In silico repurposing of antipsychotic drugs for Alzheimer’s disease

Shivani Kumar, Suman Chowdhury and Suresh Kumar*

Abstract

Background: Alzheimer’s disease (AD) is the most prevalent form of dementia and represents one of the highest unmet requirements in medicine today. There is shortage of novel molecules entering into market because of poor pharmacokinetic properties and safety issues. Drug repurposing offers an opportunity to reinvigorate the slowing drug discovery process by finding new uses for existing drugs. The major advantage of the drug repurposing approach is that the safety issues are already investigated in the clinical trials and the drugs are commercially available in the marketplace. As this approach provides an effective solution to hasten the process of providing new alternative drugs for AD, the current study shows the molecular interaction of already known antipsychotic drugs with the different protein targets implicated in AD using in silico studies.

Result: A computational method based on ligand–protein interaction was adopted in present study to explore potential antipsychotic drugs for the treatment of AD. The screening of approximately 150 antipsychotic drugs was performed on five major protein targets (AChE, BuChE, BACE 1, MAO and NMDA) by molecular docking. In this study, for each protein target, the best drug was identified on the basis of dock score and glide energy. The top hits were then compared with the already known inhibitor of the respective proteins. Some of the drugs showed relatively better docking score and binding energies as compared to the already known inhibitors of the respective targets. Molecular descriptors like molecular weight, number of hydrogen bond donors, acceptors, predicted octanol/water partition coefficient and percentage human oral absorption were also analysed to determine the in silico ADME properties of these drugs and all were found in the acceptable range and follows Lipinski’s rule.

Conclusion: The present study have led to unravel the potential of leading antipsychotic drugs such as pimozide, bromperidol, melperone, anisoperidone, benperidol and anisopirol against multiple targets associated with AD. Benperidol was found to be the best candidate drug interacting with different target proteins involved in AD.

Keywords: Drug repurposing, Alzheimer’s disease, Antipsychotic drugs, Acetylcholinesterase, Butyrylcholinesterase, Beta-secretase cleavage enzyme, Monoamine oxidase, N-Methyl-d-aspartate, Molecular docking, Schrodinger

Background

Alzheimer’s disease (AD) is the most prevalent form of dementia associated with progressive cognitive deterioration, behavioural and neuropsychiatric symptoms [1, 2]. There are approximately 35 million people worldwide and 3.7 million in India suffering from AD. About one in ten adults over 65 and almost 50% of the people above 85 years of age develops AD [3]. Currently, commercially available drugs used for symptomatic treatment of AD such as neostigmine, physostigmine, rivastigmine, donepezil, tacrine and memantine show side effects such as gastrointestinal disturbances, muscle aches, vomiting, heartburn, headache, loss of appetite, diarrhoea, loss of balance, hepatotoxicity and shorter half-life [4]. In view of these shortcomings there is continues search for new drugs with lesser side effects. In the last few years less than 25 drugs are in phase II and III clinical trials for AD, whereas more than 1700 are there for cancer therapies [5].
Drug repurposing is the process of evaluating the applicability of already known drug for their new therapeutic role. Drug repurposing has already been practiced in many therapies such as cancer, cardiovascular disease, stress incontinence, irritable bowel syndrome, erectile dysfunction, obesity, smoking cessation, psychosis, attention deficit disorder and Parkinson’s disease [6]. With already established drug compounds, the advantages are that it save time and cost on preliminary clinical trials such as chemical optimization, in vitro and in vivo screening, toxicology studies, bulk manufacturing and formulation development [7]. Whereas, a new drug candidate takes billion of dollars and at least 15 years to come in the market [8]. In fact, one of the establish drug for AD, Galanthamine, an acetylcholinesterase (AChE) inhibitor was earlier used for Poliomyelitis in Eastern Europe and then repurposed for use in AD same as Lundbeck repurposed memantine for therapeutic use in AD as Ebixa® [9, 10]. Other examples include citalopram, desvenlafaxine, and fluoxetine (Selective Serotonin Reuptake Inhibitors), levetiracetam (anti-epileptic drug), perindopril, nilvadipine, carvedilol (antihypertensive drugs), liraglutide, lixisenatide, metformin, exenatide (anti-diabetes drugs) all have shown to be significant in AD [11].

Various neuropathological symptoms of AD include deposition of senile neurotic plaques, loss of cholinergic neurons and formation neurofibrillary tangles in the central nervous system (CNS) [12]. There are many hypotheses to explain the cause of AD, such as cholinergic hypothesis, β-Amyloid hypothesis, glutamatergic and excitotoxic hypothesis, oxidative hypothesis and tau hypothesis [13].

