AlzPlatform: An Alzheimer's Disease Domain-Specific Chemogenomics Knowledgebase for Polypharmacology and Target Identification Research
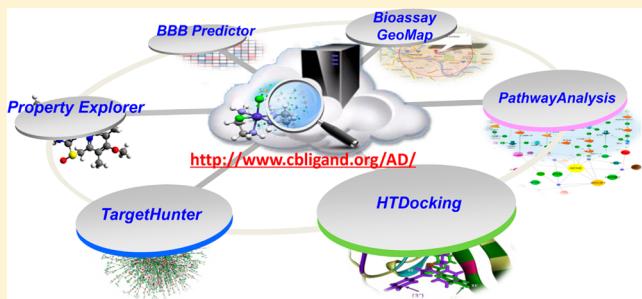
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ABSTRACT: Alzheimer's disease (AD) is one of the most complicated progressive neurodegeneration diseases that involve many genes, proteins, and their complex interactions. No effective medicines or treatments are available yet to stop or reverse the progression of the disease due to its polygenic nature. To facilitate discovery of new AD drugs and better understand the AD neurosignaling pathways involved, we have constructed an Alzheimer's disease domain-specific chemo-genomics knowledgebase, *AlzPlatform* (www.cbligand.org/AD/) with cloud computing and sourcing functions. AlzPlatform is implemented with powerful computational algorithms, includ-

ing our established TargetHunter, HTDocking, and BBB Predictor for target identification and polypharmacology analysis for AD research. The platform has assembled various AD-related chemogenomics data records, including 928 genes and 320 proteins related to AD, 194 AD drugs approved or in clinical trials, and 405 188 chemicals associated with 1 023 137 records of reported bioactivities from 38 284 corresponding bioassays and 10 050 references. Furthermore, we have demonstrated the application of the AlzPlatform in three case studies for identification of multitargets and polypharmacology analysis of FDA-approved drugs and also for screening and prediction of new AD active small chemical molecules and potential novel AD drug targets by our established TargetHunter and/or HTDocking programs. The predictions were confirmed by reported bioactivity data and our in vitro experimental validation. Overall, AlzPlatform will enrich our knowledge for AD target identification, drug discovery, and polypharmacology analyses and, also, facilitate the chemogenomics data sharing and information exchange/communications in aid of new anti-AD drug discovery and development.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegeneration and a complex multifactorial disorder among the elderly.[1] The disorder is reaching epidemic proportions with heavy social and economic costs.[2] The pathological features of AD are the loss of neurons in conjunction with the presence of oxidative stress, axonal dystrophy, senile plaques, and neurofibrillary tangles.[3] Because of its polygenic nature, AD is thought to be caused not by defects in a single gene, but instead by variations in many genes, proteins, and their complex interactions.[4] Thus, it is challenging to develop novel effective medications targeting multiple proteins in order to stop or reverse the progression of the disease.

