In Silico Analysis Towards Exploring Potential β Secretase 1 (BACE1) Inhibitors; The Cause of Alzheimer Disease

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Abstract. 1,5 benzothiazepine chalcone derivative compounds were used as ligands and docked with protein target 2XFJ code from Hydrolase enzyme crystallographic structure. Molecular Operating Environment 2020.0901 (MOE) computer program was used as software to perform docking. The aim of this research is to determine the potentiality of 1,5 benzothiazepines as β secretase 1 (BACE1) inhibitors using molecular docking studies and also to predict their toxicity using SwissADME. Based on the docking results, some promising interactions were observed between 1,5 benzothiazepines and β secretase 1 (BACE1) receptors using Verubecestat as a positive control. Compounds MA2, MA4 and MA10 shown to have the potentiality as active inhibitors for the β secretase 1 (BACE1). These three compounds have binding free energy values of -4,6302 kcal/mol, -4,4268 kcal/mol, and -5,3427 kcal/mol, respectively. In addition, they also exhibited factor of binding (i.e. a measure of the probability of tested compounds to bind with the same amino acid that the positive control, Verubecestat). The predicted toxicity for these three compounds were measured using SwissADME and the results showed that MA2, MA4 and MA10 are not toxic and they can be used as reference for designing new inhibitors for β secretase 1 (BACE1).

1. Introduction

Alzheimer disease is common cause of decline in cognitive ability. This disease is caused by a gradual and slow neurodegenerative process that will lead to age-related neuronal cell death and it is characterized by amyloid accumulation, tangling of neurofibril tissue, and synaptic dysfunction. In order to prevent and stop the death of these neurons, inhibition of secretase 1 (BACE1) can be done to prevent the accumulation of -Amyloid (Aβ) [1].

β secretase 1 (BACE1) is type 1 transmembrane protein with aspartyl protease activity, it is an enzyme that plays an important role for breakdown of Amyloid Protein Precursor (APP) to release APP fragments and also to form -Amyloid (Aβ) on nerve cell membranes [2]. In Alzheimer’s patients, APP divides incorrectly, Aβ fragments remain intact and Aβ plaques accumulate around the nerve cell membrane. This is indicated that the abnormality of the APP process, thus Aβ portion protrudes from the nerve cell membrane which then causes tangling and cell death [3].

Benzothiazepine is a seven membered heterocyclic compound containing nitrogen and sulfur elements [4]. 1,5-benzothiazepine compounds have various pharmacological activities such as anticonvulsant and antidepressant [4], anti-Tobacco Mosaic Virus [5], antifungal [6], antimicrobial [7], and also as breast anticancer 8]. The first molecules of 1,5-benzothiazepine that have been used
clinically are diltiazem, and clentiazem which have cardiovascular action and also included in the Calcium Channel Blockers (CCB) antihypertensive drug class. Several of 1,5-benzothiazepine derivatives are also used clinically for disorders of the CNS, including thiazensim, clothiapine and quetiapine [9]. Therefore, it is very interesting to explore and develop the other potential biological activity of these 1,5-benzothiazepines such as for β-secretase 1 (BACE1) inhibitors (i. e. cause Alzheimer disease).

In the drug development process, docking plays an important role in drug design [9, 10]. Molecular docking has been used to search low binding free energy and also the ligand conformations at the protein-binding sites. In addition, docking is also provided useful information for predicting the orientation of drug candidate binding to the protein target.

Several studies have been conducted to find new agents against β-secretase 1 (BACE1) that can cause Alzheimer disease. Unfortunately, thus far, chalcone-derived heterocycles like 1,5-benzothiazepines have not reported for the β-secretase 1 agents especially using the in silico study. The main aim of this study is to explore the potentiality of twelve 1,5-benzothiazepine as agents against β-secretase 1 (BACE1) and also to predict the toxicity of those compounds using SwissADME application.

