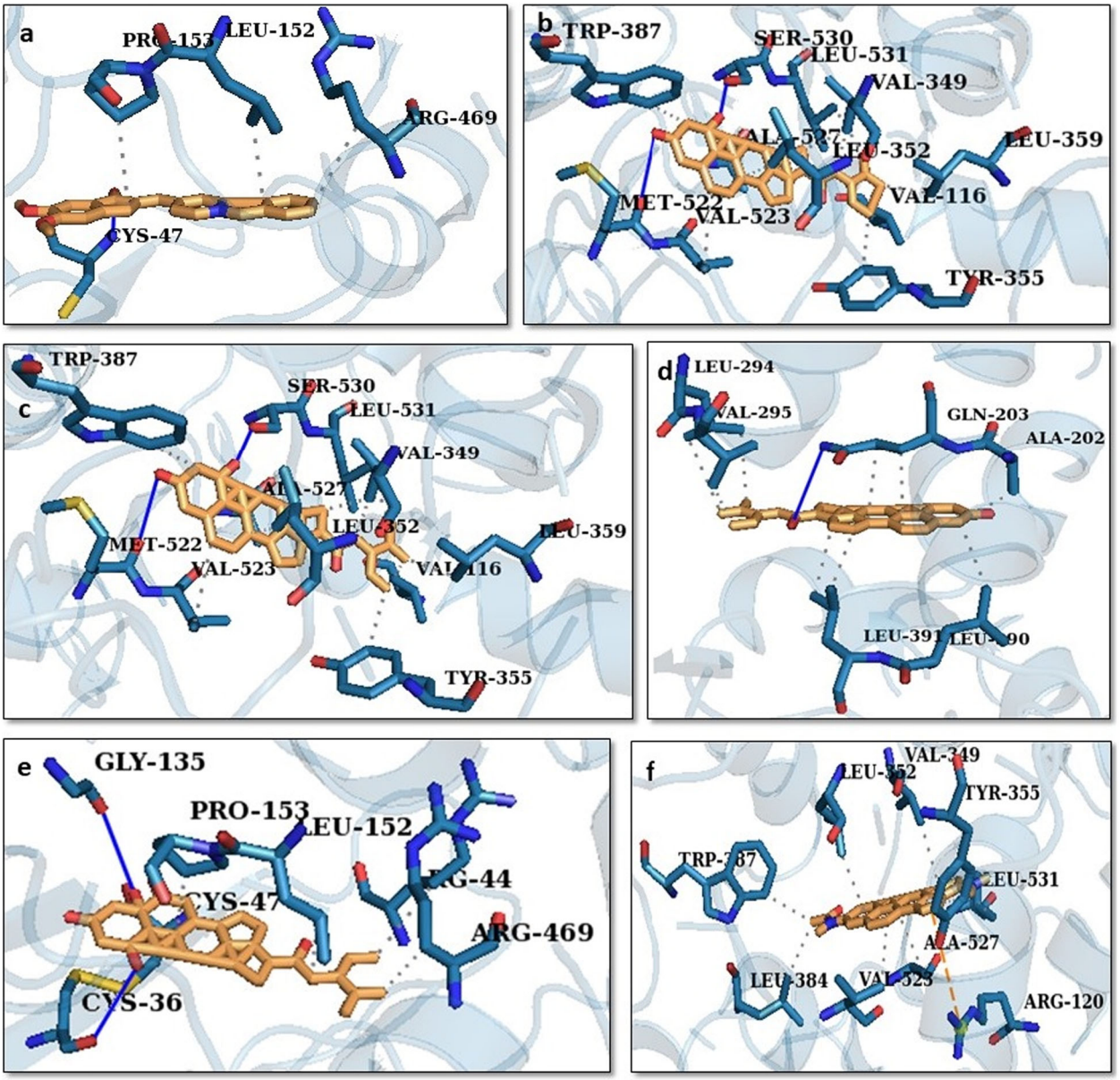

Fig. 5 **a**–**f** Amino acid interaction between cyclooxygenase-2 and **a** donepezil, **b** haloxysterol A, **c** haloxysterol B, **d** haloxysterol C, **e** haloxysterol D, **f** isosarcodine

Results and discussion

Molecular docking

The result of the binding energy of the selected alkaloids to selected vital target proteins (AChE, BChE, COX2, and MMP8) implicated in the pathogenesis of AD from the molecular docking study is shown in Table 1, with the values for compounds having higher binding affinity for the target proteins indicated in bold face. The result revealed that the standard inhibitor (donepezil) has a binding affinity of −15.1 and −12.5 Kcal/mol for AChE and BChE respectively. These Figures were surpassed by 9 out of the 33 alkaloids evaluated for anticholinesterase activity. The more negative the binding energy number, the higher the binding affinity of the alkaloid for the specific target protein. Thus, haloxysterols A, B, C and D have the most negative binding affinity for AChE (−18.7, −18.7, −18.4, and −18.6 Kcal/mol, respectively) and BChE (−15.5, −15.4, −14.9, and −15.3, respectively) compared to

