Original Research

Search for potential Alzheimer's disease therapeutics: Identification of inhibitors of amyloid oligomerization with high affinity for the zinc-binding site

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Abstract

The progression of Aβ peptide aggregation in the brain has been suggested to play a significant role in the pathogenesis and development of Alzheimer's disease. This study is intended to provide insight into the interactions between the zinc-binding site of beta-amyloids and the zinc ion itself. The absence of zinc bonded to the beta-amyloid has been shown to potentially slow down the progression of Alzheimer's disease, so the goal is to provide an analysis of available drugs that can be repurposed and could profoundly impact Alzheimer's disease treatment. We address how and with what strength the existing compounds bind with beta-amyloid, potentially replacing or blocking zinc and preventing it from attaching to the amyloid. The analysis was performed using molecular operating environment software, which, starting from a filtered database, identified the drugs most likely to bind to the zinc-binding site on beta-amyloid.

Keywords: Amyloid beta, zinc-binding site, Lipinski rule-of-five, molecular docking, Alzheimer's disease, computational prediction methodology

1. Introduction

1.1. Alzheimer's disease

Alzheimer's disease (AD), which is characterized by the loss of brain function, especially memory loss, is classified as a progressive, neurodegenerative disease that affects the cholinergic regions of the central nervous system (CNS), associated with cognitive function and spatial awareness. It is the most common cause of dementia, with an estimated 50% to 56% cases in autopsy and clinical series. The disease starts insidiously, with age being the principal risk factor. Symptoms typically begin with memory deficits, progressing to other cognitive domains with death, usually in 10 years from diagnosis.

1.2. The role of Aβ in Alzheimer's disease

Aggregation of Aβ peptide in the area surrounding neurons in the brains of AD patients has been known progression of this neurodegenerative disease plays a role in its pathophysiology. This process further leads to the formation of senile plaques, neurofibrillary tangles, neuronal cell death, and progressive dementia, causing a debilitating loss of cognitive function and, ultimately, a shorter lifespan. Aβ peptide has been identified, together with MAP-t, as the principal target for therapeutic interventions that, when controlled using pharmacological inhibitors, could lead to an efficacious treatment of AD. This goal has been so far very elusive. However, it has been determined that while the monomeric form of Aβ is relatively non-toxic when Aβ becomes aggregated into amyloid plaques, it causes neurodegenerative changes as is the case with other neurodevelopmental disorders (NDDs) such as the neuromotor Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). It has been established that the aggregated Aβ has toxic

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effects on neurons and the degree of cognitive impairment in AD is closely correlated with the amount of Aβ oligomer accumulation rather than just the total amount of Aβ. It has been concluded that two processes result in the accumulation of Aβ, namely an increase in its production coupled with a decrease in its degradation rate (Lokesh et. al., 2023).

1.3. Amyloid beta peptide

Amyloid beta peptide is a 42-amino acid peptide derived from the precursor protein, Amyloid Beta Precursor Protein (APP). The APP is a transmembrane glycoprotein that spans the membrane thickness once. It is expressed in many tissues, especially in the synapses of neurons, which plays a central role in AD pathogenesis. The gene for APP is on chromosome 21. It is cleaved by beta and gamma-secretase to release Amyloid Beta Peptide containing 40 or 42 amino acids, denoted as Aβ40 or Aβ42, respectively. Beta secretase act on APP to produce the N terminal, and Gamma secretase to produce the C terminal of the peptide. The peptide Aβ42, the main pathogenic peptide, is highly hydrophobic and tends to aggregate into oligomers and fibrils. The hydrophobic amino acids confer the hydrophobicity in the C terminal of the peptide. These fibrils are then arranged in a beta-pleated sheet and form amyloid plaques (Wang et.al., 2019)

1.4. Beta-amyloid 40 VS Beta-amyloid 42

Beta-amyloid 40 and 42 are peptides that play a crucial role in the development of AD. Beta-amyloid 40 peptide consists of 40 amino acids. It is more abundant in the brain compared to beta-amyloid 42. Aβ40 is generally considered less prone to form aggregates or plaques, one of the hallmarks of AD. Meanwhile, beta-amyloid 42 peptide is very similar to Aβ40 but is two amino acids longer, consisting of 42 amino acids. It has two additional hydrophobic residues (Ile41 and Ala42) at the C-terminus. Aβ42 is more prone to aggregate and form insoluble plaques in the brain. These plaques can disrupt communication between neurons and lead to neurodegeneration. Aβ42 is a minor component in human cerebrospinal fluid and plasma found in approximately a 1:10 ratio to the Aβ40 form. Nevertheless, Aβ42 is found three times more often in the senile plaques, characteristic of the AD cortex (Iwatsubo et.al., 1994).

The shorter and more prevalent Aβ40 form, on the other hand, seems to play a more important role in the later stages of plaque formation, as evidenced by a strong correlation between the detection of Aβ40 and the maturity of the plaques. *In vitro* experiments have clearly demonstrated that Aβ42 polymerizes much faster than Aβ40 (Jarrett et al., 1993). The amyloid hypothesis, which has been a dominant theory in Alzheimer's research, posits that the accumulation of Aβ42 and its aggregates are a key trigger in the development and progression of AD. According to this hypothesis, the abnormal accumulation of Aβ42 sets off a cascade of events that ultimately lead to neuroinflammation, oxidative stress, and the formation of neurofibrillary tangles (another hallmark of AD).