2. Methodology

2.1. Ligand Preparation

Structure of twelve ligand and also the positive control (verubecestat) was sketched using ChemDraw and then saved in .cdx format. These ligands are then entered one by one into the software MOE 2019.0101 (Chemical Computing Group), the data is saved in .mdb format. Table 1 are presented the molecular structures of twelve 1,5-benzothiazepine compounds and positive controls.

| Ne | Structure | No | Structure |
|----|-----------|----|-----------|
| MA1 | ![MA1](image1) | MA2 | ![MA2](image2) |
| MA3 | ![MA3](image3) | MA4 | ![MA4](image4) |
| MA5 | ![MA5](image5) | MA6 | ![MA6](image6) |

Table 1. Molecular structure of 1,5-Benzothiazepine and positive control (Verubecestat)
2.2. Protein Preparation

Three-dimensional structure of protein was download from protein databank (www.rcsb.org) with PDB ID 2XFJ. Then, the water molecules should be removed and followed with adding hydrogen atoms and energy minimization. The last stage is fixed with the energy minimization for backbone atoms and also alpha carbon using CHARMM27 (Chemistry at Harvard Macromolecular Mechanics) force field Protein. The protein was prepared using MOE 2019.0101 (Chemical computing group) software package and saved in .pdb format.

2.3. Molecular Docking

Molecular docking simulation was performed using MOE 2019.0101 (Chemical computing group) software package. A grid box of the protein structure was then achieved with a grid spacing of 1 Å, dimensions of 24.53 x 28.42 x 25.13 points along the x, y and z-axes and cantered on the protein active site. The lowest binding free energy was selected as the best conformation. Furthermore, complex of protein-ligand were minimized with gradient 0.01 kcal/mol/ Å using the same force field i.e. CHARMM27.
2.4. Adsorption, Distribution, Metabolism and Excretion (ADME) Prediction
ADME profiles of these twelve 1,5-benzothiazepine compounds were calculated using SwissADME (http://www.swissadme.ch/index.php) to get better insight into the physicochemical and pharmacokinetic properties as well as prediction of drug likeness of these twelve 1,5-benzothiazepine.

3. Results and Discussion

3.1. Molecular Docking
Molecular docking is one of the in silico method that is widely used to help the drug design process with the final goal is to discover new drugs. Docking allows the identification of novel compounds with therapeutic properties, predicting ligand-receptor interactions, as well as to describe structure-activity relationships [11]. Docking plays an important role in the rational drug design which is often used to predict the orientation and also the appropriate binding of small molecules to the sequence of target protein. In addition, docking can be used also to find the most optimal conformation for the protein-ligand complex. This is to ensure that the biological activity will match with the estimated value [12]. Furthermore, molecular docking is one of the best solution to reduce time and cost, as well as error factors in the drug design compared with the conventional drug design process. The conventional drug design is very complicated and requires a lot of time and money [13].

The best poses of complex ligand-protein were selected according to the lowest value of binding free energy and also Root Mean Squared Deviation (RMSD) value. Based on the docking results (Table 2), MA2, MA4 and MA10 estimated to be active as BACE1 inhibitor. All three of these compounds were able to binding well with the protein active site i.e. Thr133 and Gln134 [14]. The spatial arrangement of these compounds are depicted in Figure 1.

| No | Binding free energy (kcal/mol) | RMSD | Factor of binding | Hydrogen Bond | Van der Waals | Hydrophobic |
|----|--------------------------------|------|-------------------|----------------|---------------|-------------|
| MA1 | -4.9811 | 1.7587 | - | Val207, Gln153 | Glu62 | - |
| MA2 | -4.6302 | 1.8520 | Thr133, Gln134, Asn294, Ser386, Leu324, Glu326, Gly325 | Leu324 | Glu326 | - |
| MA3 | -4.4762 | 1.1854 | Gly238 | Asp241 | - | - |
| MA4 | -4.4268 | 1.0717 | Thr133, Gln134, Asn294, Ser386, Leu324, Glu326 | - | Glu326 | - |
| MA6 | -4.0194 | 0.8088 | - | - | - | - |
| MA7 | -4.2271 | 1.8234 | Glu268, Leu438 | Glu268 | Arg266 | - |
Superimposition is shown several residues play an important role in determining binding interactions between ligand and protein. The binding interaction of MA2, MA4, and MA10 with the active site of BACE1 indicated that the specificity of the amino acid residue Glu326 through the Van der Waals interaction and Lys382 through hydrophobic interaction. These interactions indicated that compound MA2, MA4, and MA10 are active inhibitors of BACE1 since they have similar features with the protein target. Superimposition of MA2, MA4, MA10 and the original ligand are shown in Figure 2.