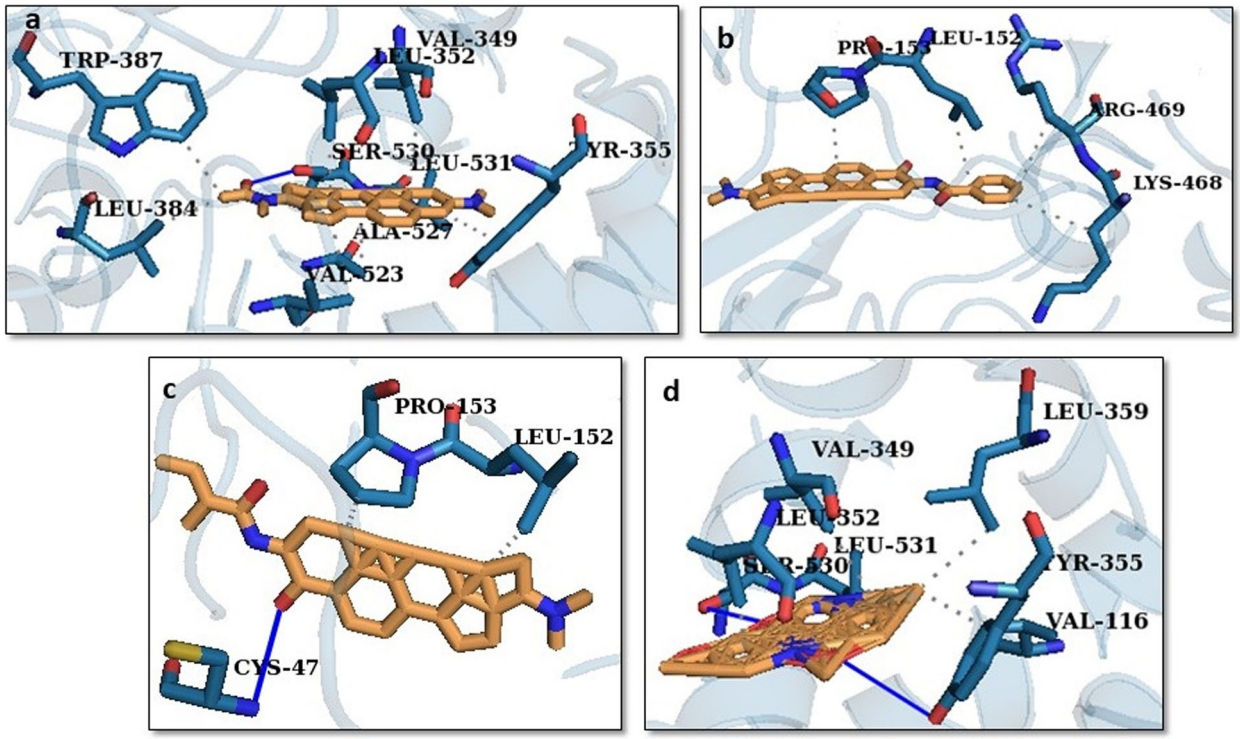

Fig. 6 **a–d** Amino acid interaction between cyclooxygenase-2 and **a** sarcodine, **b** axillaridine A, **c** sarsalignenone, **d** voacangine hydroxyindolenine

donepezil. Axillaridine A, sarsalignenone, sarcodine and isosarcodine also have higher binding energy than donepezil, with binding energy of −17.7, −17.0, −16.1, and 16.0 Kcal/mol respectively for AChE and −15.0, −14.2, −13.7, and −13.4 Kcal/mol, respectively for BChE. Voacangine hydroxyindolenine was selective for BChE with a binding affinity of −14.1 Kcal/mol. The result obtained on the ligand-protein binding interaction showed that donepezil docked in a narrow gorge (Fig. 1a) composed of several conserved amino acids, with donepezil interacting with TRYP84 and PHE330 of the anionic site of AChE via hydrophobic interaction and TYR334 of the peripheral anionic site, PAS (Fig. 1b). Similarly, haloxysterol A, B, C and D interacted with TRYP84 via a π-π stacking and PHE330 in the anionic site and a hydrophobic interaction with TYR334, and also hydrogen bond with TYR121 in the PAS region (Fig. 1c–f). However, only haloxysterol D has a hydrogen bond interaction with one of the catalytic triad residue (HIS440). Sarcodine, isosarcodine, sarsalignenone and axillaridine A exhibit similar binding pattern, creating a characteristic π-π stacking interactions with TRP84 in the catalytic site and PHE330 in the anionic site of AChE, while interactions were also observed between the compounds and PHE331 of AChE (Fig. 2a–d). The ligand-BChE binding interaction showed that donepezil and the alkaloids docked in a narrow gorge, shown in Fig. 3a. The binding of donepezil to BChE revealed hydrophobic interaction with the conserved residue (TRP82) and a hydrophobic interaction with PHE329 at the anionic site (Fig. 3b); a similar binding interaction was observed for haloxysterol D (Fig. 3f). Haloxysterols, A, B and C in addition to hydrophobic interaction with PHE329, interacted with ALA328 while an additional hydrogen bond was formed between haloxysterols B and C and HIS438 of the catalytic triad of BChE (Fig. 3c–e). A π-π stacking was observed between sarcodine /isosarcodine and TYR322, with hydrophobic interaction with anionic site residue PHE329 in isosarcodine (Fig. 4a, b). Axillaridine A and voacangine hydroxyindolenine had a π-π stacking with conserved residue TRP82, while an extra hydrophobic interaction with ASP70 in the case of the later was also observed
(Fig. 4c, d). Voacangine hydroxyindolenine had a hydrogen bond interaction with catalytic histidine residue at position 438, while sarsalignenone a π-π interaction with GLU325 in the catalytic triad of the enzyme.