It is important to note differences in the aggregation propensities of various peptides due to their different fibril structures. Aβ42 has been found through *in vitro* studies to exhibit a higher propensity to form amyloid fibrils, and it is associated with the generation of different polymorphs that represent rearrangements of its molecular structure. As a result of numerous experimental investigations Aβ40 amyloid fibrils are known to support a molecular U-shape conformation composed of two parallel β-sheets linked by a short peptide loop. Similarly, the Aβ42 fibril has been found to exhibit a structure that features two β-strands linked by a hairpin loop, resulting in the Aβ42 U-shape structure. Recently, ssNMR assays have determined that the Aβ1-42 fibril could also exist in an S-shape rather than a U-shape form (Grasso et al., 2018). Molecular modeling using sophisticated methods such as the replica-exchange molecular dynamics (REMD) has examined the stability of S-shape Aβ42, comparing the two main conformations of Aβ42 fibrils, the U-shape and S-shape. These investigations concluded that the S-shape structure offers greater conformational stability than other Aβ42 structures and found this outcome to be determined by the strong interactions involving the peptide's C-termini. Conversely, the U-shaped structure has been computationally found to contain major conformational distortions, which cause disordered assembly into oligomers. A more specific molecular explanation of the effect hinges on forming the intra-chain salt bridge connecting the side chain of residues Lys28 and Ala42. On the other hand, the S-shape structure forms only a partial distortion in the N-terminal region (involving residues Leu17 to Asp23).

This conformation is further stabilized by the hydrophobic contacts in Aβ42 that involve the residues Ile41 and Ala42 in the C-terminal region of the peptide, as compared to Aβ1-40 in which this is absent. We can conclude from these studies that the Aβ42 S-shape fibril provides a structure with the greatest stability. This is mainly generated by the formation of inter-chain hydrophobic contacts and hydrogen bonds between residues Ile41 and Ala42 in the C-terminus (Grasso et al., 2018).

1.5. Amyloid plaques

The cleavage of β-amyloid Precursor Protein (APP) by α- or β-secretase, which directs the protein to either the amyloidogenic or non-amyloidogenic pathways, respectively, determines whether Aβ is produced. The normal pathway of APP metabolism involves hydrolysis by α-secretase following the amyloidogenic pathway. The other pathway involves APP hydrolysis by β-secretase to generate sAPPβ followed by γ-secretase to produce Aβ. In healthy neurons, the Amyloid Precursor Protein, which has three domains (in the cell, in the cell membrane, and outside the cell), is digested by alpha and gamma-secretase enzymes. This digestion reaction produces some soluble polypeptides that can be broken down and recycled later in the cell. However, when the beta-secretase teams up with the gamma-secretase, the scenario goes the wrong way. This digestion reaction produces an insoluble peptide known as amyloid-beta. Amyloid-beta peptides clump together and form beta-amyloid plaques (ABP), which are deteriorative for the cell. The Aβ plaques and oligomer are potent synaptotoxins that block proteosome function, alter intracellular Ca^{2+} levels, restrain mitochondrial activity and activate the inflammatory processes (Ashrafian et.al 2021).

1.6. Effects of the occurrence of Amyloid beta plaques

The emergence of ABPs is correlated with three detrimental issues plaguing neurons in their vicinity. When ABPs are localized between two healthy neurons, their presence may lead to a disruption of the signaling process involved in neuron-neuron communication. When such neuronal signaling is impaired, this may lead to a serious loss of brain function, e.g. memory. Moreover, as recently reported in the literature, ABP could trigger an immune response with an associated inflammation, the consequence of which is often damage to the surrounding neurons. Frequently, ABPs are formed in the outer layer surrounding blood vessels in the brain in a process called angiopathy. Its result is commonly hemorrhage or even the rupture of blood vessels in the patient's brain (Ashrafian et.al., 2021).

1.7. Interaction with cations of Zinc

In most cases of amyloidogenic protein aggregation taking place during NDDs, their interactions with the cations of transition metals play a major role in their conformational transformations. These processes involve zinc, copper, and iron, which bind to the target proteins, affecting their functioning due to the associated conformational transitions. While Zn(II) and Cu(II), have been found to be abundant in the brains of healthy persons, they are normally only weakly bound to proteins. This changes markedly in an age-dependent manner in the brains of the elderly. It has been strongly suggestive because of the accumulated empirical evidence that interactions of Aβ with transition metal ions lead to, or even initiate, Aβ aggregation. Its downstream effects include neurotoxicity and the dysregulation of copper and zinc homeostasis in the brain, all of which have been linked to AD initiation and progression.

This is the basis of the hypothesis about the primary role of metal ions in the development of AD and has, therefore, led to the formulation of a therapeutic approach to managing AD based on the chelation of these ions. To support this statement, elevated Zn(II) levels have been seen in AD brains and enriched in amyloid plaques. Also, it is well-known that Aβ coordinates these metal ions within the plaques.

Further, *in vitro*, assays demonstrated that the binding of zinc ions to Aβ causes Aβ aggregation, while metal chelators cause the dissolution of aggregated Aβ. In fact, adding Zn(II) to Aβ40 solutions has reported the resultant precipitation of the peptide within milliseconds (Tõugu & Palumaa, 2012).

