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In vitro inhibitory effect of Boeserngin A on human acetylcholinerase: Understanding its potential using *in silico* ADMET studies

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ABSTRACT

Boesenbergia rotunda is a medicinal plant that used traditionally in South East Asia as a healing for various ailments including neurological disorders. Therefore, this research was designed to describe the biological evaluation pertaining to anti-human cholinesterase (hAChE) activities, molecular docking and *in silico* prediction of Boesenbergin A (BA), a natural chalcone isolated from *B. rotunda*. The anti-hAChE activity of BA was evaluated using acetylthiocholine as a substrate and 5,5-dithiobis[2-nitrobenzoic acid] (DTNB) as chromogen. Docking study with flexible boesenbergin A was done using Autodock 4.2 software and A Lamarkian Genetic Algorithm search method. ADME/Tox analysis was also performed using ADMET Descriptors software. The inhibitory activities of BA and the standard drugs tacrin and propidium iodide on human acetylcholinesterase are 70.1±5.43, 76.6±5.11 and 28.2±2.47, respectively. Molecular docking investigation showed that BA occupies the active site of hAChE and this strongly suggests that it acts as a competitive inhibitor and ultimately reduces the activity of the enzyme. BA also exhibited good ADMET properties indicating capacity for further studies towards developing this compound into a potent drug candidate for the treatment of Alzheimer's disease. The present study extends the understanding on the molecular mechanism underlying the diverse biological activities of *Boesenbergia rotunda*.

INTRODUCTION

Neurodegeneration is characterized as the dysfunctions of nerve cell and loss of neurons in the central nervous system. Alzheimer's disease (AD) is considered the top ranked neurodegenerative disease and millions of people are reported to suffer from this disturbing ailment (Darreh-Shori and Soininen, 2010; Weinreb *et al.*, 2009). Therapeutic strategies to this ailment are also based on the assumption of acetylcholinesterase inhibition (Kovacs *et al.*, 2012). Indeed, many scientific trials have been conducted in order to discover typical and non-toxic drug for the treatment of AD. The most commonly used drug for the treatment of AD is tacrine which is recognized to have so many side effects that in most cases they lead to withdrawal of the medication. Other drugs like donepezil, rivastigmine, galanthamine, caproctamine and memantine were also used for the treatment of AD, but are known for their unfavorable effects (Adewusi *et al.*, 2010; Darreh-Shori and Soininen, 2010; Kovacs *et al.*, 2012). Therefore, researchers are in continuous search for new, safe and efficient anti-AD drugs. A potential source of AChE inhibitors is provided by the abundance of plants in nature, and natural products continue to provide useful drugs and templates for the development of other compounds (Adewusi *et al.*, 2010; Erdogan Orhan, 2012). *Boesenbergia rotunda* is a medicinal plant taht usually used in Malaysia and Indonesia as food ingredients and/or condiments. Traditional healers have been utilizing the rhizomes of this plant for curative purposes against inflammation, leucorrhoea, oral diseases, cancers and neurological disorders. In addition to being used for primary health care, the rhizomes have been reported to have aphrodisiac properties (Abdelwahab *et al.*, 2011; Ching *et al.*, 2007). *B. rotunda* exhibits versatile pharmacological activities such as antimutagenesis, antimicrobial, analgesic, antipyretic, and insecticidal. Some biologically active substances have isolated from this plant such as pinostrobin, pinocembrin, chromene, panduratin C, panduratin A, cardamonin and alpinetin (Ching *et al.*, 2007; Tuchinda *et al.*, 2002). Boesenbergin A (BA) is a chalcone derivative, which was first isolated from *B. rotunda*. Chalcones are aromatic ketones that compose the basic skeleton of several biologically active compounds.

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They contain two aromatic rings with an unsaturated chain. They show antimicrobial, cytotoxic, antiparasitic and anti-inflammatory properties (Guo *et al.,* 2009). Even though other chalcones have been described in terms of their various medicinal uses, BA has not been studied extensively (Chadwick *et al.,* 2006). Thus, the current study was designed to understand the effects of BA on AchE *in vitro* and *in silico* and to understand the traditional use of *B. rotunda* as a treatment for some neurological diseases.

