Keywords: Butyrylcholinesterase, Methylrosmarinate, Enzyme inhibition, Enzyme kinetics, Molecular docking

ABSTRACT
Butyrylcholinesterase (BChE) performs a significant function in Alzheimer’s disease progression. Experimental studies have shown that the function of BChE in the attenuation of cholinergic neurotransmission is essentially altered in brains of advanced AD patients. Here, using the complimentary methods of enzyme kinetic studies, molecular modeling and protein-ligand interaction profiling, we sought to reveal the mechanistic and structural features of BChE-methylrosmarinate interactions. Molecular docking simulations revealed that methylrosmarinate dwelled well in the active centre of BChE, where it got involved in stabilizing non-covalent associations with myriad subsites. Enzyme kinetic experiments showed that the $V_{max}$ and $K_{m}$ values were 156.20 ± 3.11 U mg$^{-1}$ protein and 0.13 ± 0.01 μM, respectively. The inhibition studies showed that methylrosmarinate apparently inhibited BChE in a linear mixed manner, with an $IC_{50}$ value of 10.31 μM and a $K_{i}$ value of 3.73 ± 1.52 μM. Taken together, the extremely reduced $K_{i}$ value and the increased number of BChE–methylrosmarinate interactions presuppose that methylrosmarinate is a good inhibitor of BChE, despite the fact that the mechanism for the effect of BChE inhibition on several pathological conditions in vivo remains unexplored.

1. Introduction
Alzheimer’s disease (AD) is an advanced and irremediable disorder of the brain, which is marked by abnormal behaviors, cognitive impairment and memory loss. Although its precise mechanism is not well understood, enzymes performing critical roles in biochemical pathways pertaining the cholinergic system and AD progression are well documented [1, 2, 3].

These enzymes are the cholinesterases belonging to the carboxylic ester hydrolyase family. Two distinct cholinesterases, viz, acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) are found in vertebrates [1]. Even though these enzymes share about 50% sequence similarity, their tissue distribution, kinetic behavior and inhibitor sensitivity show a considerable variation [4, 5]. Cholinesterases regulate cholinergic neurotransmission by aiding the swift removal of acetylcholine (ACh), and the supposed cholinergic theorem propounds that reduced cholinergic neurotransmission facilitates the deepening of cognitive dysfunction in AD [6]. Other hallmarks of AD pathogenesis involve: (i) intracellular neurofibrillary tangles formation (ii) amyloid-β (Ab) peptides accumulation in neuritic plaques and (iii) loss of cholinergic neurons [7].

In healthy brain, even though AChE has a well established role of regulating cholinergic neurotransmission through the hydrolysis of neurotransmitter ACh at cholinergic synapses; the exact physiological role for BChE is yet unknown over several decades. This is because there are isolated cases of individuals who exhibit a normal phenotype, although they completely lack functional BChE gene [4]. It has however become apparent that apart from BChE putative role in the hydrolysis of exogenous esters; the neuro-peptide gut hormone, ghrelin, is likely its physiological substrate [8]. BChE also serves a secondary function to AChE by hydrolysing ACh that diffuses out of the synaptic cleft in the central nervous system (CNS) and neuromuscular junction [8]. These are in addition to the established fact that BChE protects the...
neurotransmitter role of AChE by neutralizing xenobiotics before they reach AChE [9].

Perhaps, in contrast with the aforementioned emerging functions, an emerging body of evidence points to a proximate link between BChE activity and AD [10]. In the CNS, BChE was discovered to co-localize with senile plaques. Here, it is presumed to contribute in senile plaque maturation and gradual aggregation of Aβ [11]. Some researchers have posited that BChE stimulates the transformation of innocuous plaques to diseased plaques characteristic of AD, when it becomes bound with plaques [12].

Currently, to improve cholinergic function, the main therapeutic approach is to elevate the ACh pool in the brain. And this involves the use of drugs that can inhibit cholinesterase. Unfortunately, some of these drugs trigger deleterious effects including depression, fatigue, etc [13]. These deleterious effects engendered by approved drugs utilized in AD treatment have compelled researchers to look into natural sources for safer compounds that can effectively inhibit AChE/BChE [14].