The binding of various ligands to COX-2 also revealed 9 alkaloids with remarkable binding affinities, these include haloxysterols A, B, C, D, axillaridine A, sarcodine, isosarcodine, sarsalignenone and voacangine hydroxyindolenine having more negative binding energy (−15.9, −15.9, −15.5, −16.9, −16.2, −14.6, −14.4, −15.3 and −13.8 Kcal/mol respectively) for this important protein, compared to donepezil with a binding energy of −13.7 Kcal/mol (Table 1); whereas, from the results obtained for MMP-8. Eight, out of the 33 alkaloids exhibit higher binding affinity for the

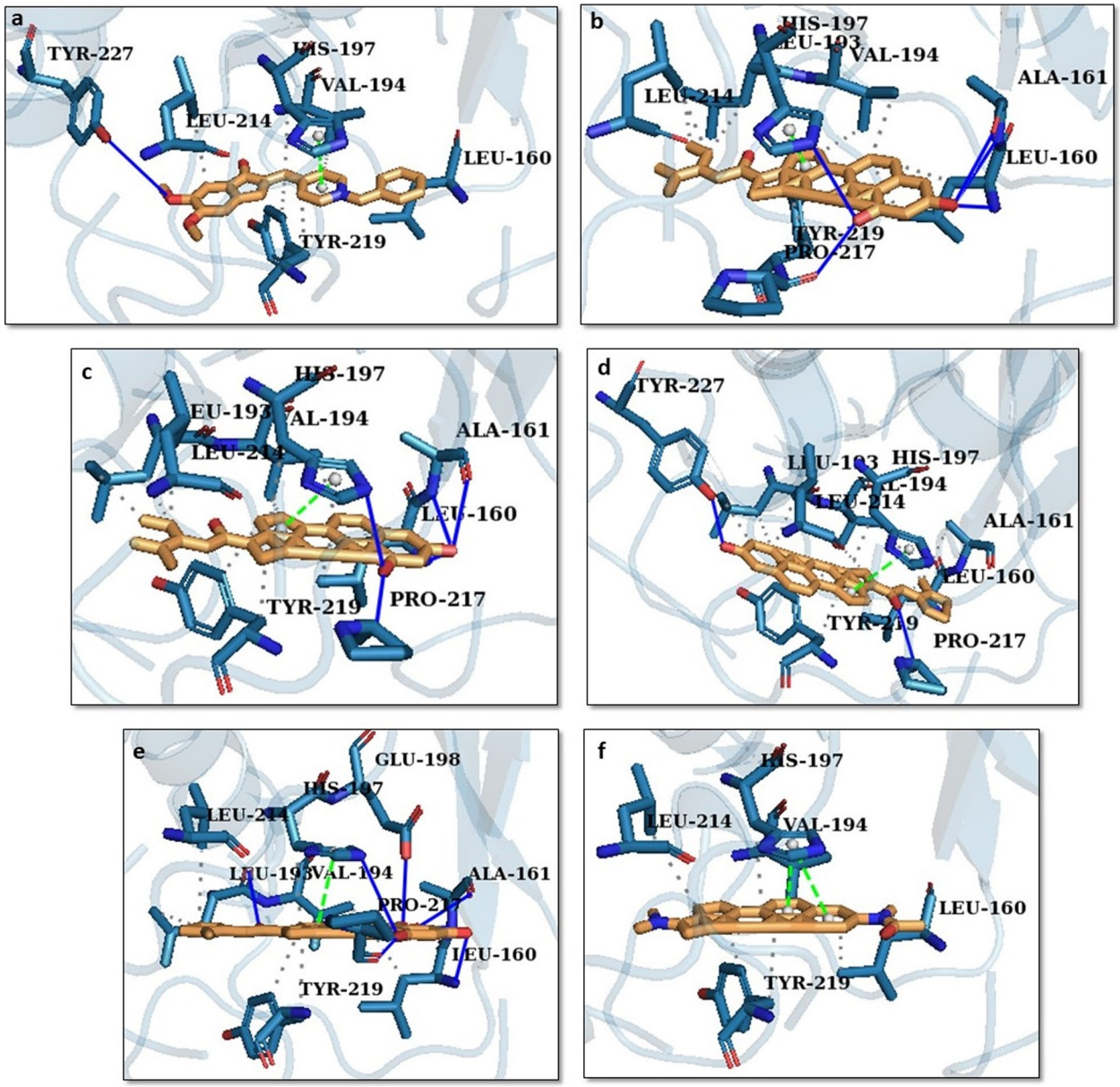

Fig. 7 **a**–**f** Amino acid interaction between neutrophil collagenase and **a** donepezil, **b** haloxysterol A, **c** haloxysterol B, **d** haloxysterol C, **e** haloxysterol D, **f** isosarcodine

protein than donepezil, these include axillaridine A, haloxysterols A, B, C, D, sarcodine, isosarcodine and voacangine hydroxyindolenine with binding energy of −18.7, −19.4, −19.4, −19.1, −20.2, −17.6, −17.6 and −18.3Kcal/mol compared to −16.0 Kcal/mol for donepezil (Table 1). 29 of the 33 compounds have reported in vitro anticholinesterase inhibitory activity expressed in (μM). Huperzine A and physostigmine, have higher pIC_{50} values (10.0 and 9.2 respectively) compared to donepezil (7.2). Sanguinine, galanthamine, 1-O-Acetyllycorine, haloxysterol B and C also have notable pIC_{50} of 7.0, 6.0, 6.1 and 6.1 respectively. In vitro cholinesterase activity of alkaloids have been previously reported (Mukherjee et al. 2007; Howes and Houghton 2009; Ahmed et al. 2013) making it ideal to explore possible role of the compounds in the treatment of other neurological disorders.