In this paper our aim is to find potential inhibitors of Aβ oligomerization by blocking the binding of Zn(II) ion using small molecules. We have used computational prediction methodology whose results and specific techniques are reported below.

2. Results

2.1. Drugs obtained from the ZINC 20 database

After filtering the ZINC 20 database with the keywords 'zinc-binding site', a database of 100 drugs was generated. All these drugs are commercially available and can bind to the zinc-binding sites of all proteins that have one.

2.2. Target selection

The role of the specific target is attributed to amyloid beta, the protein strongly associated with AD. The biological relevance of the target and its role in the disease process is confirmed since the amyloid hypothesis suggests that the accumulation of Aβ plaques in the brain is a primary event in AD pathogenesis. The experimentally determined 3D structures were obtained from the Protein Data Bank (PDB). Three structural models were found: the structural model of a 40-residue beta-amyloid fibril S-shape (6TI5), the structural model of a 42-residue beta-amyloid fibril S-shape (50QV) and the structural model of a 42-residue beta-amyloid fibril U-shape (2BEG). All three models come from *homo sapiens*. Using the Molecular Operating Environment (MOE) software, the structures of beta amyloids were visualized (**Figure 1**). Afterward, only the S-shape structures were analyzed, as the U-shape structure suffers from instability.

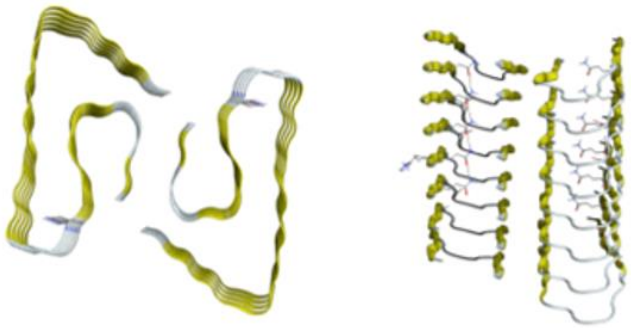

Figure 1. Visualization of beta-amyloid 42 S-shape (on the left side) and beta-amyloid 40 (on the right side) on MOE.

2.3. Definition of the zinc-binding site

Aβ can bind metal ions using several residues, including the N-terminal amine, the side chains of the carboxylic acid residues at positions 1 (Asp), 3(Glu), 7 (Asp), and 11 (Glu), and the side chains of the three His residues at positions 6, 13, and 14 (**Figure 2**) (Miller et al., 2010). These residues are all located in the 1-16 region, which is found near the central hydrophobic core (residues 17 to 21) involved in Aβ dimerization (first step of the aggregation), and thus binding of metal ions can modulate the aggregating properties of Aβ. Using MOE software, the zinc-binding sites of beta amyloids were highlighted in pink (**Figure 3**).

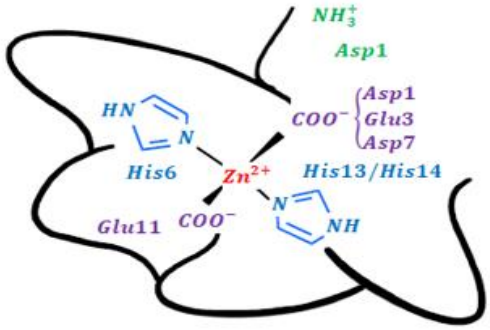

Figure 2. The zinc-binding site on Aβ.

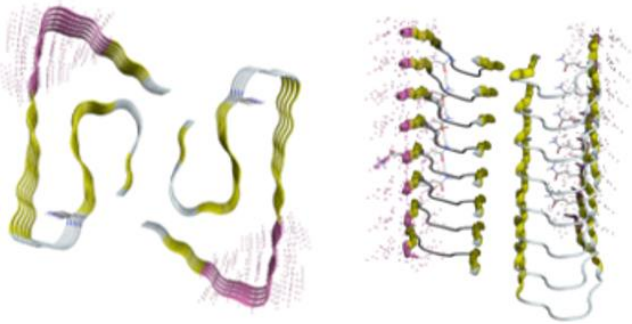

Figure 3. Visualization of the zinc-binding site, in pink, of beta-amyloid 42 S-Shape (on the left side) and of the zinc-binding site, in pink, of beta-amyloid 40 (on the right side) on MOE.

2.4. Pre-filtering

To reduce the number of compounds for further analysis, the pre-filtering was executed using MOE software. First, Lipinski's rule-of-five was considered. Following the rule, the molecular weight must be less than 500 Da to increase the permeability into the central nervous system, the logarithm of the n-octanol-water partition coefficient must be less than 5, which indicates satisfactory lipid solubility, the number of hydrogen bond donors must be less than 5 and of hydrogen bond acceptors less than 10, otherwise the penetration of biological membranes could be very difficult and reactivity too high, which would lead to promiscuous behavior. Furthermore, the extension of Lipinski's rule-of-five, called Veber's rule, was considered. The low polar surface area of less than 140 superscript base, Å, end base, to the bold 2, and reduced molecular flexibility where the number of rotatable bonds is less

than 10 are found to be important predictors of good oral bioavailability. Then, the properties of the molecules able to penetrate the blood-brain barrier were examined. A necessary but not sufficient condition is the polar surface area of the compound being smaller than 90 $Å^2$. The marketed orally active CNS drugs have a mean molecular weight value of 377 Da. The blood-brain barrier (BBB) penetration is optimal when the Log P values are in the range of 1.5 and 2.7 with the limit of the acid dissociation constant that varies between 4 and 10, number of rotatable bonds less than 5, hydrogen bond donors less than 3 and hydrogen bond acceptors less than 7. After considering both the Lipinski rule-of-five, and the Veber rules ,well as the properties of CNS active drugs, the parameters used for the computational pre-filtering in this project were implemented as: the molecular weight less than 450 Da, the number of hydrogen bond donors less than 3, the number of hydrogen bond acceptors less than 7, the number of rotatable bonds less than 8, the polar surface area less than 70 $Å^2$ and the acid dissociation constant in the range between 7.5 and 10.5. After this pre-filtering, only 13 drugs were retained