Salvia officinalis has been considered good for memory. In less severe cases of AD, its extracts and volatile oils, even in low doses, have been demonstrated to be effective in clinical trials [15]. Methylrosmarinate, a derivative of rosmarinic acid, is a polyphenolic ester of hydroxycinnamic acids with abundant presence in lemon balm, mint, basil, etc [16]. Unlike its scaffold, rosmarinic acid, there has been scanty information in the literature on the bioactive and pharmacological properties of methylrosmarinate. Further to this, it is important to assess the inhibitory potential of methylrosmarinate against horse serum butyrylcholinesterase (hBChE) in a context that combines in vitro and in silico studies.

2. Materials and methods

2.1. Chemicals and enzyme

Purified BChE formulation from horse serum, S-butyrylthiocholine iodide (BTCh) and 5,5'-dithio-2-bisnitrobenzoate (DTNB) were all obtained from Sigma-Aldrich, St. Louis, USA. Methylrosmarinate was purchased from Ennovation Chemicals LLC, NJ, USA.

2.2. BChE preparation

MOPS-KOH buffer was used for the reconstitution of the lyophilized BChE to make a concentration of 20 mM and pH 7.5. Using Kalb and Bernloehr protein assay technique, enzyme concentration was ascertained with the help of UV/Vis Spectrophotometer (ACTGene, Piscataway, USA) [17].

2.3. BChE activity determination

The activity of BChE was measured by slightly modified spectrophotometric method of Ellman et al. [18]. BTCh and DTNB were utilized as reaction substrate and chromogenic substrate, respectively, for BChE activity measurement; whilst methylrosmarinate was employed as inhibitor. All the other reagents, conditions and procedures were the same as explained in our initial publication [19]. Specific activity was determined from the following formula:

Specific activity (in U mg⁻¹ protein) = \frac{\Delta A_{412} \times V_i}{\epsilon \times V_s \times \{Protein\}}

Here, \Delta A_{412} denotes the difference in absorbance at 412, V_i is the overall reaction volume, \epsilon refers to the extinction coefficient of thionitrobenzoate, and V_s is the sample volume, whilst \{protein\} denotes the eventual BChE concentration (1.95 × 10⁻⁵ mg mL⁻¹).

2.4. Dose-dependent assessment

The methods were the same as in the preceding study, except that multiple concentrations of methylrosmarinate (0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.5 and 125 μM) was used at fixed concentration (1.0 mM) of BTCh in the presence 2.5 mM DTNB and 1.95 mg mL⁻¹ hBChE. To determine the half maximal concentration (IC₅₀), the data obtained were utilized to plot the percentage residual activity versus [methylrosmarinate] inhibition curve, from which the IC₅₀ of methylrosmarinate was obtained. The activity of BChE in the absence of methylrosmarinate was presumed 100% [20].

2.5. BChE inhibition kinetics

The reagents, conditions and procedures were similar to that of our initial publication [19], except that here the BChE activity pattern was determined by changing the concentrations of BTCh (0.05 mM–2 mM) at any of the 6 distinct fixed concentrations of methylrosmarinate (0, 0.25, 0.5, 1, 2 and 4 μM) that traversed the steepest part of the inhibition curve. Using the data obtained, Michaelis–Menten, Lineweaver–Burk, Dixon and other secondary plots were plotted, from where initial details on the inhibition mechanism was obtained [20].

2.6. Statistical analysis

Nonlinear Regression module of GraphPad Prism version 9.1.2 (226) for Windows (GraphPad Software, San Diego, California USA) was used for curve-fitting of the BChE kinetic data. Distinct mathematical models of enzyme-inhibitor interaction comprising the linear mixed type, competitive and non competitive binding associations were tested. The kinetic estimates, namely Vₘₙ, Kᵥ, Kᵢ, and α were subsequently determined.

