RESEARCH ARTICLE

In silico study of ginsenoside analogues as possible BACE1 inhibitors involved in Alzheimer's disease [version 1; peer review: 1 approved, 1 not approved]

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V1 First published: 23 Jul 2019, 8:1169
<https://doi.org/10.12688/f1000research.19775.1>

Latest published: 23 Jul 2019, 8:1169
<https://doi.org/10.12688/f1000research.19775.1>

Abstract

Background: Neurodegenerative disorders such as Alzheimer's disease show an alarming prevalence in the population, with around 50 million affected individuals worldwide, and is associated with the development of dementia, mainly affecting the elderly population. Currently, the treatments used are based on slowing the progression of the clinical symptoms of Alzheimer's; however, no specific treatment has been found that induces reversion of the disease. Natural products may induce a wide range of palliative effects, such as ginseng (*Panax ginseng*), which produces secondary metabolites called ginsenosides, which have multiple therapeutic applications, including for neurodegenerative diseases such as Alzheimer's.

Methods: A virtual screening was carried out, using the ChEMBL database to find analogs of ginsenosides based on the ginsenoside Rg1 (ChEMBL501637). Next, the molecules were optimized and their format modified. The structure of BACE1 was downloaded from the Protein Data Bank database (ID: 1FKN) and prepared for the development of molecular docking using the PyRx software. A database of the ligands was created and molecular docking experiments were carried out, obtaining affinity energy values in Kcal/mol.

Results: Of the 27 analogues studied, it was found that the molecules ChEMBL451292, ChEMBL510371 and ChEMBL503302 showed considerable affinity with BACE1 when compared with the reference molecules (verubecestat and donepezil). These affinity energy values for ChEMBL451292, ChEMBL510371 and ChEMBL503302 were -9.6, -8.1 and -7.6 Kcal/mol, respectively. Likewise, the binding site of the ligands to BACE1 were identified, with the main interactions being van der Waals and hydrogen bonding.

Conclusions: Three ginsenoside analogues showed a similar binding energy with BACE1 compared to the reference drugs. The residues

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version 1 23 Jul 2019	 view	 view

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Any reports and responses or comments on the article can be found at the end of the article.

involved in the inhibitory activity of BACE1 and the type of predominant interactions were identified, which agreed with previous reports.

Keywords

BACE1, ginsenoside, molecular docking, inhibitor, Alzheimer's disease

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Author roles: **Contreras-Puentes N:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Writing – Original Draft Preparation; **Mercado-Camargo J:** Data Curation, Formal Analysis, Methodology, Writing – Original Draft Preparation; **Alvz-Amador A:** Conceptualization, Data Curation, Investigation, Methodology, Writing – Original Draft Preparation

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Contreras-Puentes N, Mercado-Camargo J and Alvz-Amador A. *In silico study of ginsenoside analogues as possible BACE1 inhibitors involved in Alzheimer's disease* [version 1; peer review: 1 approved, 1 not approved] F1000Research 2019, 8:1169 <https://doi.org/10.12688/f1000research.19775.1>

First published: 23 Jul 2019, 8:1169 <https://doi.org/10.12688/f1000research.19775.1>

Introduction

Alzheimer's disease is a neurodegenerative condition causing changes in behavioral patterns and progressive cognitive deterioration, leading to dementia and diminished orientation and recognition abilities^{1,2}. It has been estimated that more than 45 million individuals have been diagnosed with dementia worldwide and projections for prevalence in 2050 indicate that around 130 million people will develop these types of manifestations^{3,4}.

At present, Alzheimer's disease leads to irreversible repercussions for the diagnosed population, which constitutes a problem at the public health level. Current treatments are based on the functioning of neurotransmitters such as acetylcholine, specifically as acetylcholinesterase inhibitors, preventing the degradation of this endogenous substance and favoring the stimulation of associated neurotransmitter response mechanisms^{5,6}. However, these treatments are lacking and are inefficient at significantly prolonging quality of life for patients.

Therefore, we have studied the properties of products of natural origin such as ginseng (*Panax ginseng*), specifically major secondary metabolites such as ginsenosides⁷, which are extracted from different plant components. These molecules generally contain steroid rings and have varied biological responses, such as anti-inflammatory, anti-carcinogenic and cardiovascular effects and, recently, activities at the level of nervous regulation, with potential effects on epilepsy, Parkinson's and Alzheimer's^{8–10}.