2.5. Virtual screening and docking

The Virtual Screening methodology was used as a computational technique to predict how well a given compound is likely to bind to Ab. The structure-based strategy that utilizes the information about the 3D structure of the protein target to identify small molecule ligands with high binding affinity to the target was applied. The structures of the target protein and ligand are known, which allows us to use a structure-based type of drug-discovery research This enrichment approach allows us to design a drug that fits its target very well. Molecular Docking was utilized to virtually screen the 13 drugs obtained from the previous step for potential drug candidates. Once the structure of a protein is available, the docking algorithms can be used to place the ligand and predict its most probable binding configuration to form a stable 3D protein-ligand complex. The ligands, in this case, come from the filtered database, while the receptor is the zinc-binding site. The sequence editor (SEQ button) is opened to select a specific group of atoms. After the application of 30 poses and 5 scores, two databases of 65 elements each (one for amyloid beta 1-40 and one for amyloid beta 1-42) were obtained.

2.6. Sorting

The sorting of databases using the ascending mode was applied to choose the ones with the best energy score (S). Subsequently, the top three drugs with the most negative binding energy were considered. The drugs obtained from the Molecular Docking of the Aβ40 are ZINC000000003812 and ZINC000000073940 (in two different poses). The first one is 2,6-Ditert-butyl-4-(pyrazin-2-ylamino)phenol, an orphan drug. The second one is 1-(piperidin-1-yl) propan-2-yl2,3-dihydro-1,4-benzodioxine-6-carboxylate, available for purchase from several chemical vendors. The drugs obtained from the Molecular Docking of the Aβ42 are ZINC000000073940 and ZINC000000072029 (in two different poses). The first one is the same drug obtained from the Aβ40 Docking. The second one is 3 - [(R) - (4-methoxyphenyl) - (4-methylpiperidin-1-yl) methyl] -2-methyl -1H-indole, available for purchase from several chemical vendors.

2.7. Comparison between drugs

A comparison between the drugs obtained is made in **Table 1**. Three defined ranges of Log P values are classified as low, moderate, and relatively high. The drug with a Log P of 4.5 has moderate hydrophobicity and is moderately soluble in water and organic solvents. The drug with Log P equal to 1.0719 has greater hydrophilicity, less efficient distribution in fat tissue and greater water solubility. Finally, the drug with the highest Log P is quite lipophilic and it might be well distributed in adipose tissues and has a higher affinity for lipophilic sites of action. Its elimination might take longer, however, as it might be more bound to plasma proteins. Generally, a drug with more rings may be more structurally complex than one with fewer rings. Structural complexity may influence chemical synthesis, stability, and susceptibility to chemical changes in the body. Molecules with more rings may be bulkier and have a different molecular shape than molecules with fewer rings. This may affect the ability of a drug to interact with specific binding sites on target proteins or other biological components. The number of rings can also influence the bioavailability of a drug, i.e. how quickly the drug reaches the systemic circulation after intake. More complex molecules with more rings may be more likely to undergo metabolism processes before reaching systemic circulation. Also, the number of rings may influence the ability of a molecule to cross cell membranes. A drug with the highest polar surface area (PSA) value (58.04 $Å^2$) tends to be more polar and, consequently, may be more soluble in polar solvents such as water. On the other hand, a drug with the lowest PSA value (29.46 $Å^2$) is generally less polar and may be less soluble in water. Solubility and the PSA value influence the absorption of a drug. Drugs more soluble in water may be more easily absorbed from the gastrointestinal tract, while less soluble drugs may require special delivery strategies or formulations. Solubility also affects tissue distribution. Drugs with a higher PSA value may diffuse more easily into water-based tissues, while

those with a lower PSA may have more limited distribution. The hydrogen bond acceptor number in a drug can influence several properties, including its interaction with proteins in the body. A drug with more hydrogen bond acceptors may have a higher affinity for proteins that establish such bonds and may also make the drug more soluble in polar solvents such as water. The number of donor hydrogen bonds can influence the drug's interaction with target proteins. Drugs with 2 donor hydrogen bonds may establish more such interactions, while those with 1 will establish fewer. In addition, drugs with 2 donor-hydrogen bonds may be more soluble than those with 1 donor hydrogen bonds.

2.8. Comparison between energy scores

Using MOE software, it is possible to obtain the energy score between the zinc ion and the two β amyloid structures taken into consideration, to make a comparison with the energies previously obtained as shown in **Table 2 and Table 3**.

Table 1 Comparison between the drugs: ZINC000000003812, ZINC000000073940 and ZINC000000072029.