2.7. BChE selection and preparation

The UniProt Knowledgebase was accessed and the sequence of amino acid from hSBChE (entry: PS1908) was retrieved in fasta format. This was inserted into CPHmodels Web server Version 3.2 [21]. Using human butyrylcholinesterase (hBChE), recombinant, (PDB ID: 3O9M) as template, a matching model was created for of hSBChE. The fidelity of the generated hSBChE model was evaluated with Verify3D [22]. The hSBChE receptor preparation was similar to that explained in our previous studies [19].

2.8. Methylrosmarinate selection and preparation

The standard canonical SMILES notation specific to methylrosmarinate was downloaded from the PubChem database (PubChem CID: 6479915) and inserted into Build Structure device of UCSF Chimera, Version 1.11.2 [23]. Using default settings, a high-quality and low-energy structure of methylrosmarinate was consequently created.

2.9. Molecular docking

Docking of areas of interest with 14 important sites in the active center gorge pre-identified was performed using the Patch Dock [24]. The clustering RMSD was fixed at 1.5 A and the complex type was defined as enzyme-inhibitor, in the molecular docking request form. The highest ranked (top 10) docking solutions, ranked based on their geometric score, were retrieved from the ensuing web page. This was then visualized with the aid of the PyMOL Molecular Graphics System, Version 1.8 (Schrodinger, LLC, Portland, OR, USA).

2.10. BChE-methylrosmarinate profiling

The comprehensive listing of non-covalent associations between the BChE and methylrosmarinate in the suitable docking pose was attained by PLIP [25].
3. Results and discussion

Even though its three dimensional structure is yet to be experimentally elucidated, hsBChE is an effective and well defined model of mammalian enzyme possessing extremely high level of sequence similarity with hBChE, over 90% sequence homology across 574 amino acid moieties. More so, all 14 essential residues that constitute the peripheral anionic site (PAS) (Asp70, Tyr128, Phe329), the catalytic triad (Ser198, Glu325, His438), and the acyl-binding pocket (Trp231, Leu286, Val288) are preserved in the two enzymes. This similarity with hBChE, over 90% sequence homology across 574 amino acid moieties.

In determining the kinetic behavior of BChE inhibition, the Lineweaver-Burk (double reciprocal) plot of 1/speciɛmɛnɛt activity versus 1/[methylrosmarinate] was plotted, and the results showed converging Kᵢ and μᵢ values, respectively. Three separate approaches were employed to compute the parameter estimates corresponding to competitive and noncompetitive inhibition models. These graphical deɛnɛcɛtions of the inhibition arc failed to halt at the zero mark, within the limits of experimental error, and this makes the mixed-type inhibition a blend of competitive and noncompetitive inhibition.

From the inhibition data of hsBChE, a plot of percent residual activity versus [methylrosmarinate] was made. From the ensuing graph, it was discovered that methylrosmarinate reduced the activity of BChE in a concentration-dependent fashion (Figure 1). Although methylrosmarinate reduced the activity of BChE in a dose-dependent fashion, the inhibition arc failed to halt at the zero mark, within the limits of inhibitor concentrations examined. For the measure of methylrosmarinate inhibitory potency against BChE, the IC₅₀ value was graphically determined to be 10.31 μM.

In determining the kinetic behavior of BChE inhibition, the Lineweaver-Burk (double reciprocal) plot of 1/[speciɛmɛnɛt activity] versus 1/[methylrosmarinate] was plotted, and the results showed converging lines in the 2nd quadrant, a distinct feature of linear mixed inhibition.

Specifically, methylrosmarinate decreased the value of Kₘ with a commensurate increase in the value of Vₘ. Although small, the α-value is greater than one. And this makes the mixed-type inhibition a blend of competitive and noncompetitive inhibition models. These graphical deɛnɛcɛtions are in perfect agreement with statistical studies where the parameter estimates Vₘ, Kᵢ and α were gleaned as 156.20 ± 3.11 U mg⁻¹ protein, 0.13 ± 0.01 μM, 3.73 ± 1.52 μM and 1.90 ± 0.31 μM, respectively. Three separate approaches were employed to compute the Kᵢ value. In the first approach, the experimental data was fitted to varied mathematical models of enzyme kinetic studies with the help of GraphPad Prism software version 9.1.2 (226). The GraphPad Prism analysis showed that the Kᵢ was 3.73 ± 1.52 μM. The second and third methods consisted of the determination of the Kᵢ from the replot of lineweaver burk and Dixon (Figure 3) plots [18]. The Kᵢ values were eventually found to be 4.1 μM and 4.2 μM, respectively. And the resulting mean Kᵢ was computed to be 3.94 ± 0.79 μM. Because both the Kᵢ and IC₅₀ values for methylrosmarinate fall within the micromolar levels, it is appropriate to assume that methylrosmarinate is an effective inhibitor of BChE in vitro.