**Cholinergic hypothesis**

Acetylcholine (ACh), one of the most important neurotransmitter found in CNS is hydrolyzed by cholinesterase i.e., acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes. The cognitive impairment is mainly due to loss of neurotransmitter ACh caused by reduced activity of choline acetyltransferase (ChAT), an enzyme evolved in synthesis of ACh. In view of this, the main emphasis is on anticholinergic drugs, which can inhibit both the enzymes and up-regulate the level of ACh in the CNS [14]. Studies have shown that in patients of AD, BuChE activity increases from 40 to 90%, whereas AChE activity remains the same or declines [15]. Evidences have shown that AChE and BuChE both plays an important role in accelerated pro-aggregation of β-Amyloid (Aβ) fibrils formation [16]. Cholinesterase inhibitors, which bind to the peripheral anionic site (PAS) of AChE have found to inhibit AChE-induced aggregation of Aβ fibrils [17].

**β-Amyloid cascade hypothesis**

According to this hypothesis there is an overproduction and aggregation of Aβ peptide, leading to formation of neuritic plaques in CNS [18]. Enzymes like Beta-secretase cleavage enzyme (BACE 1) and Gamma-secretase (γ secretase) are responsible for the formation of these peptides by the proteolytic cleavage of amyloid precursor protein (APP), producing two variables peptide i.e. Aβ40 and Aβ42. Both Aβ40 and Aβ42 peptide aggregation leads to neurotoxicity and neural cell death [19, 20]. Aβ1-42 polypeptide is more prone to form insoluble and toxic aggregates as compared to Aβ1-40 polypeptide [21]. BACE 1 enzyme is one of the important drug targets for the development of anti-Alzheimer’s drug because of its role in β-amylloid cascade. Inhibition of BACE 1 enzyme at the beginning of APP processing would prevent the formation of insoluble toxic Aβ aggregates that is responsible for neurodegeneration in AD.

**Oxidative hypothesis**

According to the hypothesis, the reactive oxygen species (ROS) formation increases within the mitochondria under stressful conditions and in aging, with no efficient antioxidant system it leads to the risk of developing AD. The brain of AD patients shows a significant extent of oxidative damage. ROS activates BACE 1 and γ-secretase enzymes to increase Aβ production and abnormal accumulation of Aβ fibrils in the brain of AD patients from APP. Aβ and APP may themselves also directly induce the production of ROS [22, 23]. An enzyme namely monooamine oxidase (MAO) is proposed to be involved in AD due to elevated production of ROS. MAO has two subtypes i.e. MAO A and MAO B responsible for the catalysis the deamination of xenobiotic and biogenic amines, like norepinephrine, dopamine and serotonin [24, 25]. Studies have also shown that there is an increased level of neurotransmitters in the CNS, when MAO is being inhibited [26, 27].

**Glutamatergic and excitotoxic hypothesis**

Glutamate is the major excitatory neurotransmitter, involved in synaptic plasticity, memory and learning in the cortical and hippocampal region of CNS [28, 29]. Dysfunction in the glutamatergic system has been linked with increase in oxidative stress associated with the Aβ peptide, the pathophysiological processes underlying AD. Glutamate synthesize being sensitive to oxidative stress leads to decrease in its activity and resulting in increased glutamate levels [30]. The N-methyl-d-aspartate (NMDA) receptor is a member of the family of ionotropic glutamate receptors. Its over-activation due to excessive glutamate causes continuous influx of calcium ions (Ca2+) into the nerve cells, ultimately leading to cell

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**References:**

1. Kumar et al. BMC Neurosci (2017) 18:76
2. Exenatide (anti-diabetes drugs) all have shown to be significant in AD [11].
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death [31]. Previous studies suggests that NMDA receptors are constantly hyper-activated, generating a form of 'slow excitotoxicity' at post synaptic neurons, producing a gradual neurodegenerative effect in AD patients [32]. Memantine, one of the United States Food and Drug Administration (FDA) approved drug for AD is a NMDA receptor antagonist. Therefore, NMDA receptor antagonists could be therapeutically beneficial in AD.