MATERIALS AND METHODS

Isolation of BA from *B. rotunda*

The rhizomes of *B. rotunda* were identified by a botanist at the Faculty of Science, University Putra Malaysia. Finely ground air-dried rhizomes of *B. rotunda* were extracted three times with hexane for three days each at ambient temperature. The extract was fractionated for 60 fractions. Fraction 19 yielded BA as orange needle-shaped crystals ($C_{26}H_{28}O_4$; *m/z* 404) using hexane and ethyl acetate. Spectral data was obtained using IR, ^{1}H NMR, ^{13}C NMR and LC/MS. BA was identified by comparison of its spectral data to those reported in the literature (Sudwan *et al.,* 2007). The purity of the compound was analyzed by UFLC using a Shimadzu UFLC system equipped with a PDA UV detector and Ion Trap TOF mass spectrometer.

Anti-AChE Assay

The anti-hAChE activity of BA was evaluated by Ellmann's method with slight modifications, using acetylthiocholine as a substrate (Tarek Mohamed 2011) and 5,5□f-dithiobis[2-nitrobenzoic acid] (DTNB). A mixture of sodium phosphate buffer (pH 8.0, 110 µL), sample solution (20 µL; $2X10^{-5}$M), DTNB (0.126 mM, 50 µL) and AChE enzyme (0.6 U/mL, 20 µL) was incubated in 96-well plate for 50 minutes at 37 ºC. The reaction was then initiated by the addition of acetylthiocholine iodide (0.120 mM, 50 µL) (n=3). The hydrolysis of acetylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine, at a wavelength of 412 nm every 30 s for 25 min using a 96-well microplate plate reader (TECAN Infinite M200, Mannedorf, Switzerland). BA and the standard drugs (tacrine and propidium iodide) were dissolved in analytical grade DMSO. The percent inhibition of the enzyme activity due to the presence of increasing test compound concentration was obtained from the expression; 100-(v_i/v_o x 100), where v_i is the initial rate calculated in the presence of inhibitors and v_o is the enzyme activity.

Molecular Docking Evaluation

The crystal structure of human acetylcholinesterase (hAChE) designated as 1B41 (Kryger *et al.,* 2000) was retrieved from the Protein Data Bank (PDB). Fasciculin-II as well as water molecules were removed, and polar hydrogen atoms were added to the structure. Missing atoms identified on Gln291 were added and CHARMm forcefield was applied to the structure. All of these were done using Accelrys Discovery Studio Visualizer. The chemical structure of boesenbergin A was drawn and rendered to 3D configuration using ChemBioDraw 2008 and ChemBio3D 2008 (CambridgeSoft) respectively. The structure was then minimised in HyperChem 6.0 using PM3 semi-empirical method. Minimisation was done using the Steepest Descent algorithm (1000 cycles), followed by Fletcher-Reeves (Conjugate gradient) algorithm until convergence was achieved.

A blind docking study with flexible boesenbergin A was done, using Autodock 4.2 software (Azam *et al.,* 2011). By default, solvation parameters and Gasteiger charges were assigned to all atoms of hAChE. A grid box with maximum number (126 + 1) of grid points and grid spacing of 0.547Å for all three dimensions was generated using AutoDock Tools v.1.4.2. This grid box spans the entire protein. A Lamarkian Genetic Algorithm search method was then employed for docking. The number of runs was set to 100 and the maximum number of evaluations was set to 25 million. Other parameters were set as default. From the results of blind docking, a more specific docking was consequently done by using a smaller search space which corresponded to the vicinity occupied by the most populated docked ligand cluster with the lowest mean binding energy. This grid box had similar number of grid points (126+1) but with a smaller grid spacing of 0.225Å. After the grid maps were generated, the ligand was docked again to hAChE using the same docking search method and parameters.