Evaluation of different binding poses of donepezil in COX-2 revealed a hydrophobic interaction with LEU152, PRO153, and ARG469 with an additional hydrogen bond with CYS47 (Fig. 5a). Haloxysterols A and B showed similar binding pattern, but with additional interaction with SER530 and MET522 via hydrogen bond and hydrophobic interaction with TRP387, TYR355, and VAL523 (Fig. 5b, c). A single hydrogen bond was formed between haloxysterol C and GLN203 of COX-2 while hydrophobic interaction was predominant between the compound and amino acids in COX-2

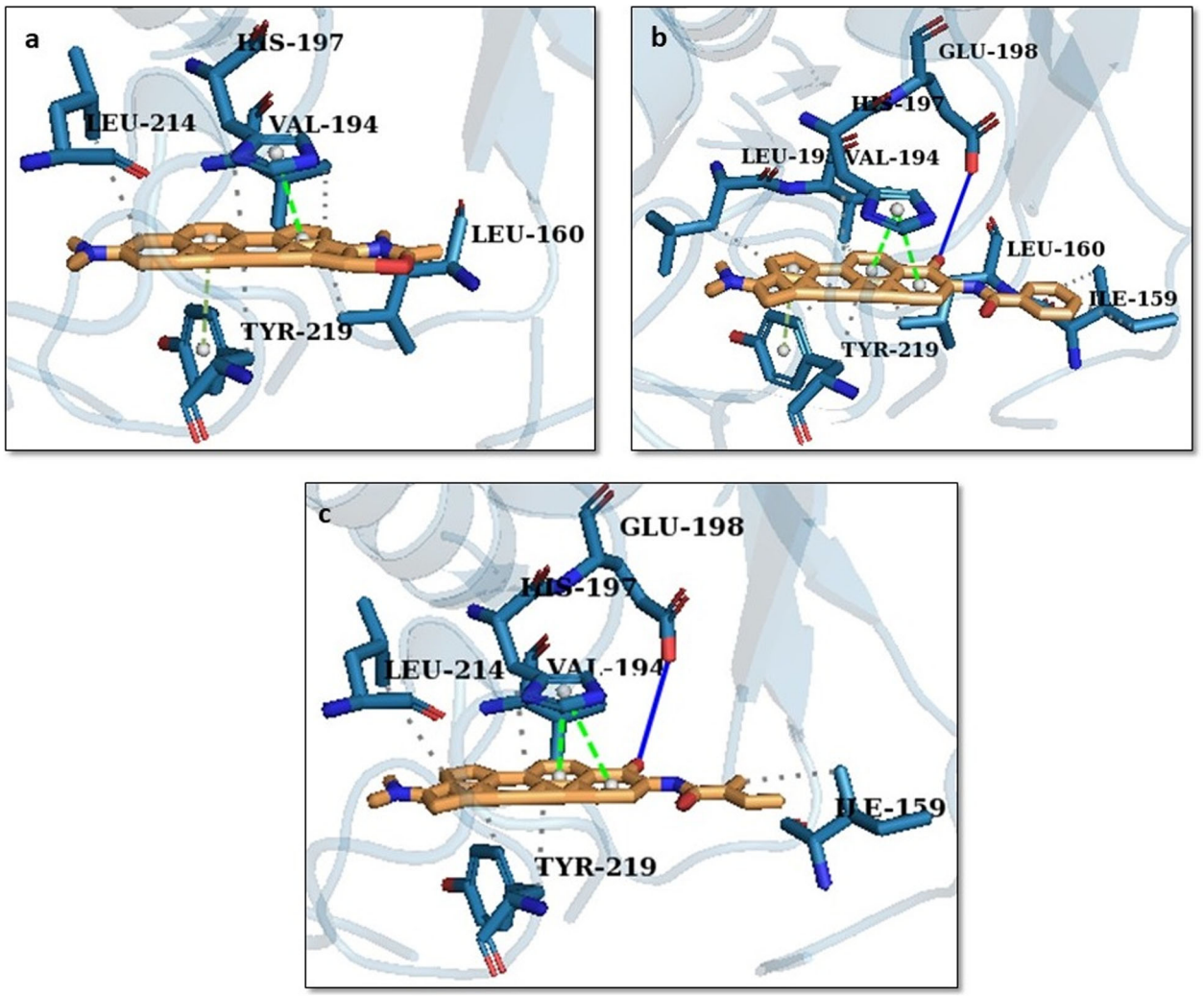

Fig. 8 **a**–**c** Amino acid interaction between neutrophil collagenase and **a** sarcodine, **b** axillaridine A, **c** sarsalignenone

binding site (Fig. 5d). A total of two hydrogen bonds were formed between haloxysterol D and CYS36 and GLY35 (Fig. 5e) while isosarcodine (Fig. 5f) and sarcodine (Fig. 6a) have a similar binding pattern to haloxysterols A and B but with isosarcodine having an extra π-cation interaction with ARG120 of COX-2. Axillaridine A had only hydrophobic interaction with amino acids in COX-2, interacting with PRO153, LEU152, ARG469 and LYS468 (Fig. 6b). Of the 9 compounds, only sarsalignenone had a similar binding pattern with donepezil in COX-2, interacting with CYS47 via hydrogen bonding as well as hydrophobic interaction with LEU152 and PRO153 (Fig. 6c). SER530 and TYR355 were seen in a hydrogen bond interaction with voacangine hydroxyindolenine in the presence of other amino acids in the binding site of COX-2 (Fig. 6d). For, MMP-8, π-π stacking exists between the compounds and HIS197 of the protein, as well as hydrophobic interactions with VAL194, and LEU214 (Figs. 7 and 8). Donepezil (Fig. 7a) and haloxysterol C (Fig. 7d) shared similar hydrogen bond interaction with TYR227 while hydrogen bond interaction with LEU160, ALA161, HIS197 and PRO217 were observed in the case of haloxysterols A, B and D (Fig. 7b, c, e).; axillaridine A (Fig. 8b) and sarsalignenone (Fig. 8c) formed hydrogen bond interaction with GLU198 of MMP-8 while sarcodine had an extra π-stacking with TYR219 (Fig. 8a). The structure of the 9 alkaloids with remarkable binding energy with acetylcholinesterase, butyrylcholinesterase, cyclooxygenase-2 and matrix metalloproteinase 8 are shown in Fig. 9. Multi-target-directed ligands for the management of AD is being explored as a viable strategy in the management of this neurodegenerative disease with phytochemicals as viable alternatives (Hu et al. 2018); since reasons such as side effects and low bioavailability limit the drugability and uses in medicine of the current drugs, necessitating a great demand to identify new inhibitors (Orhan et al. 2011; Paula et al. 2013). From the results obtained in this study, 9 anticholinesterase alkaloids (haloxysterol A, haloxysterol B, haloxysterol C, haloxysterol D, sarcodine, isosarcodine, axillaridine A, sarsalignenone and