Most of the patients having dementia suffer from behavioural and psychological symptoms of dementia (BPSD) which includes depression, aggression, apathy, delusions, agitation, euphoria, hallucinations and sleep disturbances. Antipsychotics or neuroleptics are the class of drugs that are used for the management of psychosis mainly bipolar disorder, schizophrenia and for some non-psychotic disorders including depression and anxiety [33]. There are two major groups of antipsychotic drugs namely typical and atypical antipsychotic. Typical or first generation antipsychotics inhibit dopaminergic neurotransmission whereas the atypical or second generation antipsychotics are dopamine D2 and serotonin (5-HT, 5-hydroxytryptamine) receptor antagonist [34]. Antipsychotic drugs have shown to be interacting with major 5-HT receptors such as 5-HT1A, 5-HT2A, 5-HT2C, 5-HT3, 5-HT4 and 5-HT7 receptors [35]. Antipsychotic drugs like aripiprazole, haloperidol, ziprasidone, clozapine, quetiapine are already in use for treating BPSD in AD patients. Lecozotan, a 5-HT1A receptor antagonist has cleared phase III clinical trial as cognitive enhancers in patients with AD [33, 36].

Due to the complex nature of AD, the conventional “one molecule, one target” approach may not offer the best pathway for this complex disease. Therefore, the multi-target directed strategy is much needed for disease modifying therapeutics. In view of this, present study demonstrates the interactions of screened antipsychotic drugs with the multiple targets of AD, using molecular docking and in silico ADME studies.

Methodology

Protein preparation

X-Ray crystallographic structure of all the target proteins i.e. AChE (4EY6), BuChE (1POM), MAO A (2Z5X), BACE 1 (4D8C, 3L5E) and NMDA (1PBQ) structure was downloaded from Protein Data Bank (PDB). Before performing molecular docking, all the protein structures were prepared using ‘Protein Preparation Wizard’ workflow in Schrodinger suite. This involved addition of hydrogen atoms to the protein, assignment of bond orders, and deletion of unnecessary water molecules. The important water molecules found to be interacting with the active site residues of the enzyme were retained. In case of 4EY6, seven water molecules [37]; for 1POM, two water molecules [38]; two water molecule in 4D8C, one in 3L5E [39] and one in 1PBQ [40]. No water molecules were retained in 2Z5X [41]. Side chains were added, disulphide bonds were formed, missing atoms were added and the partial charges were assigned. Energy minimization was done using OPLS_2005 (Optimized Potentials for Liquid Simulations) force field (Fig. 1). As all the downloaded proteins were co-crystallised structures, the ligand binding site were used so as to define the active site of the protein. Receptor grid generation workflow was used to define a grid (box) around the ligand, to keep all the functional residues in the grid [42].

Ligand preparation

Based on literature review, approximately 150 antipsychotic drugs were selected and their 3D structures were downloaded from Pubchem. Using Ligprep, pre-processing of the ligands were done, which includes formation of tautomers and ionization states at pH 7.0 ± 2.0 using Epik, hydrogen atoms were added, charged groups were neutralised and geometry of the ligands were optimised [43].

Molecular docking

Using glide (grid based ligand docking with energetics) algorithm, extra precision docking was performed with the prepared protein and the ligands. Structures of ligands were kept flexible to generate different conformations. OPLS force fields were used to perform these calculations [44]. All the Glide docking runs were performed on Intel® Core™ i7-3770 CPU @ 3.40 GHz of HP origin, with 4 GB RAM, Windows 8Pro operating system. All the results were analyzed in XP visualizer.

Pharmacokinetic parameters

QikProp in Schrodinger suite was used to calculate the physiochemical properties of the drugs like molecular weight of the compounds (MW), Predicted octanol/ water partition coefficient (QPlogPo/w), the number of hydrogen bond donors (donorHB), acceptors (acptHB) and percentage human oral absorption, violations of Lipinski’s rule of five were also analyzed [45].

Results

The main focus of present study is to identify antipsychotic drugs as repurposed drugs for the treatment of AD. Approximately, 150 antipsychotic drugs were screened using molecular docking. Out of the screened drugs, sulpiride, promazine hydrochloride, bromperidol, amisopirone, melperone, pimozide, benperidol, azabuperone and anisoperidone significantly interacted with selected protein targets of AD. The docking score was observed in the range of −10.927 to −14.969 in
case AChE (4EY6), −6.663 to −8.13 in case of BuChE (1P0M), −5.26 to −8.91 in MAO A (2Z5X), −5.907 to −8.513 in BACE 1 (3L5E), −3.649 to −9.076 in BACE 1 (4D8C) and −4.711 to −6.903 in NMDA (1PBQ) (Table 1). Among these antipsychotic drugs, the best hit for each target was selected on the basis of docking score and binding energy. There binding modes and the molecular interactions were also compared with potent known drugs/inhibitors in the crystal structures of the target protein. The binding energies in terms of glide energy, energy contributed due to electrostatic bonding ($\Delta G_{\text{col}}$), Van der Waals ($\Delta G_{\text{vdw}}$), hydrogen bonding ($\Delta G_{\text{Hbond}}$), interacting residues (both hydrophilic as well as hydrophobic) and their bond length of the best predicted drug with each protein target are presented in Table 2.