Properties	ZINC000000003812	ZINC000000073940	ZINC000000072029
Molecular weight (Da)	299.418	306.382	349.498
Log P	4.5208	1.0719	9.9846
Heavy atoms count	22	22	26
Rotatable bond count	4	5	4
Number of rings	2	3	4
Polar surface area ($Å^2$)	58.04	49.2	29.46
H bond acceptors count	4	5	3
H bond donors count	2	1	2

Table 2. Best energy scores (kcal/mol) obtained after docking between the beta-amyloid 40 and the top three drugs (ZINC000000003812 and two poses of ZINC000000073940) identified between the same protein with the zinc ion.

Binding Energy Score (kcal/mol)	Beta-Amyloid 40
ZINC000000003812	-6.2622
ZINC000000073940	-6.1805
Zn 2+	-1.9544

Table 3. Best energy scores (kcal/mol) obtained after docking between the beta-amyloid 42 S-shape and the top three drugs (ZINC000000073940 in two poses and ZINC000000072029) identified and between the same protein with the zinc ion.

Binding Energy Score (kcal/mol)	Beta-Amyloid 42 S-shape
ZINC000000073940	-5.1921
ZINC000000072029	-5.2767
Zn 2+	-2.0925

3. Materials and Methods

3.1. Software MOE and database

In this work, the Molecular Operating Environment (MOE) 2020.09 was utilized for 3D molecular visualization, filtering, and docking. The database chosen to extract the molecules of interest was ZINC 20, which contains commercially available chemical compounds prepared specifically for virtual screening.

3.2. Virtual screening

Virtual screening (VS) is a computational technique used in drug discovery and design to identify potential drug candidates from a large database of chemical compounds. It involves using computer algorithms and molecular modeling tools to predict how well a given compound will likely bind to a target protein or biological macromolecule associated with a specific disease. VS allows one to extract a few particularly interesting molecules from a big database of chemical compounds. In most cases, it would be too time-consuming to be carried out on millions of compounds. The first stages of VS usually include some form of filtering, and docking is performed only once the number of compounds has been reduced.

Initially, libraries are ‘‘pre-filtered’’ using a series of simple physicochemical descriptors to eliminate compounds that are not expected to become suitable as drugs. Pharmacophore analysis, neural nets, similarity analysis, scaffold analysis, and Lipinski’s rule-of-five are used at this stage. This procedure, which reduces the size of the library to a group of molecules more likely to bind the target receptor, is known as enrichment.

There are two main categories of computational techniques for VS: Ligand-based Drug Design (LBDD) and Structure-based Drug Design (SBDD) methods (**Table 4**). The LBDD approach uses information about the known ligands (small molecules that bind to the target) to predict the likelihood of a new compound binding to the target. It relies on similarity measures, such as 2D or 3D molecular fingerprints, to compare the new compounds to known active compounds. The SBDD approach relies on the 3D structure of the target protein, which can be obtained through experimental techniques such as X-ray crystallography or NMR spectroscopy. Computational docking algorithms are used to predict how well a compound is likely to bind to the target's active site.

Table 4. Differences between Structure-based Drug Design and Ligand-based Drug Design.

	Known ligand(s)
Known protein structure	Structure-based drug design (SBDD): Protein-ligand docking
Unknown protein structure	Ligand-based drug design (LBDD): Similarity searching Pharmacophore searching Quantitative structure-activity relationships (QSAR)

3.3. Lipinski's rule-of-five and Veber's rule

The Lipinski rule-of-five is an empirical set of rules for small molecule design and, principally, for those administered orally. The rule was formulated by *Christopher A. Lipinski* in 1997 based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules. To develop a set of computational tools that would best predict compound physicochemical properties, it was important to understand the range of the critical compound properties that best correlated with the survival or failure of compounds in clinical assessments. These molecular properties are the molecular weight, the lipophilicity, the hydrogen-bond donors, and the hydrogen bond acceptors.

The molecular weight (MW) shows the density, size, and volume, as well as the mass of a therapeutic agent. Reduced permeability in the gut and into the central nervous system can be correlated with increasing MW (Manuel, 1996).

Lipophilicity is a physicochemical property commonly considered to be highly relevant to the rate of absorption that describes the ability of a molecule to partition into octanol versus water (Testa et.al., 1996). The n-octanol-water partition coefficient P is a partition coefficient for the two-phase system consisting of n-octanol and water. P serves as a measure of the relationship between lipophilicity (fat solubility) and hydrophilicity (water solubility) of a substance. The value is greater than one if a substance is more soluble in fat-like solvents such as n-octanol, and less than one if it is more soluble in water. Log P is positive for lipophilic and negative for hydrophilic substances or species. In addition to high molecular weight and lipophilicity, even the innumerable amount of hydrogen-bond donor groups in a compound can decrease the ability of a molecule to permeate a membrane bilayer (Paterson et. al., 1994).