Today, molecular docking is an indispensable instrument in the design of novel and effective anticholinesterases with not only increased affinity but also selectivity. In a bid to explore the structural foundations of BChE-methylrosmarinate interaction, docking studies was conducted and methylrosmarinate spanned the active center of hsBChE. The highest ranked docking results revealed that methylrosmarinate was well accommodated within the active-centre gorge of hsBChE. The molecular docking results are strongly supported by the painstaking enzyme kinetic studies. The top-ranked docking results with the geometric score of 4666 revealing methylrosmarinate inside the active-centre gorge of BChE was chosen as the pretty much native binding pose for further evaluation (Figure 4B). In this model, the monocyclic benzene of methylrosmarinate sticks out into the acyl-binding pocket, choline-binding pocket and ends near the catalytic triad. Alternatively, the α, β unsaturated carboxylic ester core with its ring structures basically assumes the opening of the gorge in the neighbourhood of the PAS. The location of methylrosmarinate in the active-centre gorge of the enzyme is essentially maintained by hydrogen-bonding interaction with Ser287 (2.81 Å) at the acetyl binding site and GLN119 (3.33 Å) at the peripheral anionic site and also with Asn68 (2.40 Å), Glu276 (3.08 Å), Val277 (3.70 Å) and Asn289 (2.54 Å) at the entrance of the active-centre gorge.

Weaker hydrophobic associations further stabilized the relative position of methylrosmarinate with Asn68 (3.54 Å) and Val277 (3.70 Å) at the perimeter of the opening of the active-centre gorge and GLN119 (3.82 Å) of the peripheral anionic site as well as Leu286 (3.78 Å) of the acyl binding site. These are in addition to the π-stacking interaction with Phe329 (5.04 Å) at the choline binding site.

Interestingly, a careful study of the superimposed image of the modeled structure of hBChE-methylrosmarinate complex on the crystal structure of hBChE (Figure 2) revealed the methylrosmarinate inside the active-centre gorge of BChE was chosen as the pretty much native binding pose for further evaluation (Figure 4B). In this model, the monocyclic benzene of methylrosmarinate sticks out into the acyl-binding pocket, choline-binding pocket and ends near the catalytic triad. Alternatively, the α, β unsaturated carboxylic ester core with its ring structures basically assumes the opening of the gorge in the neighbourhood of the PAS. The location of methylrosmarinate in the active-centre gorge of the enzyme is essentially maintained by hydrogen-bonding interaction with Ser287 (2.81 Å) at the acetyl binding site and GLN119 (3.33 Å) at the peripheral anionic site and also with Asn68 (2.40 Å), Glu276 (3.08 Å), Val277 (3.70 Å) and Asn289 (2.54 Å) at the entrance of the active-centre gorge.

Weaker hydrophobic associations further stabilized the relative position of methylrosmarinate with Asn68 (3.54 Å) and Val277 (3.70 Å) at the perimeter of the opening of the active-centre gorge and GLN119 (3.82 Å) of the peripheral anionic site as well as Leu286 (3.78 Å) of the acyl binding site. These are in addition to the π-stacking interaction with Phe329 (5.04 Å) at the choline binding site.