Great efforts have been devoted to carrying out bioscience research with rapid accumulation of a large volume of scientific data relevant to AD. In particular, studies involved in AD neurosignaling pathways and AD-targeted new chemical ligands have been steadily proliferating.[5] The quantity and the quality
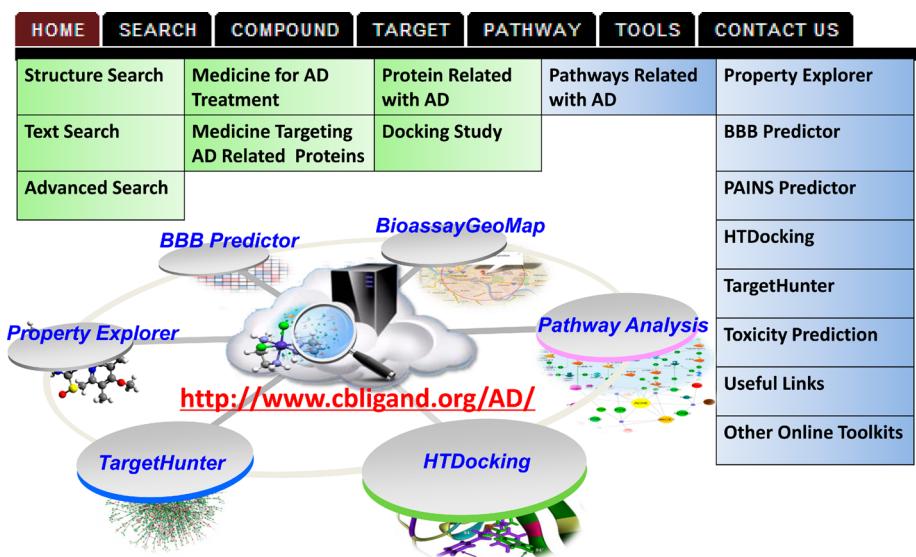
of the AD special class of molecules are expected to grow at a much faster rate in the future, thanks to rapid technology advancement in biochemistry, biophysics, medicinal chemistry, and pharmacology. Unfortunately, the venues to publicize AD-specific research have been limited to archival journals and periodicals. Although SciFinder and other databases have archived most of the documentation, the reported works are scattered. Thus, it is difficult to find, associate, and validate reported AD-related active chemical molecules and reuse the reported research results for AD target research.

Several AD-related databases have been reported to explore the molecular mechanisms, such as *AlzGene* and *Alzpathway*. The AlzGene database has been developed for investigating genetic association in the field of AD. It contains almost all genes related to AD and focuses on systematic meta-analyses of

Figure 1. Overview of AlzPlatform database featured with integrated computing and data-mining functions (www.CBLigand.org/AD).

information on AD genetic association.[6] AlzPathway, a comprehensive map of signaling pathways of AD, was constructed for exploring the AD pathogenesis.[7] However, these databases were mainly designed to investigate the pathogenic mechanisms of AD. To our knowledge, there is still no publicly available AD specific chemical genomics (or chemogenomics) database focusing on small molecules that target proteins related to AD for drug research.

Herein, an integrated cloud computing server, *AlzPlatform*, has been developed in response to the needs. The platform assembles a large repertoire of AD related chemogenomics data, including genes, protein targets, and small chemical molecules with their bioactivity records, bioassays, and references, as well as approved drugs or those in clinical trial for AD treatments. AlzPlatform enables cloud computing and sourcing services and provides powerful computational algorithms and implemented online computing programs/tools, including our established TargetHunter, HTDocking, and BBB predictor for target identification, drug repurposing, and polypharmacology analysis associated with AD (Figure 1). Therefore, AlzPlatform is a valuable platform for investigating and sharing AD targets and small chemical drug molecules at chemogenomics scale for better understanding the mechanisms of system polypharmacology in aid of new anti-AD drug discovery.

2. MATERIALS AND METHODS

2.1. Database Infrastructure. AlzPlatform was constructed based on the established molecular database prototype CBID (www.CBLigand.org/CBID),[8,9] with a MySQL (http://www.mysql.com) database and an apache (http://www.apache.org/) web server. Openbabel[10] is the search engine for chemical structures. The web interface is written in PHP language (http://www.php.net/).

2.2. Data Collection and Content. The information on protein targets and chemicals associated with AD was gathered according to the approved drugs, clinical trial drugs, and literatures from various databases, including the DrugBank, ClinicalTrials.gov, BindingDB, AlzGene, PubMed, ChEMBL, and SciFinder database. The corresponding information on signaling pathway of these targets was compiled from the

KEGG database. All the chemical structures, affinity values, and additional data including pathways, bioassays, and references were archived in relational database structure formats at the backend of the AlzPlatform database.

2.3. Web Interface. AlzPlatform provides a user-friendly interface with a powerful search engine for the detailed information on AD chemicals and targets.

(i) Keywords Search. This includes gene/protein symbol, compound name/ID, and basic pharmacological properties. Searches involving a combination of these keywords are supported without being case sensitive.