Analysis of Absorption, Distribution, Metabolism, Excretion and Toxicity properties

BA was studied for ADMET properties using ADMET Descriptors Software. This *in silico* system performs computational prediction based solely on the chemical structure of the molecule. Included are six models that provide a comprehensive analysis of ADMET characteristics: Aqueous Solubility, Blood Brain Barrier Penetration, CYP2D6 Binding, Hepatotoxicity, Intestinal Absorption and Plasma Protein Binding. ADMET Descriptors software also calculates AlogP98 and PSA_2D, which are used in plotting the confidence ellipses (Ekins *et al.,* 2005).

RESULTS

Anti-AChE Assay

The inhibitory activities of BA and the standard drugs tacrin and propidium on human acetylcholinesterase are shown in Table 1. These results indicated that the compound BA had high activity (70.1± 5.43). The standard drugs tacrin and propidium used in this study showed inhibitions of 76.6 ± 5.11 and 28.2 ± 2.47, respectively.

Molecular Modelling

The output file from the blind docking study yields the top four clusters with the lowest binding energies are also the four biggest clusters. They represent a total of 50 conformations out of the 100 generated. Most importantly, all of them share a similar

docking site; the entrance to the gorge of the protein binding site. At the deep end of the gorge is where the active site resides (Kryger *et al.*, 2000). Hence, that particular site was chosen for a more specific docking work. Results from the specific docking show that 33 conformations out of the 100 falls into a single cluster with a mean binding energy of -9.37 kcal/mol. For this particular conformation that is shown in Figure 1, a hydrogen bond forms between O17 of boesenbergin A and Ser125. Several pi-pi interactions are also present between aromatic rings of the ligand and residues Trp86, Trp286 and Tyr341 (Figure 2). Numerous hydrophobic interactions exist between boesenbergin A and Tyr72, Asp74, Trp86, Gly120, Gly121, Gly122, Tyr124, Gly126, Leu130, Trp286, Phe297, Tyr337, Phe338, Tyr341, and His447 residues, as shown in Figure 3 and 4. Van der Waals interactions are observed between O18 and C30 of boesenbergin A with Gly120, and C9 of the ligand with Tyr337 (Figure 2).

***In silico* ADMET**

In ADMET study, intestinal absorption, aqueous solubility, blood brain barriers and protein binding percentage properties of BA were investigated (Figure 5). The intestinal absorption was good and aqueous solubility was optimal. However, the blood brain barrier ratio was very high for BA. The plasma protein binding percentage was more than 90% for BA. This drug also exhibited good ADMET properties indicating scope for further studies towards developing this compound into a potent drug candidate. Results of this objective are summarized in Table 1 and Figure 5.

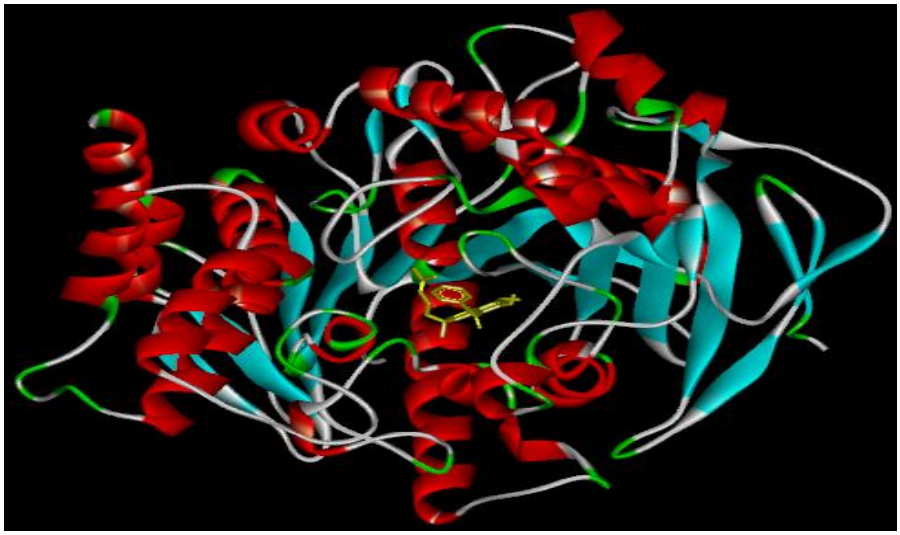

Fig. 1: Boesenbergin A (yellow stick) docked to hAChE.