Therefore, in this study, promising molecules from structural variants of ginsenosides with potential capacity of bound to proteins involved in the development of Alzheimer's disease such as β -secretase (BACE-1) were identified using interaction studies, identification of affinities, determination of binding-free energies and *in silico* methodologies based on molecular docking.

Methods

Search and selection of ligands

A search for ginsenoside Rg1 (CHEMBL501637) analogues was performed using the ChEMBL database¹¹. The name of the structure ginsenoside Rg1 (CHEMBL501637) was used to search the database. Subsequently, a filter was applied to the analogues to identify compounds with 80–90% similarity. The database provided information about the physical representation, activity charts, calculated properties and solubility of these compounds. Previous theoretical studies with ginsenosides have reported finding few structures upon screening. A preliminary set database of 50 analogue structures with the highest similarities was developed. In order to evaluate this first set of molecules, over half of the structures (27) were randomly selected using a random number generator (accession numbers for these molecules are provided as *Underlying data* and their structures as *Extended data*). Two drugs were selected as references, which were searched for using the ZINC Database¹² and PubChem, then selected and saved in MOL2 format. The selected structures were Donepezil (ZINC accession number 597013),

due to its use in pharmacological therapy for mild and moderate Alzheimer's disease. Likewise, verubecestat (PubChem accession number 51352361) was developed as a candidate molecule for the selective inhibition of BACE1.

Preparation of ligands and receptor

BIOVIA Discovery Studio software version 4.5¹³ was used to correct the structures through the conversion of the format of the structure, addition of hydrogen atoms, neutralization of the charged groups, generation of ionization states, clean geometry and, finally, the conversion of the output file into its final MOL 2 format. Subsequently, the representative crystallographic structure of BACE1 (Accession number 1FKN, resolution of 1.9 Å) was obtained from Protein Data Bank (PDB)¹⁴. For the preparation of the receptor, the protein obtained in PDB format was prepared through the addition of hydrogen atoms, the elimination of solvent molecules (water) and the allocation of partial charges using the software packages UCSF Chimera version 1.13¹⁵ and BIOVIA Discovery Studio version 4.5¹⁶.

Molecular docking

The molecular docking was performed with AutoDock Vina 4.2.1¹⁷, using a PyRx working interface¹⁸. Prior to molecular docking, a virtual screening of 27 ginsenoside analogues was carried out to determine the molecules with the best structural affinity and candidates for a potential inhibitory effect of BACE1, whereby analogues were minimized by universal force field and conjugate gradients using a default algorithm based on Open Babel tools. BACE1 and ginsenosides analogue molecules were represented in a grid space of $x = 25$ Å, $y = 25$ Å, $z = 25$ Å using a universal force field. Subsequently, each docking simulation used a single ginsenoside analogue molecule and the software selected the top eight conformations with the largest change in free energy of binding, which were classified according to the energy level of each conformation and according to root-mean-square-deviation value obtained. The result of each docked molecule was determined according to the docking energy of the receptor-ligand complex. The top conformations of the structures with BACE1 were obtained in pdbqt format, using PyMOL software version 2.3.2¹⁹ for its visualization and conversion into PDB format. BIOVIA Discovery Studio version 4.5 was used to determine interaction forces and amino acids.

Results

Docking interactions between the ginsenoside analogues with the highest binding affinity with BACE1 are shown in Figure 1A–E. The six analogues show binding energies of ≥ 7.5 Kcal/mol, affinity values that are comparative to those of the reference molecules. Equally, reference drugs, inhibitors of BACE1 and of acetylcholinesterase (AChE), were used in the study are shown in Figure 2A and 2B. Different types of interactions were observed between analogues and the residues of the pocket formed in the BACE1 enzyme, such as van der Waals, H-bonding (conventional and carbon), π -sigma, alkyl and π -alkyl. Likewise, common amino acids involved in binding with ginsenosides analogues such as L30, D32, S35, Y71, T72, Q73, I110, D228, T231 and T232 were identified. The binding

Figure 1. Molecular interactions of ginsenoside analogues and β -secretase 1 (BACE1 **A**. CHEMBL451292 analogue binding to BACE1. **B**. CHEMBL510371 analogue binding to BACE1. **C**. CHEMBL503302 analogue binding to BACE1. **D**. CHEMBL201643 analogue binding to BACE1. **E**. CHEMBL448647 analogue binding to BACE1. **F**. CHEMBL508182 analogue binding to BACE1.