(ii) Structure Query. This provides two types of search functions: substructure and similarity. JME is used as the input interface,[11] and OpenBabel is the search engine at the backend.[12,13] In the structure search window, users can either sketch the structure in the JME interface or upload a file containing a small chemical molecule. After submission, the search is performed by OpenBabel at the server side and the results will be returned to the client side by loading a new page, including ID, structure of compound, target name, and the corresponding links to the literature.

2.4. Chemoinformatics Tools. Target identification and drug design with desired properties are top priorities for medicinal chemists. The data on chemogenomics and cheminformatics collected in AlzPlatform provide a valuable opportunity to explore the underlying targets; absorption, distribution, metabolism, and excretion (ADME) and toxicity prediction; and also calculation of molecular properties and drug-likeness. As such, state-of-art machine learning algorithms and chemoinformatics tools have been deployed on the platforms for facilitating AD drug design and target identification as briefed below.

TargetHunter. TargetHunter was implemented in AlzPlatform to provide online computing algorithm to predict the possible targets or off-targets of compounds. A detailed description of the algorithm has been published.[9] The basic principle of the TargetHunter program is based on a known medicinal chemistry concept: structurally similar compounds have similar physical properties that may result in similar biological profiles. This predicts the targets of a query compound by use of the powerful data-mining algorithm (TAMOSIC),

which assigns the targets associated with the most similar compounds of a query chemical as the predicted targets. TargetHunter is a powerful cloud computing tool with attractive features: (i) ease of use; (ii) query data retrieval function; (iii) user choices of desired fingerprints and databases; (iv) high accuracy; and (v) Bioassay finder implemented BioassayGeoMap function to find the authors who have published a bioassay for validation. Such a tool will assist researchers to develop bioactive compounds for research on AD target. TargetHunter is available at http://cbligand.org/TargetHunter.

HTDocking. In addition to the ligand-based TargetHunter tool online, we have also established a high-throughput docking (HTDocking, http://www.cbligand.org/AD/docking_search. php) program. It is a web-based computing tool that automates docking procedure to search for protein targets and to explore interactions between compound and protein. In the current version of AlzPlatform database, crystal structures of proteins related to AD have been collected from the Protein Data Bank (PDB) to build an AD domain-specific subset. AutoDock Vina is used as the docking engine at the backend.[14] Water molecules and ligands were removed, hydrogen atoms were added, and the active sites of each protein were defined by the residues around the cocrystallized ligands or generated using the AutoDock utility scripts. AutoDock Vina can provide 3−5 predicted binding affinity values (ΔG values) from different docking poses for each compound in a binding pocket of a protein. In our HTDocking program, we only consider the best binding affinity value which is further transformed as docking score. The docking score is calculated as pK_i, where $pK_i = -\log(\text{predicted } K_i)$ and the predicted $K_i = \exp^{(\Delta G \times 1000/(1.9871917 \times 298.15))}$. The docking score of a queried compound from each protein structure is used to assess and rank the potential protein partners or targets.

Blood−Brain Barrier (BBB) Predictor. The blood−brain barrier (BBB) is the bottleneck in AD drug development and is the single most important factor limiting the future growth of neurotherapeutics.[15] Considering this, a BBB predictor was specially designed to classify whether a compound can cross the blood−brain barrier (BBB+) or not (BBB−). This predictor was built by applying the support vector machine (SVM)[16] and LiCABEDS[17] algorithms on four types of fingerprints of 1593 reported compounds.[18] The BBB predictor is available at http://www.cbligand.org/BBB/.

In addition, AlzPlatform provides toxicity prediction with the Toxtree package[19] (http://cbligand.org/Tox), an online service for removal of false positive results[20] (http://cbligand.org/PAINS), and property calculator for the calculation of molecular properties, such as molecular weight, formula, number of rotatable bonds, hydrogen bond donors and acceptors, polar surface area, xLogP, and Lipinski's rule of five.[21] The properties calculator was implemented with the CDK package[22] and is available at http://www.cbligand.org/cbid/Property_Explorer.php. Furthermore, links are provided for quickly accessing chemoinformatics resources, such as actelion's property explore for ADME prediction and calculation of molecular properties and drug-likeness with Molinspiration. Thus, AlzPlatform acts as a chemoinformatics hub to other tools and databases, which can facilitate researchers in AD drug development and target identification.