An orally active drug-like compound should satisfy most, if not all, of the criteria formulated in the Lipinski rule-of-five: 5 or fewer number of H- bond donors, 10 or fewer H-bond acceptors, a molecular weight (MW) up to 500 Da, and octanol-water partition coefficient (Log P) equal to 5 or less (Turner & Agatonovic-Kustrin,2007). To improve the predictions of drug-likeness, the rules have spawned many extensions, such as Veber's Rule. The polar surface area and the number of rotatable bonds are important predictors of good oral bioavailability (Daniel et al., 2002). The polar surface area (PSA) or topological polar surface area (TPSA) of a molecule is defined as the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, also including their attached hydrogen atoms. PSA is a commonly used medicinal chemistry metric for optimizing a drug's ability to permeate cells. Molecules with a polar surface area greater than 140 $Å^2$ tend to be poor at permeating cell membranes. The number of rotatable bonds (RBN) is the number of bonds that allow free rotation around themselves. They are defined as any single bond, not in a ring, bound to a nonterminal heavy atom. Excluded from the count are amide C-N bonds because of their high rotational energy barrier (Daniel et. al., 2002). Reduced molecular flexibility, as measured by the number of rotatable bonds (10 or fewer), and low polar surface area (no greater than 140 $Å^2$) are found to be important predictors of good oral bioavailability, independent of molecular weight (Daniel et. al 2002).

3.4. BBB penetration

Lipinski's rule-of-five raised awareness of the importance of ADME (absorption, distribution, metabolism, and elimination) and physicochemical properties for the success of drug discovery (Zhang & Wilkinson, 2007). It is critical to use the appropriate descriptors, as the presence of BBB reduces the effective drug delivery to the brain and the potential benefit coming from the administration of the medication. Only small molecules are generally able to penetrate the BBB, therefore a necessary but not sufficient condition is the surface area of the compound being smaller than 80 $Å^2$. The upper limit for PSA for a molecule to penetrate the brain is around 90 $Å^2$. Furthermore, CNS (Central Nervous System) drugs have significantly reduced molecular weights compared with other therapeutics. For marketed CNS drugs, the mean value of MW is 310, compared with a mean MW of 377 for all marketed orally active drugs (Leeson & Davis, 2004). Lipophilicity is another descriptor identified as important for CNS penetration. For several classes of CNS active substances, it was found that blood-brain barrier penetration is optimal when the Log P values are in the range of 1.5-2.7, with a mean value of 2.1 (Hansch et al., 1977). Moreover, it was demonstrated that possessing a positive charge at pH 7–8 tends to favor brain permeation (Clark, 2003). Strong bases and acids are thereby pretty much precluded from BBB penetration. The established limit of the acid dissociation constant (pKa) varies between 4 and 10 (Fischer & Gottschlich, 1998). CNS drugs have significantly fewer rotatable bonds than other drug classes. Most centrally acting compounds have rotatable bond count of five or less (Leeson & Davis, 2004). Furthermore, compounds with high hydrogen bond-forming potential have minimal distribution through the BBB (Pardridge, 1998). Increasing hydrogen bonding decreases BBB penetration. The limits of the hydrogen bond donor count less than 3 and

hydrogen bond acceptor count less than 7 were accepted thanks to the results obtained from the Wenlock marketed drug dataset (Wenlock, 2003).

3.5. Docking and sorting

After the execution of Lipinski's rule-of-five, 13 drugs were obtained, and docking was applied between the new database and the zinc-binding site of both β amyloid 40 and 42. The zinc-binding site, whose identification is based on the literature, results from the selection of His6 - His14 residues, both in Aβ42 and Aβ40. After the application of 30 poses and 5 scores, two databases of 65 elements each were obtained. Afterward, the sorting of databases using the ascending mode was applied to choose the top 3 compounds with the best energy score (S).

The drugs are compared according to their main characteristics: Log P, heavy atom count, ligand efficiency (LE), rotatable bond count, number of rings, Fsp3, polar surface area, hydrogen bond acceptors' count and hydrogen bond donors' count. The n-octanol-water partition coefficient is frequently referred to by the symbol P. Values for Log P typically range between -3 (very hydrophilic) and +10 (extremely lipophilic/hydrophobic)[1]. In the early stage of the drug discovery process, lipophilicity is often ranked as one of the most important physicochemical properties to screen lead compounds (Manuel et.al., 1996). It is an important characteristic of any chemical because it largely determines a chemical's fate inside a living organism[2]. According to Lipinski's rule-of-five, an oral drug should have a Log P value of less than 5, ideally between 1.35 and 1.8, for good oral and intestinal absorption.

The heavy atom count of a molecule is defined as the total number of non-hydrogen atoms within the chemical structure[3]. The ligand efficiency is defined as the binding free energy of the ligand-target complex divided by the number of heavy atoms (Manuel et.al., 1996). For rotation angle counting any non-ring single bond connected to a non-terminal, non-hydrogen atom is included in the score except for the C-N amide bonds due to their high barrier to rotation[4]. A higher number of aromatic rings has a negative impact on solubility, while increasing protein binding, CYP450 inhibition, and hERG inhibition, which is not simply due to changes in size or lipophilicity (Daniel et. al 2002; 2015-2023). It was concluded that the fewer the number of aromatic rings contained in an oral drug candidate, the more likely that candidate is to be developed into a drug entity. In addition, more than three aromatic rings in a molecule correlate with poorer compound developability and, thus, an increased risk of attrition in development. It is demonstrated that even within a defined lipophilicity range, increased aromatic ring count leads to decreased aqueous solubility (Daniel et. al., 2002).

Regarding Aβ 40, the first drug obtained is ZINC000000003812 (orphaned), with the molecular formula C18H25N3O (**Table 5** and **Figure 4**). As far as this drug is concerned, it has few known characteristics.