Validation of docking protocol

For all the target proteins, the ligand in native co-crystallised structure, was extracted and was re-docked with the cavity of the respective protein molecule in order to validate the reliability, reproducibility and docking calculations. It was observed that the co-crystallised ligand was almost superimposing with the respective docked conformation of the ligands (Fig. 2). As a common rule, the scoring function is successful, if the bound ligands conformation in the experimental downloaded structure
Docking score of the top nine interacting drugs with multiple target proteins

| Name of the ligand | Docking score | BuChE (1P0M) | MAO A (2Z5X) | BACE 1 (3L5E) | BACE 1 (4D8C) | NMDA (1PBQ) |
|--------------------|---------------|--------------|--------------|---------------|---------------|-------------|
| Positive control   | Donepezil     | 11.02        | 7.246        | 6.123         | 6.80          | 4.029       |
| Sulmepride         | 11.058        | 7.246        | 6.349        | 4.831         | 4.425         | 5.907       |
| Promazine hydrochloride | 11.074     | 5.907        | 6.784        | 4.711         | 6.524         | 4.167       |
| Bromperidol        | 10.927        | 6.576        | 7.486        | 5.247         | 6.067         | 6.169       |
| Anisopirone        | 11.001        | 6.083        | 8.169        | 6.903         | 6.075         | 6.854       |
| Meluperone         | 11.423        | 7.249        | 6.524        | 6.067         | 6.169         | 6.854       |
| Pimozide           | 14.969        | 8.386        | 8.968        | 6.854         | 6.169         | 6.854       |
| Benperidol         | 14.425        | 6.742        | 9.076        | 6.778         | 6.169         | 6.854       |
| Azabuperone        | 11.882        | 7.584        | 4.167        | 6.51          | 6.169         | 6.854       |
| Anisoperidone      | 7.989         | 8.513        | 7.11         | 5.196         | 6.854         | 6.854       |

Table 1 Dock score of the top nine interacting drugs with multiple target proteins

The catalytic site of human AChE is 20 Å deep which has the catalytic site of human AChE (Ser203, His447 and Glu334), acyl-binding pocket (Phe295 and Phe297) at the base of the gorge, oxygen hole (Gly120, Gly121, and Ala204), quaternary ammonium binding locus (Trp86) and lastly, PAS (Tyr72, Asp74, Tyr124, Trp286 and Tyr341) in mammals, which clusters at the entry of active site gorge [47]. In case of AChE, the best docking was observed with pimozide with dock score of −14.969 and glide energy of −50.362 kcal/mol and that of known inhibitor donepezil was −11.02 and glide energy was −27.944 kcal/mol. Pimozide forms hydrogen bonding with Tyr124, Phe295 and Ser293, pi–pi stacking with Trp286 (PAS residue), Phe338 and salt bridge with Tyr341 (PAS residue). Polar interactions were observed with catalytic residues Ser203 and His324 (Tables 1, 2 and Fig. 3).

Molecular interaction of the bromperidol with butyrylcholinesterase

The catalytic site of human BuChE has an active site of 20 Å; its catalytic site has three residues (Ser198, His438, Glu325), choline binding site or the cation–r site (Trp82), oxygen hole (Gly116, Gly117, Ala199), acyl binding site (hydrophobic pocket) (Leu286, Val288) and PAS (Asp70) [48]. Bromperidol showed best docking score with BuChE of −8.111 and glide energy of −42.936 kcal/mol and of the known inhibitor rivastigmine was −3.123 and glide energy of −35.510 kcal/mol. It forms hydro bond with Pro285, pi–pi stacking with Phe329 and polar interactions with catalytic site residues Ser198, His438 (Tables 1, 2 and Fig. 4).

Molecular interaction of the melperone with monoamine oxidase

MAO A has hydrophobic cavity which has a volume of ~400 Å. Depending upon the conformation of Phe208, MAO A can be a large single cavity or a bipartite cavity but in this case it does not work as gating residue. The enzyme has conserved active site residues which includes a pair of Tyr of the “aromatic sandwich” and a Lys hydrogen bonded to the N(5) position of the flavin i.e. Lys305 in MAO A. There are other non-conserves active site residues mainly Asn181 and Ile180 in MAO A. The major determinant in controlling the differential inhibitor and substrate specificities of these enzymes is Phe208–Ile335 in MAO A [49]. In case MAO A, the least docking score was of −8.91 with melperone and glide energy with −29.292 kcal/mol and in the case of known inhibitor, marplan, the docking score was −8.777 and glide energy was −39.947 kcal/mol. Docking results showed both hydrogen bonding and pi-cation with Phe208 (Tables 1, 2 and Fig. 5).