2.5. Chemicals and Reagents. Huperzine A was purchased from J&K Scientific Ltd. (Beijing, China). Methyl sandaracopimarate (MS), a known diterpenoid compound, was isolated and identified from the extract of seeds of *Platycladus orientalis.*

2.6. *Caenorhabditis elegans* Strains and Maintenance. The *Caenorhabditis elegans* (*C. elegans*) strains Cl4176, also the *Escherichia coli* OP50 strain, were obtained from the *Caenorhabditis* Genetics Center (CGC; University of Minnesota, Minneapolis, MN). The transgenic nematode CL4176 strain, as an AD model,[23] is a temperature-sensitive mutant strain that expresses human $A\beta_{1-42}$ when it reaches nonpermissive temperatures. The expression of $A\beta_{1-42}$ in muscle cells causes paralysis in these mutants. The nematodes were maintained and assayed on nematode growth medium (NGM) agar plates with *Escherichia coli* OP50 at 16 °C. All worms used were raised from eggs obtained after sodium hypochlorite treatment of hermaphrodites.[24]
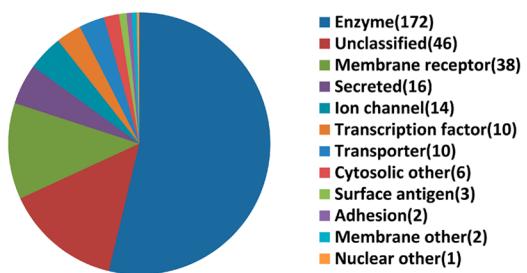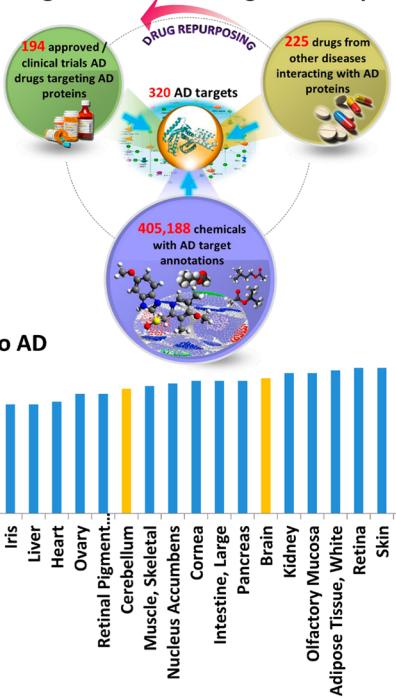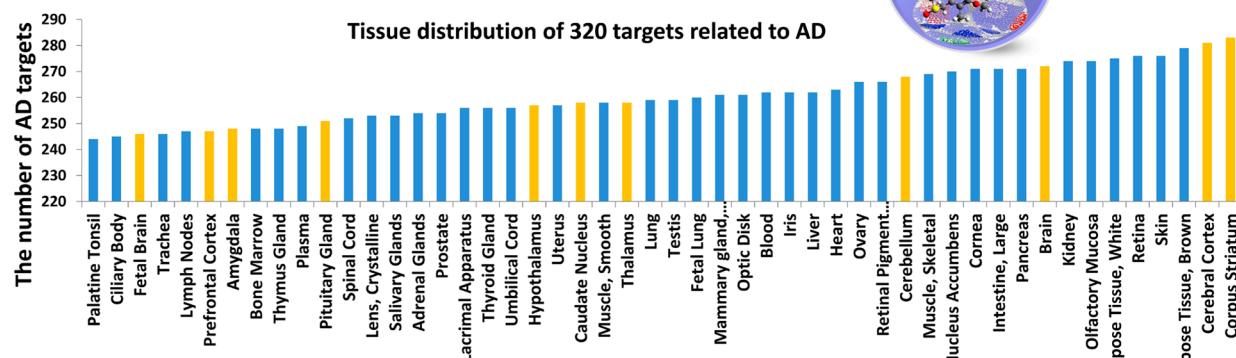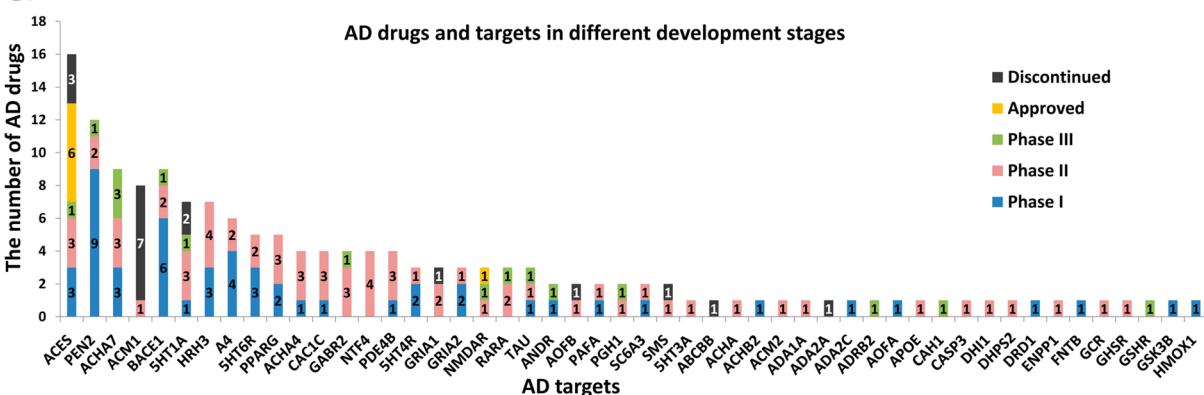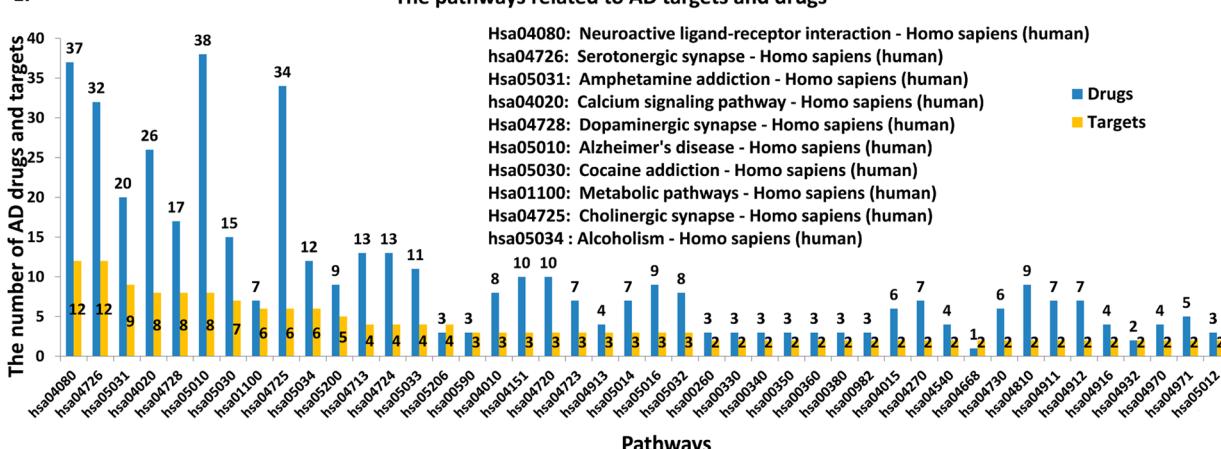
2.7. Paralysis Assay. The paralysis assay was measured according to the method described previously,[25] with slight modifications. The strain CL4176 maintained at 16 °C was egg-synchronized onto the 35 mm culture plates with or without methyl sandaracopimarate (300 μM). Transgene expression was induced by increasing the temperature from 16 to 26 °C. Induction occurred 36 h after egg laying and lasted until the last worm became paralyzed. For the paralysis assay, the survival of worms was determined by touch-provoked movement.[26] Worms were scored as paralyzed when they failed to respond to repeated touching with a platinum wire. Every experiment was conducted three times in a double-blind manner.