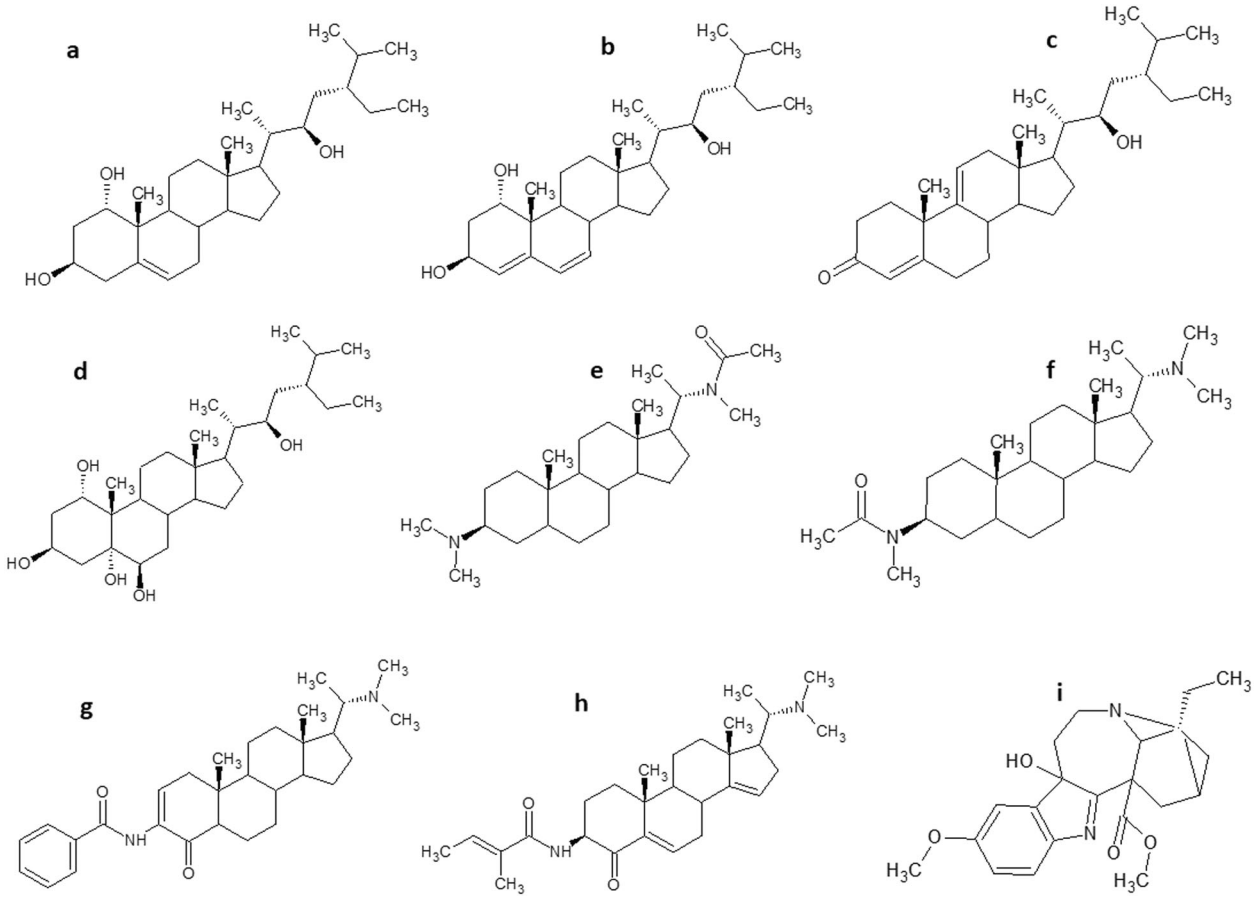

Fig. 9 **a**–**i** structures of alkaloids with remarkable binding energy with acetylcholinesterase, butyrylcholinesterase, cyclooxygenase-2 and matrix metalloproteinase 8: **a** haloxysterol A, **b** haloxysterol B, **c** haloxysterol C, **d** haloxysterol D, **e** sarcodine, **f** isosarcodine, **g** axillaridine A, **h** sarsalignenone, **i** voacangine hydroxyindolenine

voacangine hydroxyindolenine) with remarkable inhibitory tendency towards COX-2 and MMP-8 were identified from a set of 33 selected alkaloids. Studies have shown that COX-2 may have a central role in neurodegeneration, supporting clinical evaluation of selective COX-2 inhibitors as neuroprotective agents in AD (Aisen 2002). COX-2 expression is increased in frontal cortex of the brain of AD subjects (Pasinetti and Aisen 1998). Therefore, ability of the selected alkaloids to inhibit COX-2 may decrease the neurodegeneration observed in AD brain. Matrix metalloproteases play a significant role in diseases of the central nervous system (CNS) because they mediate disruption of the blood brain barrier, regulate extracellular matrix protein destruction and remodeling as well as tissue inflammation in response to oxidative stress (Yong et al. 2001). Upregulation of MMPs is has been reported in several neurological disorders including AD (Yong et al. 2001) prompting the search for new inhibitors of MMPs. The ability of haloxysterols A, B, C, D, sarcodine, isosarcodine, axillaridine A, sarsalignenone, voacangine hydroxyindolenine to inhibit MMP-8 with higher binding affinity compared to Donepezil (a standard drug) may proof significant in the quest for new treatments for AD.