Molecular interaction of the benperidol and anisoperidone with beta-secretase cleavage enzyme

Ligand interaction in BACE 1 depends on the active site residues conformation, which consisted of catalytic dyad (Asp32, Asp228), 10 s loop composed of residues 9–14, flap consisting of amino acids 67–77 and
all other residues within 8 Å from aspartates. The flap occurs in three form close form, close to open form and transition form. Therefore, to avoid biasness we chose two BACE 1 complex, 3L5E (transition form) and 4D8C (open form) [19]. With BACE 1 (4D8C), benperidol showed that highest docking score of −6.84 and glide energy of −54.082 kcal/mol, known inhibitor LY2886721 had docking score of −6.80 and glide energy of −35.835 kcal/mol. In this case, hydrogen bonding was observed with Thr72 and Phe108 whereas salt bridge with Asp32 and Asp217 (catalytic residues) (Tables 1, 2 and Fig. 6). Anisoperidone was best docked with BACE 1 (3L5E) with a score of −8.513 and glide energy of −43.063 kcal/mol whereas the known inhibitor LY2886721 had dock score of −6.123 and glide energy of −35.835 kcal/mol. It forms hydrogen binding with

| Target | Drug name | Glide energy (kcal/mol) | ΔG_{edw} (kcal/mol) | ΔG_{ecol} (kcal/mol) | Xphbond (kcal/mol) | Interacting residues/molecule | Bond length (Å) |
|--------|-----------|-------------------------|---------------------|----------------------|-------------------|-----------------------------|---------------|
| AChE   | Pimozide* | −50.362                 | −39.030             | −11.332              | 0.700             | Tyr124 (H bond)             | 2.05          |
|        |           |                         |                     |                      |                   | Trp286 (π–π stacking)       | 3.72          |
|        |           |                         |                     |                      |                   | Ser293 (H bond)             | 2.64          |
|        |           |                         |                     |                      |                   | Phe295 (H bond)             | 1.96          |
|        |           |                         |                     |                      |                   | Phe338 (π–π stacking)       | 5.18          |
|        |           |                         |                     |                      |                   | Tyr341 (n-cation)           | 4.81          |
|        |           |                         |                     |                      |                   |                            |               |
|        | Donepezil | −27.944                 | −25.03              | −2.91                | 0.000             | Trp86 (n–n stacking)        | 3.98587       |
|        |           |                         |                     |                      |                   | Trp86 (n–n stacking)        | 4.2134        |
|        |           |                         |                     |                      |                   |                            |               |
| BuChE  | Bromperidol* | −42.936               | −38.35              | −4.584               | −0.7              | Pro285 (H bond)             | 2.04008       |
|        |           |                         |                     |                      |                   | Phe329 (n–π stacking)       | 5.42016       |
|        |           |                         |                     |                      |                   |                            |               |
|        | Rivastigmine* | −35.110               | −30.006             | −4.850               | 0                  | Tyr332 (n–n stacking)       | 3.13          |
|        |           |                         |                     |                      |                   |                            |               |
| MAO A  | Melperone* | −29.292                 | −25.99              | −3.301               | 0                  | Phe208 (H bond)             | 2.06394       |
|        |           |                         |                     |                      |                   | Phe208 (n-cation)           | 4.06326       |
|        | Marplan*  | −36.965                 | −32.950             | −4.015               | −0.689            | Gln215 (H bond)             | 2.26          |
|        |           |                         |                     |                      |                   | Asn181 (H bond)             | 2.23          |
|        |           |                         |                     |                      |                   | Tyr407 (n–n stacking)       | 3.65          |
| BACE 1 (3L5E) | Anisoperidone* | −43.063            | −34.559             | −8.505               | −0.99             | Trp137 (H bond)             | 1.9793        |
|        |           |                         |                     |                      |                   | Tyr259 (H bond)             | 2.5579        |
|        |           |                         |                     |                      |                   | Tyr132 (n-cation)           | 5.55641       |
|        |           |                         |                     |                      |                   | Asp93 (salt bridge)         | 4.41983       |
|        |           |                         |                     |                      |                   | Gln134 (H bond)             | 2.19          |
|        |           |                         |                     |                      |                   | Lys168 (H bond)             | 2.73          |
|        |           |                         |                     |                      |                   | Phe169 (n–n stacking)       | 5.29          |
| BACE 1 (4D8C) | Benperidol* | −54.082               | −42.994             | −11.088              | −1.254            | Thr72 (H bond)              | 2.09515       |
|        |           |                         |                     |                      |                   | Phe108 (H bond)             | 2.13012       |
|        |           |                         |                     |                      |                   | Asp32 (salt bridge)         | 4.14004       |
|        |           |                         |                     |                      |                   | Asp217 (salt bridge)        | 4.27946       |
|        | LY2886721* | −35.835                 | −34.465             | −1.370               | −0.934            | Thr72 (H bond)              | 1.88          |
|        |           |                         |                     |                      |                   | Gln73 (H bond)              | 2.12          |
|        |           |                         |                     |                      |                   | Thr220 (H bond)             | 2.30          |
| NMDA   | Anisopirol* | −36.533                | −34.095             | −2.437               | −1.134            | Phe246 (H bond)             | 1.91824       |
|        |           |                         |                     |                      |                   | Trp223 (n–n stacking)       | 5.25223       |
|        | DCKA*     | −43.865                 | −28.096             | −15.769              | −3.422            | Pro124 (H bond)             | 2.148         |
|        |           |                         |                     |                      |                   | Thr126 (H bond)             | 1.740         |
|        |           |                         |                     |                      |                   | Arg131 (two H bond)         | 1.756         |
|        |           |                         |                     |                      |                   |                            | 1.822         |
Trp137, Trp259; pi-cation with Tyr132 and salt bridge with Asp93 (Tables 1, 2 and Fig. 7).