3.2. Adsorption, Distribution, Metabolism and Excretion (ADME) Prediction
ADME profile is also known as the pharmacokinetic profile [15]. ADME can reduce the risk in the later stages of drug development and also to optimize the screening process [16]. ADME calculations related to Lipinski’s rule that can be used to predict whether a compound is toxic or not to be used as a drug candidate. The five Lipinski rules have some criteria the maximum molecular weight (MW) is 500, the octanol-water partition coefficient (log P) does not exceed five [17], the maximum number of donor hydrogen bonds is 5, the maximum acceptor hydrogen bond is 10 and also has a polar surface area less than 140. The three active compounds based on the docking results, namely MA2, MA4, and MA10 also follow the five Lipinski rules, thus all of the three compounds were worthy of being candidates for drug molecules. Table 3 presented data from SwissADME

| Compound     | Score  | MW   | P  | Donor Hbonds | Acceptor Hbonds | Polar Surface Area |
|--------------|--------|------|----|--------------|-----------------|-------------------|
| MA2          | -4.8323| 1,2501 | - | Glu62, Asp214 | Glu62, Asp214 | -                 |
| MA4          | -4.6789| 1,5523 | - | Glu62, Asp214 | Glu62, Asp214 | -                 |
| MA10         | -5.3427| 0.8605 | - | Glu326, Lys382 | Glu326, Lys382 | -                 |
| MA11         | -5.2931| 2,1583 | - | Pro152, Glu62 | Pro152, Glu62 | -                 |
| MA12         | -5.1605| 1,0430 | - | Val207, Glu62 | Val207, Glu62 | -                 |
| Verubecestat | -5.0353| 1,3711 | - | Asn294, Glu326 | Asn294, Glu326 | -                 |
| Native ligand| -7.3479| 1,4314 | - | Thr293, Glu326, Glu371, Lys382 | Thr293, Glu326, Glu371, Lys382 | -                 |
Figure 1. spatial arrangement of (a) MA2 (b) MA4 (c) MA10 (d) positive control (e) native ligand with the protein
Figure 2. Superimposition of the potential inhibitor BACE1 MA2 (green), MA4 (yellow), MA10 (blue) dan ligand asal (purple)

| Profil          | MA2     | MA4     | MA10    |
|-----------------|---------|---------|---------|
| Mw (g/mol)      | 331,43  | 375,44  | 363,90  |
| Consensus log P | 3,13    | 3,41    | 3,76    |
| Hidrogen donor  | 1       | 1       | 0       |
| Hidrogen akseptor| 2       | 4       | 1       |
| Rotable bond    | 2       | 2       | 2       |
| Druglikeness    | yes     | yes     | yes     |

4. Conclusion
Compounds MA2, MA4 and MA10 shown to have the potentiality as active inhibitors for the β secretase 1 (BACE1). These three compounds have binding free energy values of -4.6302 kcal/mol, -4.4268 kcal/mol, and -5.3427 kcal/mol, respectively. In addition, they also exhibited factor of binding (i.e., a measure of the probability of tested compounds to bind with the same amino acid that the positive control, Verubecestat). The predicted toxicity for these three compounds were measured using SwissADME and the results showed that MA2, MA4 and MA10 are not toxic and they can be used as reference for designing new inhibitors for β secretase 1 (BACE1).

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