Interestingly, a careful study of the superimposed image of the modeled structure of hBChE-methylrosmarinate complex on the crystal structure of hBChE (Figure 2) revealed the methylrosmarinate inside the active-centre gorge of BChE was chosen as the pretty much native binding pose for further evaluation (Figure 4B). In this model, the monocyclic benzene of methylrosmarinate sticks out into the acyl-binding pocket, choline-binding pocket and ends near the catalytic triad. Alternatively, the α, β unsaturated carboxylic ester core with its ring structures basically assumes the opening of the gorge in the neighbourhood of the PAS. The location of methylrosmarinate in the active-centre gorge of the enzyme is essentially maintained by hydrogen-bonding interaction with Ser287 (2.81 Å) at the acetyl binding site and GLN119 (3.33 Å) at the peripheral anionic site and also with Asn68 (2.40 Å), Glu276 (3.08 Å), Val277 (3.70 Å) and Asn289 (2.54 Å) at the entrance of the active-centre gorge.

Weaker hydrophobic associations further stabilized the relative position of methylrosmarinate with Asn68 (3.54 Å) and Val277 (3.70 Å) at the perimeter of the opening of the active-centre gorge and GLN119 (3.82 Å) of the peripheral anionic site as well as Leu286 (3.78 Å) of the acyl binding site. These are in addition to the π-stacking interaction with Phe329 (5.04 Å) at the choline binding site.

Interestingly, a careful study of the superimposed image of the modeled structure of hBChE-methylrosmarinate complex on the crystal
structure of hBChE-BTch complex showed that little or no steric clash existed between methylrosmarinate and BTCh (Figure 4D). This suggests that both methylrosmarinate and BTCh can simultaneously coexist within the active-centre gorge of hBChE.

The involvement of methylrosmarinate in several associations with the acyl binding Leu286 and choline-binding Phe329 may explain the very reason behind the reduced affinity of BChE for BTCh. On the other hand, its hydrogen-bonding association with the active site Ser198 may justify the reduced rate of transformation of BTCh. Therefore, linear mixed model is calculated to arise as the predominant pattern of inhibition with regard to BChE–methylrosmarinate interaction. Taking account of the noncholinergic route of AD, methylrosmarinate binding to the outer rim of the PAS of BChE might hinder Aβ peptides heterologous association with BChE.

Considering BChE-linked pathological conditions, AD possess a special significance on the basis of its therapeutic ramifications. The symptoms of AD are believed to be connected with reduced levels of neuronal ACh caused by the depletion of cholinergic neurons [13, 26]. While the amounts of BChE are increased in AD, AChE levels are decreased [27]. Neuritic plaques have been discovered to be connected with increased levels of BChE and so are neurofibrillary tangles [12, 28]. BChE activity has been identified to be markedly raised from about 41 % to 80 % in brain regions of patients with complicated AD. Contrastingly, there is a significant decrease of about 60% in activity of AChE in troubled brain areas of AD patients [29].

Also, there are a myriad of factors that suggest that BChE may be looked at as a possible target for AD therapy. (i) BChE possesses a longer half-life compared to AChE [30]. (ii) The monomeric G1 class of BChE,
which predominates in the growing brain, is elevated in patients with AD [30]. (iii) High levels of ACh inhibit AChE, but BChE is unaffected [30]. (iv) Inhibition of BChE stimulates a concentration-dependent rise in the amount of ACh [31]. (v) Deleterious effects are discovered after treatment of AChE-deficient mice with inhibitors BChE [32].

Therefore, BChE performs a regulatory function in the hydrolysis of ACh, making it an alternative to AChE, and thus therapeutic candidates that basically inhibit BChE may have therapeutic effects on AD.

4. Conclusion

All experimental and computational evidence in this research suggests methylrosmarinate as an effective linear mixed inhibitor of Butyrylcholinesterase. Hence, we advance that methylrosmarinate may promote cholinergic transmission by lowering the hydrolysis of ACh by BChE. Methylrosmarinate might also reduce the deposition of Aβ fibrils in the brain by forming an integument over the peripheral anionic site and averting the interaction of Aβ peptide with this infamous area of BChE. However, this potentiality needs to be verified in future in vivo studies.