Pharmacokinetic properties of selected alkaloids

The results obtained for the ADME study on the 9 compounds with remarkable binding energy for the selected proteins are shown in Table 2. Interestingly, the result indicated that the 9 compounds are potential oral drug candidates. All but haloxysterol C has no Lipinski violation i.e., they all have molecular weight of less than 400 Daltons, less than 10 hydrogen bond acceptor, less than 5 hydrogen bond donor and octanol/water partition coefficient (MLogP) of less than 5 (haloxysterol C had an MLogP of 5.6). Also, all nine compounds have less than 10 rotatable bonds and topological surface area (TPSA) less than 140 $Å^2$ signifying that none of the compounds violated Veber's rule. Lipinski examined orally active compounds to define physicochemical ranges for high probability to be an oral drug (i.e., the drug-likeness). This popular *Rule-of-five* delineated the relationship between pharmacokinetic and physicochemical parameters (Lipinski 2000). Lipinski's rule states that, generally, an orally active drug has no more than one violation of the following criteria: (1) Not >5 hydrogen bond donors (nitrogen or oxygen atoms with one or more

Table 2 Physicochemical properties of alkaloids with remarkable binding energy with acetylcholinesterase, butyrylcholinesterase, cyclooxygenase-2 and matrix metalloproteinase 8

S/N	Compounds	Molecular Weight	H-bond acceptor	H-bond donor	MLogP	Lipinski's violations	Rotatable bonds	TPSA (Å^2)	Veber's violation	WLOGP	BBB permeation
1	Haloxysterol A	446.71	3	3	4.92	0	6	60.69	0	5.97	Yes
2	Haloxysterol B	444.69	3	3	4.82	0	6	60.69	0	5.74	Yes
3	Haloxysterol C	426.67	2	1	5.60	1	6	37.30	0	7.12	No
4	Haloxysterol D	480.72	5	5	3.39	0	6	101.15	0	4.13	No
5	Sarcodine	402.66	2	0	4.74	0	4	23.55	0	5.44	Yes
6	Isosarcodine	402.66	2	0	4.74	0	4	23.55	0	5.44	Yes
7	Axillaridine A	462.67	3	1	4.48	0	5	49.41	0	5.70	Yes
8	Sarsalignenone	438.65	3	1	3.90	0	5	49.41	0	5.07	Yes
9	Voacangine hydroxyindolenine	384.47	6	1	1.97	0	6	71.36	0	1.78	Yes

MLOGP Octanol/Water Partition Coefficient

TPSA Topological Polar Surface Area

WLOGP Lipophilicity

hydrogen atoms). (2) Not >10 hydrogen bond acceptors (nitrogen or oxygen atoms) (3) A molecular mass <500 Daltons and (4) Anoctanol-water partition coefficient log P not greater than 5. The nine compounds with higher negative binding affinity have all their parameters within the acceptable range stated by Lipinski i.e., none of the compounds have more than one violation of the Lipinski rule of five. Also, the compounds were evaluated using Verber's rule taking into account the polar surface area and the number of rotatable bonds (Veber et al. 2002). None, out of the 9 alkaloids violated the Veber's rule which further strengthen their potential as oral drug candidates.

Moderately polar (TPSA < 79 Å^2) and relatively lipophilic (WLOGP from 0.4 to 6.0) compounds have a high probability to access the central nervous system (CNS) (Daina and Zoete 2016). Consequently, only haloxysterol C (TPSA = 101.15 Å^2) and haloxysterol D (WLOGP = 7.12) (Table 2) violated this rule while the seven other compounds may transverse the BBB. This was depicted in an egg-yolk-like model where seven of the compounds aggregated in a sphere with the two non BBB permeant (BBB-) outside the sphere (Fig. 10). However, only six spheres are visible inside the box because sarcosine and isosarcosine appear as a single sphere since they have exactly the same WLOGP, TPSA and MLogP. The Blood Brain Barrier (BBB) can be regarded as a cover protecting the brain by a "physical" barrier and a "biochemical" barrier consisting of enzymatic activities and active efflux (Daina and Zoete 2016). Thus, BBB serves as the most important barrier between CNS and systemic circulation (Goodwin 2005). Despite the massive effort in CNS drug research in both academia and industry, there has been no parallel effort in CNS drug delivery, which is peculiar owing to the existence of the BBB (Pardridge 2009); therefore, the ability of the selected alkaloids to cross the blood brain barrier may prove significant in the treatment of Alzheimer's disease.

Based on the remarkable binding affinity of the anticholinesterase alkaloids for both COX-2 and MMP-8 (indicated by their high negative binding energies relative to donepezil), their properties of oral drug likeness (no violation of Lipinski's and Veber's rules) and their blood brain barrier crossing potentials, they qualify for further study as drug candidates for AD.

Conclusion

Alzheimer's disease is a neurodegenerative disorder wherein the death of neuronal cells in the brain causes loss of memory and cognitive decline that negatively affect quality of life and expectancy. Due to the complexity of the causes of AD, new agents that are based on different mechanisms are needed. Computer-based study and drug design has now become an important approach for the discovery of novel drugs. Results obtained from this study revealed that 9 alkaloids, earlier identified as having anticholinesterase activity have now been identified as orally drugable, COX-2 and MMP-8 multitarget inhibitors and also possess the ability to cross the blood brain barrier, with the exception of haloxysterols C and D. This study highlighted alkaloids that should be examined further, using different experimental models, to validate their promising potential as lead compounds in the management of AD and other related neurodegenerative diseases.