**Molecular interaction of the anisopirol with N-methyl-d-aspartate receptor**

For protein 1PBQ, crucial interacting residues with co-crystallised ligand DCKA are Pro124, Ser180, Thr126 and Arg131. Hydrophobic pocket of 1PBQ has following amino acid residues: Phe16, Phe92, Trp223 and Phe250 [50]. Anisopirol showed the best docking score of $-6.903$ and glide energy of $-36.533$ kcal/mol whereas, DCKA showed a docking score of $-14.084$ and glide energy of $-43.865$ kcal/mol. Hydrogen bonding was observed between anisopirol and Phe246 whereas pi–pi stacking with Trp223, polar interactions with Thr126 and Ser180 whereas hydrophobic interactions are observed with Pro124, Phe92 of 1PBQ (Tables 1, 2 and Fig. 8).

**Prediction of pharmacokinetic properties**

Pharmacokinetics depends upon the molecular descriptors of the drug candidate. In silico prediction of absorption, distribution metabolism and excretion (ADME)
properties have become important in drug selection and to determine its success for human therapeutic use. Therefore, these physio-chemical descriptors were calculated so as to determine the ADME properties of the drugs. Lipinski’s rule of five is based on the observation that drugs with molecular weight of 500 Da or less, donorHB ≤ 5, acceptorHB ≤ 10 and QPlogP/o/w ≤ 5 are orally administered drugs. Molecules which are not following more than one of these four rules may have problem with bioavailability. None of the drugs in present study violated the Lipinski’s rule of five. Percentage human oral absorption was calculated to predict the oral absorption on the scale of 0–100%. More than 80% are thought to have as high absorption whereas any
compound with less than 25% is poor. According to this
criterion; all the drugs have medium to high oral absorp-
tion ranging from 53.364 to 100% (Table 3).

**Discussion**

Over the past years, de novo drug discovery has faced
some serious issues due to its cost and time consumption.
While the investment has increased in the pharmaceutical companies, the number of new approved drugs has been stationary; therefore, in silico drug repurposing is an efficient and encouraging tool for discovering new uses from already existing drugs. There are various examples of repurposed drugs which were discovered by computational approach and are now being used in other diseases. For example, Raltegravir, originally an HIV-1 integrase inhibitor is now used as adjuvant therapy in cancer [51] and Valsartan, an angiotensin receptor blocker is now being used for AD [52]. Complex pathophysiology of AD suggests multi-target strategies and...

Fig. 7 The lowest energy conformation of docking result of Benperidol with BACE 1 (4D8C)

Fig. 8 The lowest energy conformation of docking result of Anisopirol with NMDA (1PBQ)
drugs with polypharmacological interactions. In the present study, we are focusing on the conventional (AChE, BuChE and NMDA) as well as new experimental (BACE 1, MAO A) targets of AD. For each receptor, about three to five drug poses were analysed to identify the pose with least docking score and the minimum binding energy. The docking score of top nine interacting antipsychotic drugs with all the target protein (AChE, BuChE, BACE 1, MAO A, and NMDA) were studied and their pharmacologically active protein target was also predicted (Tables 1 and 4).