Declarations

Author contribution statement

Sani Muhammad Uzairu: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Yahaya Tijani: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Madu Adamu Gadaka: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Figure 4. Docking results of methylrosmarinate in the active-centre gorge of hsBChE. (A) The molecular structure of methylrosmarinate was built using UCSF Chimera, Version 1.11.2 [23]. (B) The most suitable orientation of methylrosmarinate relative to hsBChE. Methylrosmarinate colored in tan is depicted by stick, and BChE shown as surface contours is depicted in CYMK color coding. (C) Interactions between hsBChE and methylrosmarinate. (D) Superimposition of the BChE–methylrosmarinate complex on the human BChE-BTCh complex (PDB ID: 1P0P). Methylrosmarinate and BTCh depicted by sticks are shown in tan and forest green color codings, respectively. The entire images were depicted using Chimera Version 1.11.2 [23].
Babagana Modu; Umar Abdullahi Zakariya: Performed the experiments; Analyzed and interpreted the data.
Miriam Watafua; Hadiza Ali Ahmad: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Aminu Ibrahim; Aliyu Daja: Analyzed and interpreted the data; Wrote the paper.
Hassan Zanna; Abdullahi Balarabe Sallau: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement
Data will be made available on request.

Declaration of interest’s statement
The authors declare no conflict of interest.

Additional information
No additional information is available for this paper.