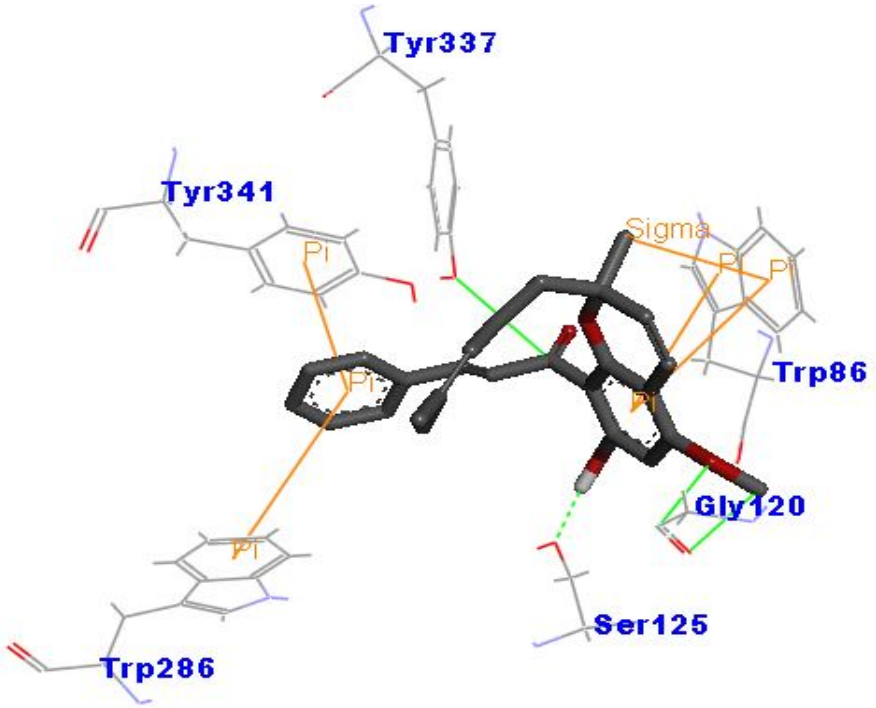

Fig. 2: A 3D representation showing the multiple pi-pi and pi-sigma interactions (orange lines), hydrogen bond (green dotted line), and Van Der Waals interactions (green line) between boesenbergin A with residues at the binding site of hAChE.