The other drugs were also found to be display good molecular interaction with certain protein. For example, Pimozide showed best interaction with AChE, Bromperidol with BuChE, Melperone with MAO A, Anisopirol with NMDA, Anisoperidone with BACE 1 (3L5E) and Benperidol with BACE 1 (4D8C). In accordance to this approach, Benperidol, a butyrophenone derivative, was found to be the best candidate drug based on its dock score (Table 1), glide energy and molecular interactions with all target protein (Table 5 and Fig. 9). The second best candidate, Melperone was of same class of compound as that of Benperidol, suggesting potent role of their basic skeletal structure in the interaction with the target proteins. For proteins MAO A and BACE 1, Melperone showed lower docking score as compared to Benperidol. This observation suggests that further modification in their basic structure and formation of some new analogs may enhance their multi potent anti-Alzheimer’s activity.

A lead molecule is considered to be a successful oral drug when it is quickly and completely absorbed from the gastrointestinal tract, distributed to the specific site in the body where it has to act, metabolised in such a way that it doesn’t immediately abolish its activity and should eliminate from the body without causing any harm to any organ. Because of poor pharmacokinetics, half of the developed drugs fail to reach markets. Benperidol and Melperone didn’t violated Lipinski’s rule of five and showed 86.914 and 96.488% human oral absorption.

With in silico repurposing approach, there can be a possibility of false positive hits during screening and also the activity of the candidate drug molecules may vary in the in vitro or in vivo systems. Therefore, to validate their potency further in vitro and in vivo studies are needed to be performed.

**Conclusion**

AD is a complex neurodegenerative disease involving multiple targets such as AChE, BuChE, BACE 1, MAO A and NMDA. Several molecules have been developed against these targets to alleviate the symptoms and having disease modifying effects. In spite of the laborious efforts, presently very few drugs are in the pipeline due to the limitation associated with molecule to satisfy ADME profile. Due to these limitations the present study explored the repurposing of already known antipsychotic drugs, which means their pharmacokinetics, toxicology profile, formulation development and bulk manufacturing have already been done hence saving cost and time. Of all the antipsychotic drugs studied, Benperidol was found to be the best candidate for the cholinergic (AChE

| S. no | Name of the drug         | Molecular weight | QPlogPo/w | DonorHB acptHB | Percent human-oral absorption | Violation of Lipinski’s rule |
|-------|--------------------------|------------------|-----------|----------------|------------------------------|-------------------------------|
| 1     | Sulmepride               | 242.031          | 1.46      | 1              | 2                            | 82.403                       | 0                             |
| 2     | Promazine hydrochloride  | 214.221          | 0.867     | 4              | 6.9                          | 53.364                       | 0                             |
| 3     | Bromperidol              | 223.069          | 4.368     | 1              | 4.75                         | 100                          | 0                             |
| 4     | Anisopirol               | 358.455          | 4.047     | 1              | 5.45                         | 100                          | 0                             |
| 5     | Melperone                | 263.354          | 2.944     | 0              | 4                            | 96.488                       | 0                             |
| 6     | Pimozide                 | 461.555          | 6.413     | 1              | 4                            | 96.301                       | 1                             |
| 7     | Benperidol               | 381.449          | 3.45      | 1              | 6                            | 86.914                       | 0                             |
| 8     | Azabuperone              | 290.38           | 1.905     | 0              | 6                            | 79.665                       | 0                             |
| 9     | Anisoperidone            | 335.445          | 4.126     | 0              | 4.75                         | 100                          | 0                             |

**Table 3 Molecular descriptor values of the top interacting antipsychotic drugs**
### Table 4 Top interacting drug with its pharmacological known and predicted protein target

| Drug name | IUPAC name | Drug structure | Known pharmacological target | Predicted drug target |
|-----------|------------|----------------|-------------------------------|-----------------------|
| Pimozide  | 3-1-[4,4-bis(4-fluorophenyl)butyl]-piperidin-4-yl]-1H-benzimidazol-2-one | ![Drug Structure](image1) | Dopamine D2 receptor [53] | AChE |
| Bromperidol | 4-[4-(4-bromophenyl)-4-hydroxy-2-piperidin-1-yl]-1-(4-fluorophenyl)butan-1-one | ![Drug Structure](image2) | Dopamine D2 receptor [54] | BuChE |
| Melperone | 1-(4-fluorophenyl)-4-(4-methylpiperidin-1-yl)butan-1-one | ![Drug Structure](image3) | Dopamine D2/D3 and 5-HT<sub>2A</sub> antagonist [55] | MAO A |
| Anisoperidone | 1-(4-methoxyphenyl)-4-(4-phenyl-3,6-dihydro-2H-pyridin-1-yl)butan-1-one | ![Drug Structure](image4) | Dopamine D2 and 5-HT2A receptors [56] | BACE 1 (3L5E) |
| Benperidol | 3-1-[4-(4-fluorophenyl)-4-oxobutyl]piperidin-4-yl]-1H-benzimidazol-2-one | ![Drug Structure](image5) | Dopamine D2 antagonist [57] | BACE 1 (4DBC) |
| Anisopirol | 1-(4-fluorophenyl)-4-[4-(2-methoxyphenyl)piperazin-1-yl]butan-1-ol | ![Drug Structure](image6) | Dopamine receptor [58] | NMDA |
Table 5  Molecular interaction of Benperidol with different protein targets