2.8. Cell Culture and Transient Transfection. HepG2 cells were plated at a density of 2×10^6 cells on a 48 well plate 24 h before transfection. Plasmids were transfected using *Trans-IT* LT (Mirus, Madison, WI) according to the manufacturer's protocol. To evaluate the binding of methyl sandaracopimarate to PPARγ, triplicate transfections were performed using the following plasmids: pCMX-tk-PPRE-LUC (400 ng), pCMX-PPARγ (200 ng), pCMX (200 ng), and (Beta galactosidase) β-gal (50 ng). At 24 h after transfection, the cells were treated with Rosiglizone (10 μM), vehicle, or the methyl sandaracopimarate at increasing concentrations (0.1, 1, 10, 50, 100, and 500 μM). At 24 h after the compound treatment, the cells were lysed and the Luciferase signal was quantified using a standard luminometer (Perkin-Elmer).The Luciferase signal was normalized to β-gal signal.[27] All transfections were performed at least three times.
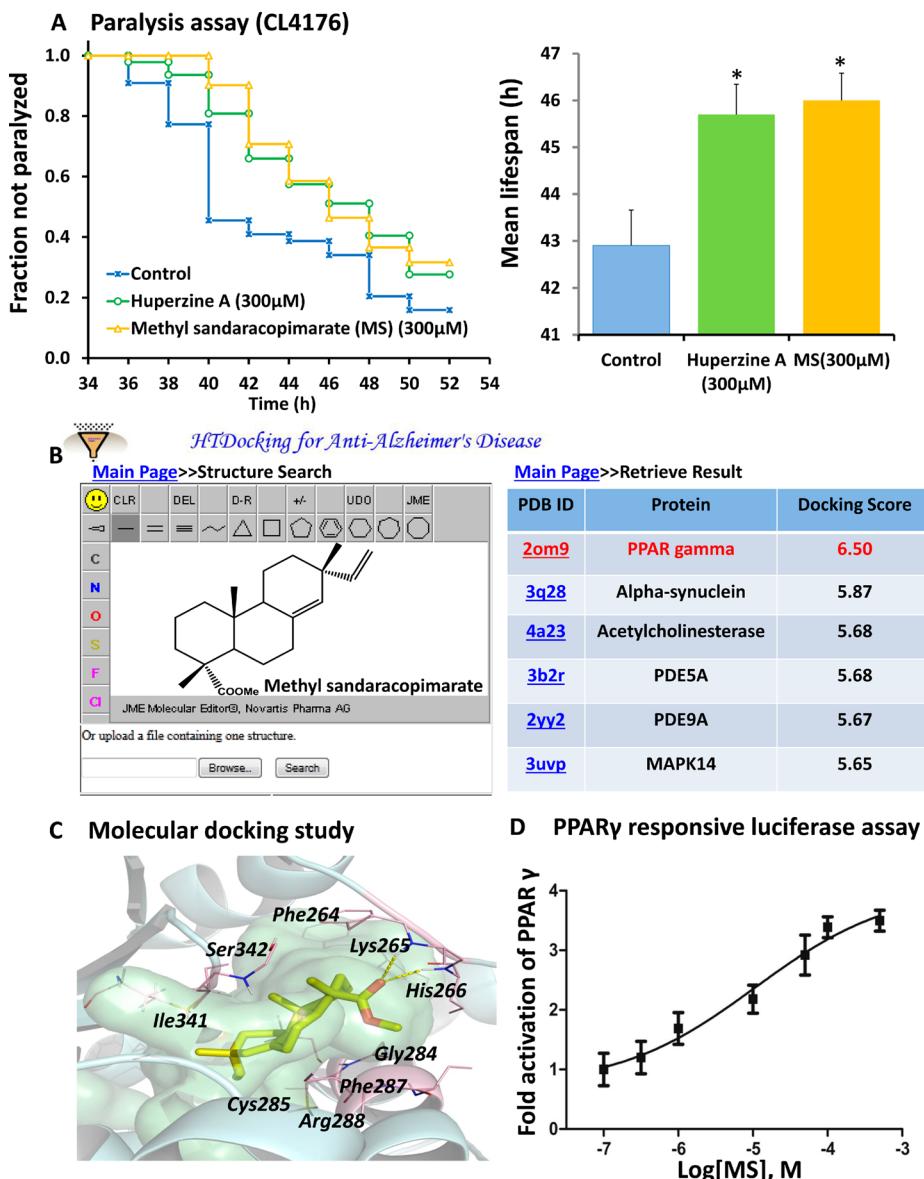
3. RESULTS