Table 5. Chemical characteristics of ZINC000000003812.

Properties	ZINC000000003812 (C18H25N3O)
Molecular weight (Da)	299.418
LogP	4.5208
Heavy atoms count	22
Rotatable bond count	4
Number of rings	2
Polar surface area (Å^2)	58.04
Hydrogen bond acceptors count	4
Hydrogen bond donors count	2

[1] Partition Coefficient Lipinski's (2024, April 17) in Wikipedia. https://en.wikipedia.org/wiki/Partition_coefficient.

[2] Understanding Lipinski's rule-of-five (2024, May 28) in Wikipedia. https://en.wikipedia.org/wiki/Lipinski%27s_rule_of_five

[3] Ibid.

[4] Ibid.

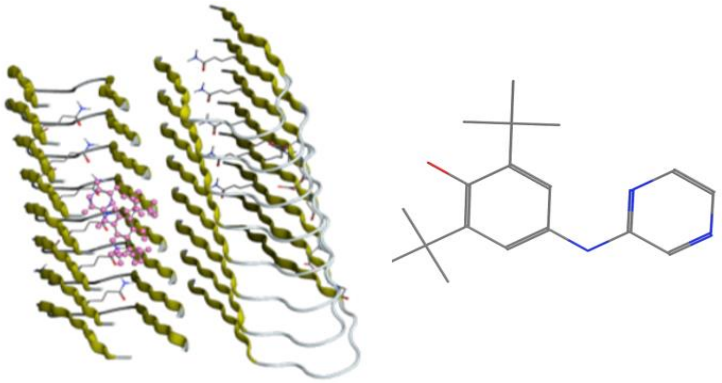

Figure 4. Beta amyloid 40 linked to ZINC000000003812 (on the left side) and chemical structure of the drug ZINC000000003812 (on the right side).

Fsp3, the fraction of carbon atoms that are sp3 hybridized, is a 2D descriptor used as a surrogate for three-dimensionality. It is expressed as a value between zero and one. Compounds with higher values of Fsp3 appear to exhibit higher solubility, lower melting points, less promiscuity, less protein binding, and less CYP450 inhibition[5].

The polar surface area of a molecule is defined as the surface sum over all polar atoms or molecules, primarily oxygen and nitrogen, including their attached hydrogen atoms. PSA is a commonly used medicinal chemistry metric for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared ($Å^2$) tend to be poor at permeating cell membranes. For molecules to penetrate the blood–brain barrier, a PSA less than 90 $Å^2$ is usually needed (Daniel et. al 2002).

The hydrogen bond acceptors' count is the total number of NH and OH bonds (Timothy et al., 2009). It is generally assumed that hydrogen bond donors and acceptors impact passive diffusion across cell membranes, a fundamental event during drug absorption and distribution (Daniel et. al., 2002) The hydrogen bond donors' count is the total number of N or O atoms[6].

After docking, two databases with 65 drugs each were obtained (one for Aβ1-40 and one for Aβ42) and then sorted by increasing binding energy. Then, the first three drugs with the most negative binding energy are considered.

The second and third drug is ZINC000000073940, represented in 2 poses, with molecular formula C17H23NO4 (**Table 6** and **Figure 5**).

Table 6. Characteristics of ZINC000000073940.

Properties	ZINC000000073940 (C17H23NO4)
Molecular weight (Da)	306.382
LogP	1.0719
Heavy atoms count	22
Rotatable bond count	5
Number of rings	3
Polar surface area ($Å^2$)	48
Hydrogen bond acceptors count	5
Hydrogen bond donors count	1

[5] Molecular Descriptors Ligand Efficiency Metrics Table, HA (Number of heavy atoms) (2024) in *RGD Science*. https://www.rgdscience.com/index.php/molecular-descriptors-ligand-efficiency-metrics/

[6] Polar surface Area (2024, April 10) in Wikipedia. https://en.wikipedia.org/wiki/Polar_surface_area

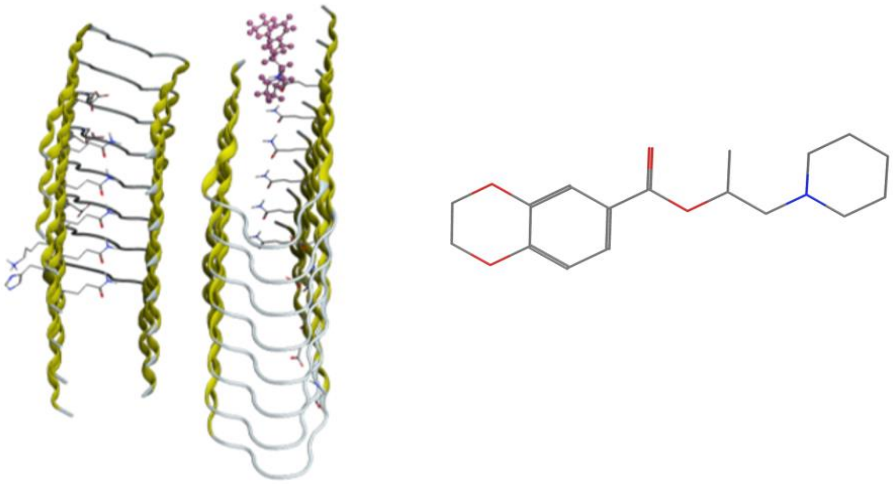

Figure 5 Beta amyloid 40 linked to ZINC000000073940 (on the left side) and chemical structure of the drug ZINC000000073940 (on the right side).