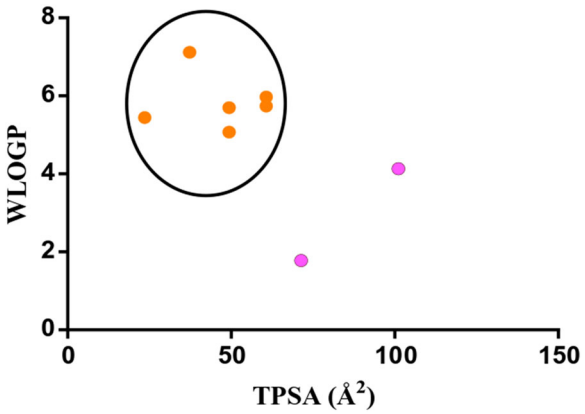

Fig. 10 Blood brain barrier properties of anticholinesterase alkaloids with significant remarkable binding affinity to cyclooxygenase-2 and matrix metalloproteinase 8. 7 permeants (BBB^+, orange dots inside black sphere), 2 non permeants (BBB-, purple dots)

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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References

Ahmed F, Ghalib RM, Sasikala P, Ahmed KM (2013) Cholinesterase inhibitors from botanicals. Pharm Rev 7(14):121

Ahmed E, Nawaz SA, Malik A, Choudhary MI (2006) Isolation and cholinesterase-inhibition studies of sterols from Haloxylon recurvum. Bioorg Med Chem Lett 16:573–580

Aisen PS (2002) Evaluation of selective COX-2 inhibitors for the treatment of Alzheimer's disease. J Pain Symptom Manag 23(4): S35–S40

Andrade MT, Lima JA, Pinto AC, Rezende CM, Carvalho MP, Epifanio RA (2005) Indole alkaloids from Tabernaemontana australis (Müell. Arg) Miers that inhibit acetylcholinesterase enzyme. Bioorg Med Chem 13(12):4092–4095

Ata A, Iverson CD, Kalhari KS, Akhter S, Betteridge J, Meshkatalsadat MH, Orhan I, Sener B (2010) Triterpenoidal alkaloids from Buxus hyrcana and their enzyme inhibitory, anti-fungal and anti-leishmanial activities. Phytochemistry 71:1780–1786

Atta-ur-Rahman ZU, Khalid A, Anjum S, Khan MR, Choudhary MI (2002) Pregnane-type steroidal alkaloids of Sarcococca saligna: A new class of cholinesterase inhibitors. Helv Chim Acta 85:678–688

Camps P, Formosa X, Galdeano C, Gómez T, Muñoz-Torrero D, Ramírez L, Viayna E, Gómez E, Isambert N, Lavilla R, Badia A (2010) Tacrine-based dual binding site acetylcholinesterase inhibitors as potential disease-modifyinganti-Alzheimer drug candidates. Chem-biol Inter 187(1–3):411–415

Choudhary MI (2001) Bioactive natural products as a potential source of new pharmacophores. A theory of memory. Pure Appl Chem 73(3):555–560

Daina A, Michielin O, Zoete V (2014) ILOGP: A simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. J Chem Inf Model 54 (12):3284–3301

Daina A, Michielin O, Zoete V (2017) SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7:42717

Daina A, Zoete V (2016) A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. ChemMedChem 11(11):1117–1121

Dall'Acqua S (2013) Plant-derived acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer disease. Bot: Targets Ther 3:19–28

Elgorashi EE, Stafford GI, Van Staden J (2004) Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. Planta Med 70(03):260–262

Giovannini MG, Scali C, Biotech S, Bellucci A, Pepeu G (2003) Experimental brain inflammation and neurodegeneration as model of Alzheimer's disease: Protective effects of selective COX-2 inhibitors. Int J Immunopathol Pharm 16(2):19–28

Gleeson M, Hersey A, Hannongbua S (2011) In silico ADME Models: A General Assessment of their Utility in Drug Discovery Applications. Curr Top Med Chem 11(4):358–381

Goodwin JT (2005) In silico Predictions of Blood-Brain Barrier Penetration: Considerations to "Keep in Mind.". J Pharm Exp Ther 315(2):477–483

Hoerr R, Noeldner M (2002) Ensaculin (KA-672. HCl): A Multitransmitter Approach to Dementia Treatment. CNS Drug Rev 8:143–158

Howes MJ, Houghton PJ (2009) Acetylcholinesterase inhibitors of natural origin. Int J Biomed Pharm Sci 3(SI1):67–86

Hu X, Dong W, Cui Z, Gao C, Yu Z, Yuan Q, Min Z (2018) In silico identification of AChE and PARP-1 dual-targeted inhibitors of Alzheimer' s disease. J Mol Model 24:150–158

Hussain G, Rasul A, Anwar H, Aziz N, Razzaq A, Wei W, Ali M, Li J, Li X (2018) Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. Int J Bio Sci 14(3):341

Karczmar A (1998) Invited review. Anticholinesterases: Dramatic aspects of their use and misuse. Neurochem Int 32(5-6):401–411

Kavanagh S, Gaudig M, Van Baelen B, Adami M, Delgado A, Guzman C, Jedenius E, Schäuble B (2011) Galantamine and behavior in Alzheimer disease: analysis of four trials. Acta Neurol Scand 124:302–308

Khalid A, Zaheer-Ul-Haq, Ghayur MN, Feroz F, Atta-Ur-Rahman Gilani AH, Choudhary MI (2004) Cholinesterase inhibitory and spasmolytic potential of steroidal alkaloids. J Steroid Biochem Mol Biol 92(5):477–484

Kim MH, Kim SH, Yang WM (2014) Mechanisms of action of phytochemicals from medicinal herbs in the treatment of Alzheimer's disease. Planta Med 80:1249–1258

Kim DK, Lee KT, Baek NI, Kim SH, Park HW, Lim JP, Shin TY, Eom DO, Yang JH, Eun JS (2004) Acetylcholinesterase inhibitors from the aerial parts of Corydalis speciosa. Arch Pharmacal Res 27(11):1127