AlzPlatform (www.cbligand.org/AD/) archived 928 genes, 320 AD related proteins, 194 AD drugs approved and in clinical trials, and 405 188 chemicals associated with 1 023 137 records of reported AD bioactivities from 38 284 AD corresponding bioassays and 10 050 references.

Figure 1 shows an overview of the web-interfaced molecular information database for AD with implemented chemoinformatics computing tools and programs (www.CBLigand.org/AD). The current version of AlzPlatform consists of varieties of AD related proteins (a total of 320): (i) 172 enzymes such as acetylcholinesterase (ACES), monoamine oxidase B, angiotensin-converting enzyme, and cyclooxygenase-2; (ii) 38 membrane receptors, such as serotonin receptors, C−C chemokine receptors, and beta adrenergic receptors; (iii) 14 ion channels, such as glutamate *N*-methyl-D-aspartate (NMDA) receptors and neuronal acetylcholine receptors; and (iv) 96 other proteins (Figure 2A). For most of these targets, their RNA can be detected in the brain. For example, at least 283 of them are expressed in the corpus striatum and 281 are expressed in the cerebral cortex (Figure 2B). Besides the 194
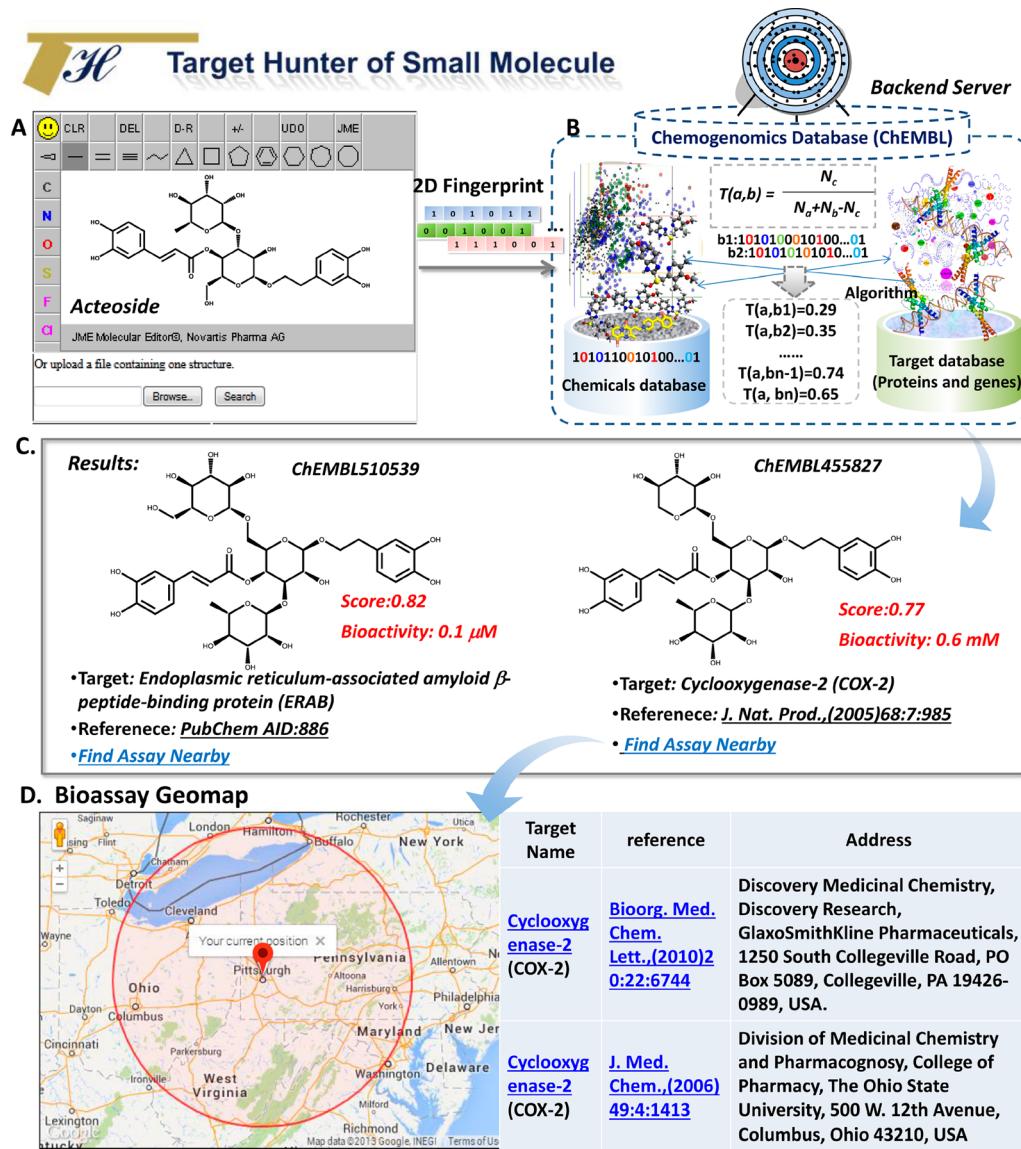
Figure 2. Chemogenomics data archived in AlzPlatform. (A) Summary of AD-related targets. (B) Tissue distribution of targets associated with AD. The yellow lines denote that these tissues are located in the central nervous system. (C) Drugs and compounds associated with AD targets. (D) AD drugs in different development phases and their corresponding targets. These approved and clinical trials AD drugs were classified by different phases with distinct colors. The yellow and black lines indicate the approved and discontinued AD drugs, respectively. The blue, pink, and green lines denote clinical trial drugs in phases I, II, and III, respectively. (E) AD targets and their drugs were plotted according to the pathways the targets involved. The blue and yellow bars indicate drugs and targets, respectively.