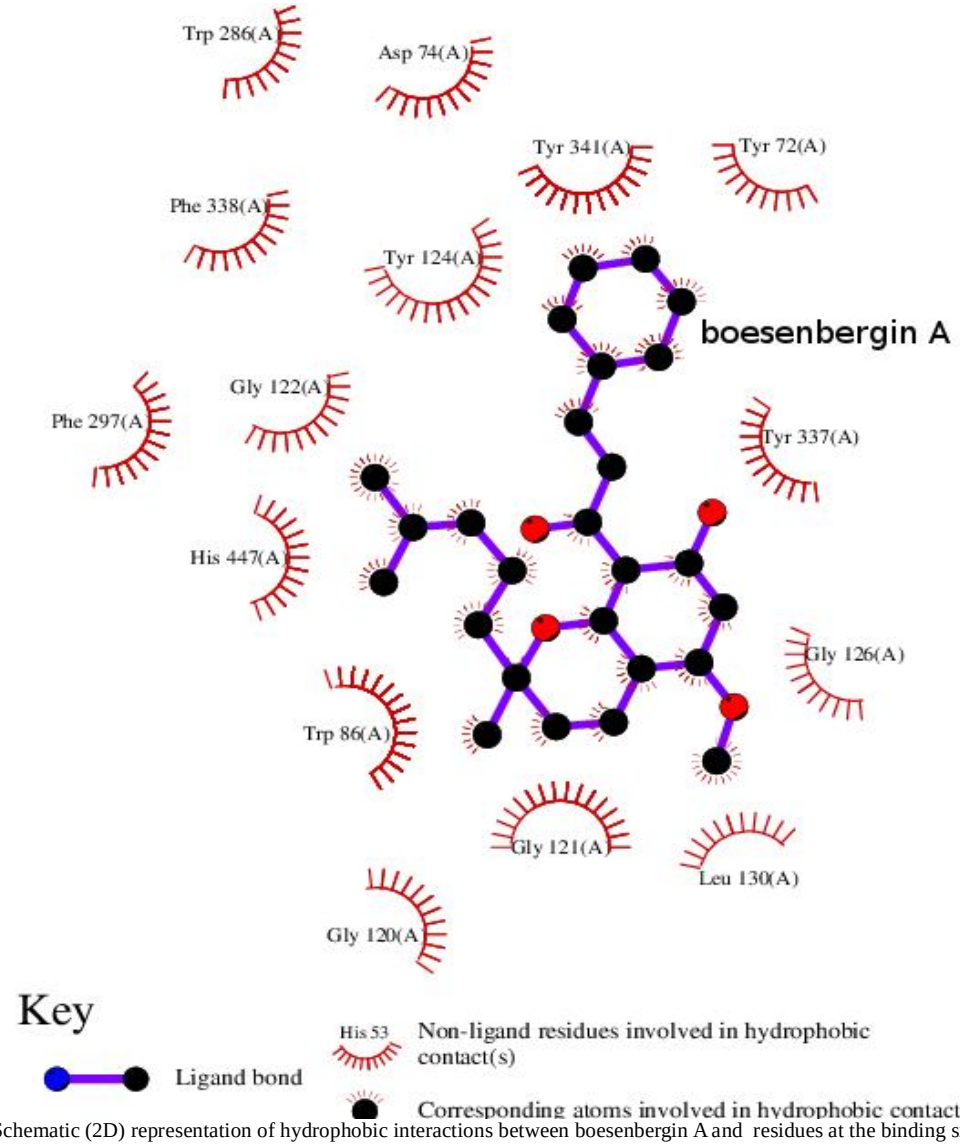

Fig. 3: Schematic (2D) representation of hydrophobic interactions between boesenbergin A and residues at the binding site of hAChE.

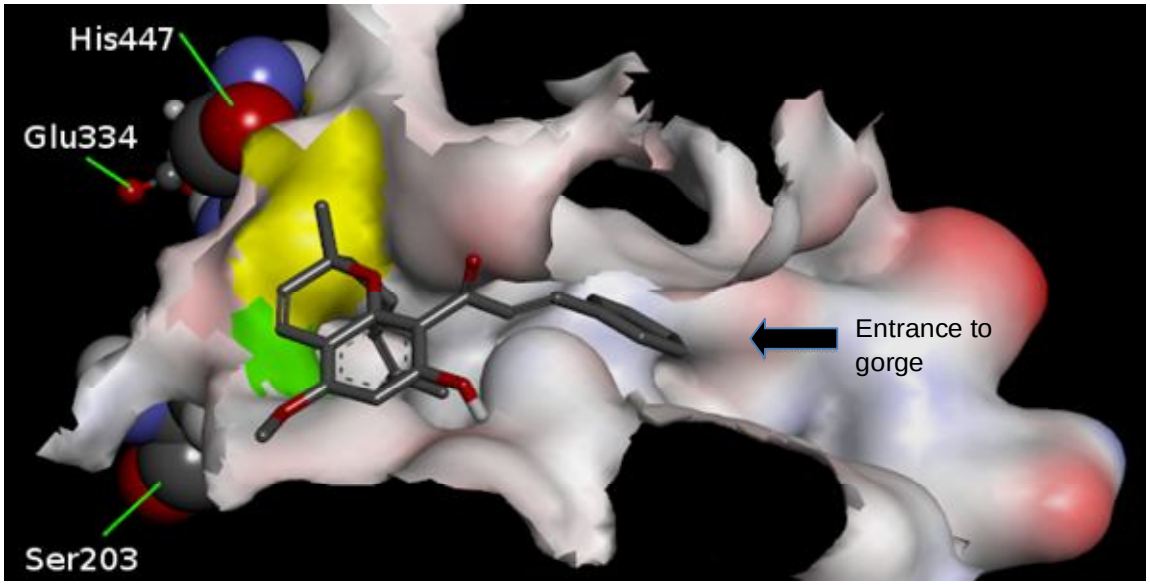

Fig. 4: A cross-sectional surface diagram illustrating the hAChE gorge surrounding boesenbergin A. His447 and Ser203 are represented in CPK model while Glu334 is represented in ball and stick model. The yellow and green surfaces belong to His447 and Ser203 respectively. The narrow entrance can be clearly seen at the middle of the diagram and it leads from right to left into the active site.