Krátký M, Štěpánková Š, Vorčáková K, Švarcová M, Vinšová J, Decker M (2016) Novel cholinesterase inhibitors based on O-aromatic N, N-disubstituted carbamates and thiocarbamates. Molecules 21(2):191

Kumar GP, Khanum F (2012) Neuroprotective potential of phytochemicals. Pharmacogn Rev 6(12):81. 6(12):81

Lin H, Li Q, Gu K, Zhu J, Jiang X, Chen Y, Sun H (2017) Design of multi-target agents for the treatment of Alzheimer's disease based on tacrine. Curr Top Med Chem 17(27):3000–3016

Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. J Pharm Toxicol Methods 44 (1):235–249

López S, Bastida J, Viladomat F, Codina C (2002) Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. Life Sci 71(21):2521–9

Mohsen UA (2012) Studies on imidazopyridine derivatives as acetylcholinesterase inhibitors. Clin Exp Health Sci 2(3):119

Möller HJ, Graeber M (1998) The case described by Alois Alzheimer in 1911 Historical and conceptual perspectives based on the clinical record and neurohistological sections. Eur Arch Psychiatry Clin Neurosci 248:111–122

Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007) Acetylcholinesterase inhibitors from plants. Phytomedicine 14 (4):289–300

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: An Open chemical toolbox. J Chemin- 3(10):33

Orhan IE, Orhan G, Gurkas E (2011) An Overview on Natural Cholinesterase Inhibitors - A Multi-Targeted Drug Class - and Their Mass Production. Mini-Rev Med Chem 11:836–842

Orhan I, Şener B, Choudhary MI, Khalid A (2004) Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. J Ethnopharmacol 91 (1):57–60

Pardridge WM (2009) Alzheimer's disease drug development and the problem of the blood-brain barrier. Alzheimer's Dement 5 (5):427–432

Park CH, Kim SH, Choi W, Lee YJ, Kim JS, Kang SS, Suh YH (1996) Novel anticholinesterase and antiamnesic activities of dehydroevodiamine, a constituent of Evodia rutaecarpa. Planta Med 62(05):405–9

Pasinetti GM, Aisen PS (1998) Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. Neuroscience 87(2):319–24

Paula A, Belén M, Julia M, Paola N, Cavallaro V (2013) Natural AChE Inhibitors from Plants and their Contribution to Alzheimer's Disease Therapy. Curr Neuropharmacol 11:388–413

Peress N, Perillo E, Zucker S (1995) Localization of tissue inhibitor of matrix metalloproteinases in Alzheimer's disease and normal brain. J Neuropathol ExpNeurol 54:16–22

Plummer SM, Holloway KA, Manson MM (1999) Inhibition of cyclooxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kB activation via the NIK/IKK signalling complex. Oncogene 18:6013–6020

Pochetti G, Montanari R, Gege C, Chevrier C, Taveras AG, Mazza F (2009) Extra binding region induced by non-zinc chelating inhibitors into the S1′ subsite of matrix metalloproteinase 8 (MMP-8). J Med Chem 52:1040–1049

Rasool M, Arif Malik SW, Tul-Ain Q, Jafar TH, Rasool R, Kalsoom A, Ghafoor MA, Sehgal SA, Gauthaman K, Naseer MI, Al-Qahtani MH (2018) In-Silico Characterization and In-vivo Validation of Albiziasaponin-A, Iso-Orientin, and Salvadorin Using a Rat Model of Alzheimer's Disease. Front Pharm 9:1–15

Rosenberg GA (2009) Matrix metalloproteinases and their multiple roles in neurodegenerative diseases. Lancet Neurol 8(2):205–216

Sánchez-Linares I, Pérez-Sánchez H, Cecilia JM, García JM (2012) High-Throughput parallel blind Virtual Screening using BINDSURF. BMC Bioinforma 13(14):S13

Selvaraj C, Tripathi SK, Reddy KK, Singh SK (2011) Tool development for Prediction of pIC 50 values from the IC 50 values-A pIC 50 value calculator. Curr Trends Biotechnol Pharm 1(5):2

Song JH, Yu JT, Tan L (2015) Brain-Derived Neurotrophic Factor in Alzheimer's Disease: Risk, Mechanisms, and Therapy. Mol Neurobiol 52(3):1477–1493

Sugimoto H, Yamanishi Y, Iimura Y, Kawakami Y (2000) Donepezil hydrochloride (E2020) and other acetylcholinesterase inhibitors. Curr Med Chem 7:303–339

Sultana N, Sultana R (2009) A new lanostane triterpene from skimmia laureola. Z Nat 64(4):459–463

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem 31(2):455–461

ul Haq Z, Uddin R (2011) Structure Based 3D-QSAR Studies on Cholinesterase Inhibitors. In Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets. IntechOpen https://doi.org/10.5772/17891

Veber DF, Johnson SR, Cheng H, Smith BR, Ward KW, Kopple KD (2002) Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. J Med Chem 45:2615–2623

Venkatesan R, Ji E, Kim SY (2015) Phytochemicals that regulate neurodegenerative disease by targeting neurotrophins: a comprehensive review. BioMed Res Int 2015:814068. https://doi.org/10.1155/2015/814068

Woodling NS, Colas D, Wang Q, Minhas P, Panchal M, Liang X, Mhatre SD, Brown H, Ko N, Zagol-Ikapitte I, van der Hart M (2016) Cyclooxygenase inhibition targets neurons to prevent early behavioural decline in Alzheimer's disease model mice. Brain 139(7):2063–2081

Yong VW, Power C, Edwards DR (2001) Metalloproteinases in biology and pathology of the nervous system. Nat Rev Neurosci 2(7):502