Regarding Aβ 42, the first tree drugs after sorting are taken into consideration. The first drug is ZINC000000072029 with molecular formula C23H28N20 (**Table 7** and **Figure 6**). The second and third drug is ZINC000000073940 in two different poses, with molecular formula C17H23NO4 (**Table 6** and **Figure 7**).

Table 7. Characteristics of ZINC000000072029.

Properties	ZINC000000072029 (C23H28N2O)
Molecular weight (Da)	349.498
LogP	3.9846
Heavy atoms count	26
Rotatable bond count	4
Number of rings	4
Polar surface area ($Å^2$)	29.46
Hydrogen bond acceptors count	3
Hydrogen bond donors count	2

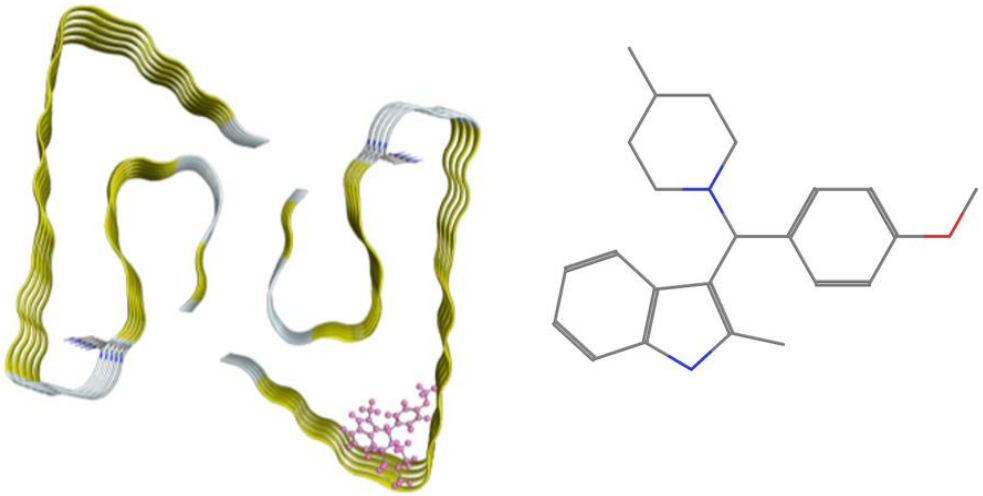

Figure 6. Beta amyloid 42 S-shape linked to ZINC000000073940.

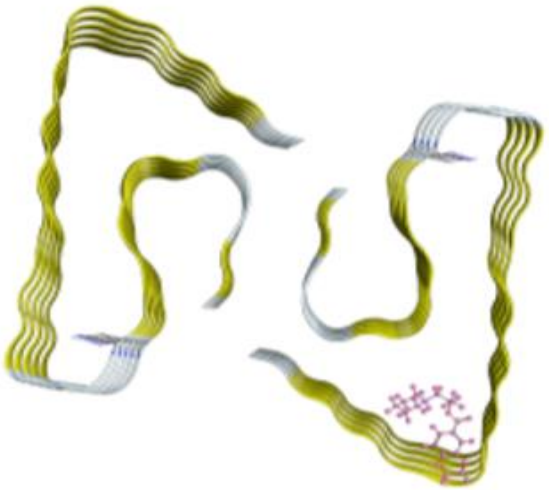

Figure 7. Beta amyloid 42 S-shape linked to ZINC000000073940.

4. Discussion

From the data that are highlighted in **Table 2** and **Table 3**, it appears that the drug-amyloid binding energy value is more negative (or higher in modulus) than the zinc-amyloid binding energy. Although this is a rather weak consideration, it can be inferred that the drug can replace zinc in the binding site on the amyloid peptide. If so, the substitution of the drug for zinc could block the formation of amyloid plaques between neurons. This consideration must be taken cautiously as we are not provided with sufficient information about the analyzed compounds from *in vitro* or *in vivo* assays.

It is important to also consider another type of drug that can help to block the zinc ion in Alzheimer's disease but in a different way. PBT2 is a safe-for-human-use zinc ionophore and an experimental drug candidate. It is a second generation 8-hydroxyquinoline analog intended to be a successor to clioquinol as a potential drug for the treatment of Alzheimer's disease and Huntington's disease. PBT2 is a metal protein-attenuating compound (MPAC) being developed PBT2 prevents Aβ accumulation and concurrently restores copper and zinc homeostasis in neurons. While an increase in the metal levels in the aging brain accelerates plaque formation, PBT2 is aimed to significantly translocate copper and zinc ions into neurons from their extracellular surroundings, hence preventing or slowing down metal-mediated Aβ aggregation. Therefore, PBT2 acts as a Cu and Zn ionophore, not as a chelator. In accordance with what has been analyzed in this report, it is possible that, in the future, these drugs may complement the use of PBT2 in treating AD. It is worth noting that PBT2 is based on a zinc-scaling mechanism, unlike the mechanism on which the drugs under study in this project are based, which mechanism is substitution. Based on what has just been stated, it is impossible to compare PBT2 and the drugs we have analyzed, as they are characterized by different mechanisms of action.

Data Availability Statement: All the data used in this paper can be provided upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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