affinity of selected ginsenosides, the BACE1 inhibitor and the acetylcholinesterase inhibitor with BACE1 are shown in Table 1, where it can be seen that the compound CHEMBL451292, CHEMBL510371, CHEMBL503302, CHEMBL201643, CHEMBL448647 and CHEMBL508182, showed the highest energy affinity, with values of -9.6, 8.1, 7.6, 7.5, 7.5 and 7.5 Kcal/mol, respectively. On the other hand, the reference drugs

showed energies of binding 9.8 Kcal/mol for verubecestat and 8.3 Kcal/mol for donepezil. The hydrophobic residues Q12, I110, G11, T231, T232, I118, L30, G34, S35, D32, and N233 were found to strengthen the interactions between BACE1 and ginsenosides analogues through van der Waals interactions. It was also found that hydrogen bonding interactions between the active site of the BACE1 enzyme and ginsenoside analogues involved

Figure 2. Molecular interactions of a selective β -secretase 1 (BACE1) inhibitor and acetylcholinesterase inhibitor with BACE1. **A.** Verubecestat (inhibitor) binding to BACE1. **B.** Donepezil binding to BACE1.

Table 1. Molecular interactions of the BACE1 active site with the BACE1 inhibitor (Verubecestat), AChE inhibitor (Donepezil) and selected ginsenosides with highest binding energies.

Ligands	Binding energy (Kcal/mol)	Type of interaction	Interacting residues
CHEMBL451292 (A)	-9.6	Van der Waals, H-bond, π -sigma	L30, D32, G34, S35, Y71, T72, F108, I118, Y198, I226, D228, G230, T231, T232, N233, V332
CHEMBL510371 (B)	-8.1	Van der Waals, H-bond, π -sigma, alkyl, π -alkyl	Q12, L30, D32, S35, Y71, T72, Q73, F108, I110, W115, I118, D228, T232, N233, R235, G264, R307, F322
CHEMBL503302 (C)	-7.6	Van der Waals, H-bond, π -alkyl	G11, L30, D32, G34, Y71, T72, Q73, G74, F108, I110, W115, I118, D228, G230, T231, T232, N233, R235, R307, S325
CHEMBL201643 (D)	-7.5	Van der Waals, H-bond, π -sigma, alkyl, π -alkyl	Q12, G13, L30, D32, G34, S35, T71, T72, W115, Y198, I226, D228, G230, T231, N233, R307, L321, F322, S325
CHEMBL448647 (E)	-7.5	Van der Waals, H-bond	G11, G34, S35, Y71, T72, Q73, L107, I118, D228, G230, T231, N233, R235, R307, F322, S325
CHEMBL508182 (F)	-7.5	Van der Waals, H-bond, π -alkyl	G34, S35, Y71, T72, Q73, I110, Y198, I226, D228, G230, T231, T232, G264, E265, R307, L321, T324
Donepezil	-8.3	Van der Waals, H-bond, π -alkyl, π - π -T-shaped	G11, Q12, L30, D32, Y71, Q73, I110, K115, I118, G230, T232, N233, R235, G264, R307, L321, A323
Verubecestat	-9.8	Van der Waals, H-bond, π - π stacked	Q12, L30, D32, Y71, T72, Q73, G74, F108, I110, W115, I118, I226, D228, T329, T231, T232, V332

BACE1, β -secretase 1; AChE, Acetylcholinesterase.

residues D32, Q73, N233, G264, R307, R235, T72, F322, K321, T231 and T329.

Discussion

It has been reported that ginsenosides demonstrate a variety of biological activities and due to this, have been progressively included as alternative in cardiovascular, antioxidant and anticancer therapies. Their recently discovered activities at the neuronal level establish a reason for the study of these metabolites in regards to neurodegenerative alterations such as Alzheimer's disease^{4,20}, which are not fully understood at the molecular level.

For example, the study of ginsenosides as anti-Alzheimer's agents, specifically as potential BACE1 inhibitors²¹. Therefore, some authors have proposed experimental studies that indicate the potential role of these compounds as promising elements in the inhibition of BACE1 and as future molecules for the treatment of this pathology. Therefore, as an alternative to a molecular study and before the empirical approaches, *in silico* studies have been implemented to reveal the capacity binding and affinity of these metabolites and BACE1, studying the interaction and affinity energy of 27 randomly selected ginsenoside analogues.