References
[1] A. Nordberg, C. Ballard, R. Bullock, T. Darreh-Shori, M. Somogyi, A review of butyrylcholinesterase as a therapeutic target in the treatment of Alzheimer’s disease, Prim. Care Companion J. Clin. Psychiatry 15 (2013) 1–23.
[2] Z. Bingel, et al., Antidiabetic, anticholinergic and antioxidant activities of aerial parts of shaggy bindweed (Convulvulus betonicifolia Miller subsp.)-profiling of phenolic compounds by LC-HRMS, Heliyon 7 (2021), e06986.
[3] H. Kuzhtat, et al., LC-HRMS profiling, antidiabetic, anticholinergic and anti-oxidant activities of aerial parts of kinkor (Ferulago stellata), Molecules 26 (2021) 2469.
[4] C.N. Pope, S. Brimijoin, Cholinesterases and the fine line between poison and remedy, Biochem. Pharmacol. 153 (2018) 205–216.
[5] K.R. Dave, A.R. Syal, S.S. Katayre, Tissue cholinesterases. A comparative study of their kinetic properties, Zeitschrift fur Naturforch. - Sect. C J. Biosci. 55 (2000) 100–108.
[6] X. Chen, et al., Late Paleozoic magmatism and porphyry copper metallogenesis in Balkhash metallogenic zone, Kazakhstan, central Asia, Jilin Daxue Xuebao (Diquj Kexue Ban)/J. Jilin Univ. Earth Sci. Ed. 43 (2013) 734–747.
[7] H.W. Querfurth, F.M. Laferla, Alzheimer’s Disease, 2010, pp. 329–344.
[8] S. Brimijoin, V.P. Chen, Y. Pang, L. Geng, Y. Gao, Physiological roles for butyrylcholinesterase: a BChE-ghrelin axis, Chem. Biol. Interact. 259 (2016) 271–275.
[9] G. Johnson, S.W. Moore, Why has butyrylcholinesterase been retained? Structural and functional diversification in a duplicated gene, Neurochem. Int. 61 (2012) 783–797.
[10] N.H. Greig, D.K. Lahiri, K. Sambamurti, Butyrylcholinesterase: an important new target in Alzheimer’s disease therapy, Int. Psychogeriatr. 14 (2002) 77–91.
[11] P. Gómez-Ramón, M.A. Morán, Ultrastructural localization of butyrylcholinesterase in senile plaques of the brains of aged and Alzheimer disease patients, Mol. Chem. Neuropharmacol. 30 (1997) 161–173.
[12] A.L. Guillotet, J.F. Smiley, D.C. Minh, M.M. Mesulam, Butyrylcholinesterase in the life cycle of amyloid plaques, Ann. Neurol. 42 (1997) 909–918.
[13] R.M. Lane, S.G. Potkin, A. Enz, Targeting acetylcholinesterase and butyrylcholinesterase in dementia, Int. J. Neuropsychopharmacol. 9 (2006) 101–124.
[14] N. Wexlaki, A. Kuciu, A. Kiss, Screening of traditional European herbal medicines for acetylcholinesterase and butyrylcholinesterase inhibitory activity, Acta Pharm. 60 (2010) 119–128.
[15] T. Iwamoto, D. De Filippis, G. Esposito, A. D’Amico, A.A. Izzo, The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-β peptide-induced neurotoxicity, J. Pharmacol. Exp. Therapeut. 317 (2006) 1143–1149.
[16] L. Lin, et al., Comparative evaluation of rosmarinic acid, methyl rosmarinate and pedalin isolated from Rabdosia serra (MAXIM.) HARA as inhibitors of tyrosinase and α-glucosidase, Food Chem. 129 (2011) 884–889.
[17] V.F. Kalb, R.W. Bernlohr, A new spectrophotometric assay for protein in cell extracts, Anal. Biochem. 82 (1977) 362–371.
[18] A. Muller, F. Adler, C. Ziomek, K. Klotz, L. Ivers, T. Seibert, G. Naumann, H. Stosiek, J. Stelzer, High-Resolution Imaging in Three-Dimensional Live Cells by Confocal Microscopy, Nat. Methods 4 (2007) 607–610.
[19] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H.J. Wolfson, PatchDock and SymmDock: servers for rigid and symmetric docking, Nucleic Acids Res. 33 (2005) W443–W447.
[20] I.H. Segel, Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems Horizons in Biochemistry and Biophysics, vol. 1, John Wiley & Sons, 1975, pp. 220–221, 60.
[21] M. Nielsen, C. Lindegaard, O. Lund, T.N. Petersen, OPModes-3.0-remote homology modeling using structure-guided sequence profiles, Nucleic Acids Res. 38 (2010) 576–581.
[22] R. Luty, J. Bowei, D.Verify3D, Einesberg, Assessment of protein models with three-dimensional profiles, Methods Enzymol. 277 (1997) 396–404.
[23] L. Xia, L. Zheng, J.L. Zhou, Effects of ibuprofen, diclofenac and paracetamol on the life cycle of amyloid plaques, Ann. Neurol. 42 (1997) 909–918.
[24] I.O. Den VeldeE, F.C. Stam, Some cerebral proteins and enzyme systems in The Rodent Brain: A Comparative and Developmental Study, Amsterdam, Lisse, 1985, pp. 261–271.
[25] T. Iuvone, D. De Filippis, G. Esposito, A. D’Amico, A.A. Izzo, The spice sage and its active ingredient rosmarinic acid protect PC12 cells from amyloid-β peptide-induced neurotoxicity, J. Pharmacol. Exp. Therapeut. 317 (2006) 1143–1149.
[26] L. Lin, et al., Comparative evaluation of rosmarinic acid, methyl rosmarinate and pedalin isolated from Rabdosia serra (MAXIM.) HARA as inhibitors of tyrosinase and α-glucosidase, Food Chem. 129 (2011) 884–889.
[27] N.H. Greig, D.K. Lahiri, K. Sambamurti, Butyrylcholinesterase: an important new target in Alzheimer’s disease therapy, Int. Psychogeriatr. 14 (2002) 77–91.
[28] A. Muller, F. Adler, C. Ziomek, K. Klotz, L. Ivers, T. Seibert, G. Naumann, H. Stosiek, J. Stelzer, High-Resolution Imaging in Three-Dimensional Live Cells by Confocal Microscopy, Nat. Methods 4 (2007) 607–610.
[29] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H.J. Wolfson, PatchDock and SymmDock: servers for rigid and symmetric docking, Nucleic Acids Res. 33 (2005) W443–W447.
[30] I.H. Segel, Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady State Enzyme Systems Horizons in Biochemistry and Biophysics, vol. 1, John Wiley & Sons, 1975, pp. 220–221, 60.
[31] M. Nielsen, C. Lindegaard, O. Lund, T.N. Petersen, OPModes-3.0-remote homology modeling using structure-guided sequence profiles, Nucleic Acids Res. 38 (2010) 576–581.
[32] R. Luty, J. Bowei, D.Verify3D, Einesberg, Assessment of protein models with three-dimensional profiles, Methods Enzymol. 277 (1997) 396–404.