DISCUSSION

The current study was designed to investigate the anti-human cholinesterase inhibitory effects of BA. BA was isolated from *B. rotunda*, a South-eastern plant that used traditionally to cure some neurological disorders. The results of this study show that BA ($2X10^{-5}$M) has a significant inhibition towards human type AchE (70.1± 5.43). There are certain natural and synthetic AchE inhibitors which will prevent the cause of AD by inhibiting AchE (Giacobini, 2004). A previous study showed that naturally occurring compounds could strongly inhibit acetylcholinesterase enzyme (Reiner *et al.,* 2000). A blind docking work was initially performed to locate the possible binding sites of boesenbergin A to hAChE. After the best binding location is identified, a more specific docking study was performed within the binding spatial vicinity to obtain a more accurate ligand conformation and binding energy. This *in silico* study has shown that, like tacrine, boesenbergin A binds itself to the active site of hAChE. Acetylcholines are hence denied access to the active site and this could ultimately cause the inhibition of the protein's hydrolase activity (Cravatt *et al.,* 1996).Boesenbergin A forms interactions with several amino acid residues that are important for the protein function. His447 and Ser203 for instance, are two out of the three residues that make up the catalytic triad of hAChE. His447 forms hydrophobic interaction with boesenbergin A while for Ser203, it is effectively blocked by the ligand that is in its proximity. Figure 4 clearly show how boesenbergin A occupies the gorge while denying acetylcholines access through a single narrow opening that leads into the active site. Table 2 summarises the various interactions that exist between boesenbergin A and the residues from hAChe active site (Wiesner *et al.,* 2007).

Table. 2: The residues from each hAChE active site sub-sites and its interaction with boesenbergin A. Residues that are proximate to the ligand are those that has at least one atom within 5Å distance from a nearest atom of the ligand.

Active site subsite	Residue	Interaction with boesenbergin A
Catalytic triad	Ser203	Proximity
	Glu334	-
	His447	Hydrophobic
Oxyanion hole	Gly121	Hydrophobic
	Gly122	Hydrophobic
	Ala204	Proximity
Anionic subsite	Trp86	Hydrophobic
	Tyr133	Proximity
	Gly202	Proximity
	Gly448	Proximity
	Ile451	-
Acyl binding pocket	Trp236	-
	Phe295	Proximity
	Phe297	Hydrophobic
	Phe338	Hydrophobic
Peripheral anionic subsite	Asp74	Hydrophobic
	Tyr124	Hydrophobic
	Ser125	Hydrogen bond
	Trp286	Hydrophobic
	Tyr337	Van Der Waals
	Tyr341	Hydrophobic
Other residues of omega loop	Thr83	-
	Asn87	-
	Pro88	-

Residues Asp74 and Tyr124 are suggested to be of great importance to the activity of the protein. Mutation from Asp74 to Tyr74, for instance, is said to destroy the negative charge at the entrance of the gorge, thus affecting the capability of the protein to recruit acetylcholine (cation) to the active site (Wiesner *et al.,* 2007). Similarly, the presence of boesenbergin A at that site effectively causes a steric interference that masks the electrostatic properties of these residues. More importantly, the fact that boesenbergin A occupies the active site of hAChE strongly suggests that it acts as a competitive inhibitor and ultimately reduces the activity of the enzyme (da Silva *et al.,* 2012; Miyazawa *et al.,* 1997).

Calculating ADMET descriptors early in the development of a drug is important to help avoid having to eliminate compounds with unfavorable ADMET characteristics later in the development process, preferably before synthesis (Hop, 2012; Vangala *et al.,* 2012). The current study has predicted human intestinal absorption (HIA) after oral administration. Intestinal absorption is defined as a percentage absorbed rather than as a ratio of concentrations (cf. blood-brain penetration). A well-absorbed compound is one that is absorbed at least 90% into the bloodstream in humans.