The molecular docking experiments in this study revealed that the higher energy ginsenosides not studied previously demonstrate a considerable affinity with BACE1, specifically the analogues CHEMBL451292, CHEMBL510371 and CHEMBL503302, with binding energies of -9.6, -8.1 and -7.6 Kcal/mol, respectively. Amino acid residues such as D32, Q73, G230, N233, and R235 were identified as being associated with the formation of hydrogen bonds. Likewise, van der Waals hydrophobic interactions were established by residues L30, G34, S35, T72, F108, I110, I118, T231, T232, R307, K321 and S325, as reported in the study by Choi *et al.* 2016, which established a strong association between these interactions and the sugars of the molecular structures, which bear a great resemblance to the results obtained for the analogues with higher affinities²². On the other hand, similar interactions with the reference molecules (BACE1 and AChE inhibitors) with common residues identified in the present study and reported previously, such as D32, F108, I118, T232, N233 and K321, were demonstrated, with energy values of -9.8 and -8.3 Kcal/mol for the BACE1 and AChE inhibitors, respectively. It was indicated that the analogues CHEMBL451292 and CHEMBL510371, when interacting with BACE1, demonstrated a similar affinity to the reference drugs, with a higher affinity than donepezil and a similar affinity to Verubecestat, agents that are relevant to the current modern treatments against Alzheimer's disease. Donepezil is used in the usual treatment of the disease in its early and middle phases²³ and, despite it having been established that its mechanism of action is based on the inhibition of AchE, our study established that said molecule exhibited binding to active site residues of BACE1, as shown in Figure 2B. On the other hand, Verubecestat is an experimental drug for the treatment of early stage disease, which was developed as an inhibitor of BACE1. During its development it showed interfering activity in the formation of the amyloid cascade and in avoiding the aggregation effect of the β -amyloid plaques. However, during the clinical phase, the study was discontinued due to safety concerns about observed alterations in the liver^{24,25}.

Therefore, the use of computational tools for screening of new therapeutic agents with potential pharmacological activity

could contribute to the elucidation at the molecular level of the mechanism of action of ginsenosides, as a group of promising metabolites for the prevention and treatment of Alzheimer's disease.

Data availability

Underlying data

Figshare: Ginsenoside analogs as possible BACE1 inhibitors. <https://doi.org/10.6084/m9.figshare.8408549.v1>²⁶

This project contains the following underlying data:

- In silico study of Results_DOCKED_27LIGANDS.xlsx (analogue and drug structures listed in .xlsx format)

Extended data

Figshare: Ginsenoside analogs as possible BACE1 inhibitors. <https://doi.org/10.6084/m9.figshare.8408549.v1>²⁶

This project contains the following extended data:

- 597013_donepezil_out.pdb (donepezil structure in PDB format)
- 51352361_uff_E=743.53_out.pdbqt (verubecestat structure in PDBQT format)
- Structures of the 27 ginsenoside analogues in PDB format

Data are available under the terms of the [Creative Commons Zero "No rights reserved" data waiver](#) (CC0 1.0 Public domain dedication).

Grant information

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

Universitary Corporation Rafael Nuñez and University of Cartagena.

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Current Peer Review Status:

Version 1

Reviewer Report 22 November 2019

<https://doi.org/10.5256/f1000research.21694.r56034>

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Unfortunately, there are many issues with this paper and the underlying studies, to the extent that the authors really need to rethink the entire programme of research reported.

The authors have the very laudable objective, of identifying potential new treatments for Alzheimer's disease. They focus on identifying inhibitors of the BACE1 enzyme. This is a drug target that has been the subject of intense activity over the past 10+ years in Pharma, biotech and academic drug discovery. Many inhibitors of BACE1 have been reported in the literature and in patents together with multiple crystal structures and associated data. A quick search in ChEMBL indicates >8700 inhibitors for human BACE1 and 385 crystal structures in PDB. Furthermore, multiple compounds have progressed into clinical trials, including several into Phase III. Unfortunately, however, these have been unsuccessful, to the extent that the so-called "amyloid hypothesis" is under question.

Overall, the authors unfortunately do not appear to be aware of, let alone to have made much use, of the available data. They are vague on key aspects of their computational protocols. They do not cite relevant literature, nor do they appear to be aware of key limitations with some of their approaches. There is no experimental validation of their computational predictions. I will now address in more detail some of these issues.