|                | ACHE (4EY6) | BuChE (1P0M) | MAO A (2Z5X) | BACE 1 (4D8C) | BACE 1 (3LSE) | NMDA (1PBQ) |
|----------------|-------------|--------------|--------------|---------------|---------------|-------------|
| Glide energy   | 46.208      | 44.437       | 54.432       | 54.082        | 48.264        | 37.114      |
| Interacting    | Trp286 (n-π  | Ser287 (H    | Phe208 (H    | Thr72 (H      | Trp137 (H     | Asn128 (H   |
| residue        | stacking)   | bond)        | bond)        | bond)         | bond)         | bond)       |
|                | Tyr337 (n-π  | Phe329 (n-π  | Phe208 (H    | Phe108 (H     | Tyr132 (n     |             |
|                | stacking)   | stacking)    | bond)        | bond)         |-cation)       |-cation)     |
|                | His447 (n-π | Trp231 (n-π  |              |               |               |             |
|                | stacking)   | stacking)    |              |               |               |             |
|                | Ser293 (H   |              |              |               |               |             |
|                | bond)       |              |              |               |               |             |
|                | Phe295 (H   |              |              |               |               |             |
|                | bond)       |              |              |               |               |             |
|                |              |              |              |               |               |             |

- 46.208 Trp286 (n-π stacking)
- 44.437 Ser287 (H bond)
- 54.432 Phe208 (H bond)
- 54.082 Thr72 (H bond)
- 48.264 Trp137 (H bond)
- 37.114 Asn128 (H bond)

- Tyr337 (n-π stacking)
- Phe329 (n-π stacking)
- His447 (n-π stacking)
- Ser293 (H bond)
- Phe295 (H bond)
- Phe208 (H bond)
- Phe208 (n-cation)
- Thr72 (H bond)
- Phe108 (H bond)
- Tyr132 (n-cation)
- Asp32 (salt bridge)
- Asp217 (salt bridge)
- Trp137 (H bond)
- Arg189 (n-cation)
- Asp217 (salt bridge)
- Asn128 (H bond)
and BuChE), monoaminergic (MAO A), glutamatergic system (NMDA) and beta-secretase cleavage enzyme (BACE 1). Hence, in silico drug repurposing has been able to identify promising results which might be useful therapeutically in AD.

**Fig. 9** Molecular interaction of the Benperidol with target proteins (a 4EY6, b 1P0M, c 1PBQ, d 4D8C, e 3L5E, f 2Z5X)

**Abbreviations**
AD: Alzheimer’s disease; AChE: acetylcholinesterase; CNS: central nervous system; ACh: acetylcholine; BuChE: butyrylcholinesterase; ChAT: choline acetyltransferase; PAS: peripheral anionic site; BACE 1: beta secretase cleavage enzyme 1; γ-Secretase: gamma secretase; Aβ, β-amyloid; APP: amyloid precursor protein; ROS: reactive oxygen species; MAO: monoamine oxidase; NMDA: N-methyl-d-aspartate; Ca²⁺: calcium ions; FDA: Food and Drug Administration,
USA; BPSD: behavioural and psychological symptoms of dementia; S-HT: S-hydroxytryptamine receptors; PDB: Protein Data Bank; OPLS: optimized potentials for liquid simulations; Glide: grid based ligand docking with energetics; CPU: Central Processing Unit; RAM: Random Access Memory; MW: molecular weight; QPlogPo/w: predicted octanol/water partition coefficient; donorH: number of hydrogen bond donors; acceptorH: number of hydrogen bond acceptors; RMSD: root mean square deviation; ΔGdiss: energy contributed due to electrostatic bonding; ΔGphdiss: energy contributed due to Van der Waals bonding; Xphbond: energy contributed due to hydrogen bonding; ADME: absorption, distribution, metabolism and excretion.

Authors’ contributions
SK and SK* conceived and designed the project. SK and SC performed the experiments and analysed the results. All authors wrote the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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