The intestinal absorption model includes 95% and 99% confidence ellipses in the ADMET_PSA_2D, ADMET_AlogP98 plane. The ellipses define regions where well-absorbed compounds are expected to be found: 95% of well-absorbed compounds are expected to fall within the 95% ellipse, while 99% of well-absorbed compounds should fall within the 99% ellipse. Note that the location of any particular compound does not necessarily imply whether it will be well, moderately, or poorly absorbed (Paixão *et al.,* 2012; Zhang *et al.,* 2012). In general, however, absorption tends to drop off quite rapidly outside the 95% ellipse. There are four prediction levels [0 =Good, =Moderate, 2=Poor, 3=Very Poor. These levels are defined by the 95% (blue line) and 99% (magenta line) confidence ellipsoids in Figure 5. The upper limit of PSA_2D value for the 95% confidence ellipsoid is at 131.62, while the upper limit of PSA_2D value for the 99% confidence ellipsoid is at 148.12.

CONCLUSION

This paper extends the understanding on the molecular mechanism underlying the biological activities of *Boesenbergia rotunda* and its bioactive compound, BA. Furthermore, it supports the pharmacological basis that this finger root has been used as traditional medicine for the treatment of neurological disorders. BA also exhibited good ADMET properties indicating capacity for further studies towards developing this compound into a potent drug candidate. The *in silico* study suggests that boesenbergin A may inhibit the activity of human acetylcholinesterase by occupying the active site, acting as a competitive inhibitor. Its binding may also affect the capability of the enzyme to recruit acetylcholine because of its interactions with several residues from the peripheral anionic subsite.

Conflict of Interests

Authors declare no conflict of interest.

REFERENCES

Abdelwahab, S.I., Mohan, S., Abdulla, M.A., Sukari, M.A., Abdul, A.B., Taha, MME., Syam, S., Ahmad, S., Lee, KH. The methanolic extract of Boesenbergia rotunda (L) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property in vivo: Possible involvement of indirect antioxidant action. J ethnopharmacol. 2011;137: 963–970.

Adewusi, E.A., Moodley, N., Steenkamp, V. Medicinal plants with cholinesterase inhibitory activity: A Review. J Medicinal Plant Res. 2010; 9: 8257-8276.

Azam, F., Prasad, M.V.V., Thangavel, N., Ali, HI. Molecular docking studies of 1-(substituted phenyl)-3-(naphtha [1, 2-d] thiazol-2-yl) urea/thiourea derivatives with human adenosine A2A receptor. Bioinform. 2010;6: 330-337.

Chadwick, L., Pauli, G., Farnsworth, N. The pharmacognosy of *Humulus lupulus* L.(hops) with an emphasis on estrogenic properties. Phytomed. 2006;13: 119-131.

Ching, A.Y.L., Wah, T.S., Sukari, M.A., Lian, G.E.C., Rahmani, M., Khalid, K. Characterization of flavonoid derivatives from Boesenbergia rotunda (L.). Malays J Anal Sci. 2007;11: 154-159.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., Gilula, NB. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature. 1996; 384: 83-87.

da Silva, J.I., de Moraes, M.C., Vieira, L.C.C., Corrêa, A.G., Cass, Q.B., Cardoso, CL. Acetylcholinesterase capillary enzyme reactor for screening and characterization of selective inhibitors. J Pharmaceut Biomed Anal. 2012; 12: 234-240.

Darreh-Shori, T., Soininen, H. Effects of cholinesterase inhibitors on the activities and protein levels of cholinesterases in the cerebrospinal fluid of patients with Alzheimers disease: A review of recent clinical studies. Current Alzheimer Res. 2010; 7:67-73.

Ekins, S., Nikolsky, Y., Nikolskaya, T. Techniques: application of systems biology to absorption, distribution, metabolism, excretion and toxicity. Trends Pharmacol Sci. , 2005; 26, 202-209.