1. A quick search using the ChEMBL interface suggests that there are 474 Ginsenoside analogues using the default ChEMBL similarity search method and threshold (85%). This appears to be much more than the authors report (further, "80-90% similarity" is too vague a description of their approach).
2. These 474 analogues have >4000 reported activities in ChEMBL. However, none of these are against BACE1. Thus, there is no evidence from the literature/ChEMBL that ginsenoside or its analogues bind to BACE1 (nor would there be any particular reason to believe that they are active at this target, on the basis of their chemical structure)

3. Two other drugs were selected, one a known BACE1 inhibitor and the other an AChE inhibitor. I can understand the logic for including the former, but why was the AChE inhibitor included? I cannot find any data in ChEMBL to suggest that donepezil has any activity against BACE1. Is this therefore included as a "known inactive" to provide a comparison?
4. There is a crystal structure available of verubecestat bound to BACE1 (PDB code 5hu1). The authors seem unaware of this fact and choose to use a very early BACE1 structure for their docking studies. They make no reference to this known structure when presenting the results of their own docking studies for this molecule. Moreover, the docking mode they obtain is completely different to the known crystal structure... key interactions with the catalytic asp residues are not present and indeed the whole binding mode looks to be wrong. If the authors cannot demonstrate that their docking protocol reproduces known structures I would have serious concerns about all of the subsequent work and the conclusions drawn.
5. A long-standing, and still largely unsolved problem in "docking and scoring" is that the scoring methods in current use are very rarely able to reproduce experimentally measured binding affinities. I would thus have absolutely no confidence in the "binding energies" reported by the authors. The authors provide no supporting evidence nor citations to previous work that validates their docking and scoring protocol in the context of known experimental data. The calculated free energy differences between the known inhibitor verubecestat and the AChE inhibitor donepezil from their docking calculations is ~1.5kcal/mol. This would translate to just over a 10-fold difference in binding constant. Given that verubecestat has reported pIC50 affinities in the 8.3-9.4 range, this would suggest that donepezil should be a reasonable inhibitor of BACE1. There is no literature evidence that I can find to support this. Given all of this, I can only conclude that all of the reported "free energies of binding" in this paper are wholly erroneous and moreover the docking modes on which they are based are similarly suspect.
6. Irrespective of the issues described above, any predictions made using computational methods should be tested in the laboratory. Thus, we should not be seeing papers such as this which only contain computational predictions (and certainly not in a reputable journal such as f1000). Rather, we need to see experimental validation, by testing some or all of the molecules described in an appropriate assay (possibly also supported by crystal structure determination).
7. There are also a number of smaller problems with the paper including typographical issues (e.g. the caption to figure 2 defers to "BASE1"). In view of the above significant and major problems I will not capture any more of these in this report.

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: drug discovery, computer modelling and simulation, cheminformatics, structure-based design

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 19 August 2019

<https://doi.org/10.5256/f1000research.21694.r52068>

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Zhe Wang

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Contreras-Puentes *et al.*, searched the database for the analogues of ginsenoside, and identified three compounds that strongly bound to BACE1 by structure simulation. The bindings of the compounds with BACE1 were compared with that of other known BACE1 inhibitors including verubecestat and donepezil, an acetylcholine transferase inhibitor with some inhibitory effect on BACE1 activity. BACE1 residues involved in the bindings and the forces mediating the bindings were characterized.

The compounds identified in this paper can be tested for the effects on BACE1 functions, both *in vitro* and *in vivo*. While bindings of compounds with enzymes may result in a suppression of enzyme activities, an activating effect cannot be ruled out. If the compounds do inhibit beta-cleavage of APP by BACE1, their applications in AD prevention and therapy could be explored.

A recent clinical trial showed that verubecestat, a potent BACE1 inhibitor, failed to correct AD symptoms. BACE1 is required for a number of neuronal activities, and the inhibition of BACE1 may cause severe side effects. In such a context, compounds specifically targeting beta-cleavage of

APP by BACE1 without affecting other BACE1 functions are desired, and these compounds could be useful for AD prevention. Hence, the compounds identified in this paper could also be tested for specificity.

It would be more informative if the authors could analyse the bindings of these compounds with BACE2 that is a close homolog of BACE1, and involved in APP processing and neuronal apoptosis. To our knowledge, all known BACE1 inhibitors also inhibit BACE2, despite of their higher preference for BACE1.

7.5kcal/mol was set as the energy threshold for compound screening. It would be better to justify this threshold.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Alzheimer's disease, biochemistry, cell biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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