Erdogan Orhan, I., 2012. Current Concepts on Selected Plant Secondary Metabolites With Promising Inhibitory Effects Against Enzymes Linked to Alzheimers Disease. Current Medicinal Chemistry 19, 2252-2261.

Giacobini, E. Cholinesterase inhibitors: new roles and therapeutic alternatives. Pharmacol. Res. 2004;50: 433-440.

Guo, W., Kong, E.H., Meydani, M. Dietary polyphenols, inflammation, and cancer. Nutr. Cancer 2009;61: 807-810.

Hop, CECA. Role of ADME Studies in Selecting Drug Candidates: Dependence of ADME Parameters on Physicochemical Properties. Encyclopedia of Drug Metabolism and Interactions. 2012.

Kovacs, J., Pakaski, M., Juhasz, A., Feher, A., Drotos, G., Fazekas, C.O., Horvath, T.L., Janka, Z., Kalman, J. Achetylcholinesterase (AChE) inhibition and serum lipokines in Alzheimer's disease: friend or foe? Neuropsychopharmacol Hungarica. 2012;14: 19-27.

Kryger, G., Harel, M., Giles, K., Toker, L., Velan, B., Lazar, A., Kronman, C., Barak, D., Ariel, N., Shafferman, A. Structures of recombinant native and E202Q mutant human acetylcholinesterase complexed with the snake-venom toxin fasciculin-II. Acta Crystallographica Section D: Biol Crystall. 2000;56: 1385-1394.

Miyazawa, M., Watanabe, H., Kameoka, H. Inhibition of acetylcholinesterase activity by monoterpenoids with ap-menthane skeleton. J Agri Food Chem. 1997; 45:677-679.

Paixão, P., Gouveia, L.F., Morais, J.A.G. Prediction of the human oral bioavailability by using in vitro and in silico drug related parameters in a physiologically based absorption model. Inter J of Pharmaceutics. 2012;12:123-129.

Reiner, E., Radic, Z., Giacobini, E., Mechanism of action of cholinesterase inhibitors. Cholinesterases and Cholinesterase Inhibitors. 2000;12:103-119.

Sudwan, P., Saenphet, K., Aritajat, S., Sitasuwan, N. Effects of *Boesenbergia rotunda* (L.) Mansf. on sexual behaviour of male rats. Asian J Androl. 2007; 9:849-854.

Tarek Mohamed , X.Z., Lila K. Habib , Jerry Yang , Praveen PN. Design, synthesis and structure–activity relationship (SAR) studies of 2,4-disubstituted pyrimidine derivatives: Dual activity as cholinesterase and Ab-aggregation inhibitors. Bioorg. Medicinal Chem. 2011; 23:2269-2281.

Tuchinda, P., Reutrakul, V., Claeson, P., Pongprayoon, U., Sematong, T., Santisuk, T., Taylor, WC. Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia pandurata*. Phytochem. 2002;59:169-173.

Vangala, S., Pinjari, J., Patole, P., Ravindran, S., Gangal, R., Wangikar, P., Basu, S., Ahmed, T., Rastogi, H. Translational Drug Discovery Research: Integration of Medicinal Chemistry, Computational Modeling, Pharmacology, ADME, and Toxicology. Encyclopedia of Drug Metabolism and Interactions. 2012.

Weinreb, O., Mandel, S., Bar-Am, O., Yogev-Falach, M., Avramovich-Tirosh, Y., Amit, T., Youdim, MBH. Multifunctional neuroprotective derivatives of rasagiline as anti-Alzheimer's disease drugs. Neurother. 2009;6:163-174.

Wiesner, J., Kriz, Z., Kuca, K., Jun, D., Koca, J. Acetylcholinesterases-the structural similarities and differences. J Enzyme Inh Medicinal Chem. 2007;22: 417-424.

Zhang, Y., Liu, H., Jiao, Y., Yuan, H., Wang, F., Lu, S., Yao, S., Ke, Z., Tai, W., Jiang, Y. De novo design of N-(pyridin-4-ylmethyl) aniline derivatives as KDR inhibitors: 3D-QSAR, molecular fragment replacement, protein-ligand interaction fingerprint, and ADMET prediction. Molec Div. 2012;12:1-16.

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