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Figure 3. Target identification and experimental validation for a bioactive natural product. (A) Time course of Aβ-induced paralysis in the transgenic *C. elegans*.CL4176 treated with standard nematode growth medium (NGM) and methyl sandaracopimarate (MS). Huperzine A was used as a positive control. (B) The chemical structure query window for AD targets prediction of MS by HTDocking server. (C) Molecular docking study of MS in the active site of PPARγ (PDB: 2OM9). The residues interact with MS through hydrophobic and hydrogen bonds. Yellow dotted lines denote hydrogen bonds and key residues are labeled in black. (D) The predicted target was further validated by in vitro PPARγ responsive luciferase assay. MS activates PPARγ in a concentration dependent manner with EC_{50} value of 15 μM. The results represent mean ±SD of values. The significance of differences from normal control group is at *$p < 0.05$.

drugs for AD clinical treatments, AlzPlatform also contains 225 additional drugs that are reported to interact with AD-related proteins but are used for treatment of other diseases. Some of these drugs may have the potential to be repurposed for AD research or treatment. Moreover, small molecules and their bioactivities against these targets could be used for systematic in silico screening of anti-AD lead compounds (Figure 2C). The statistics on these AD drugs, in different development phases, were plotted according to their interacting targets. As shown in Figure 2D, AD drugs approved by the FDA interact with hands of targets, including ACES and NMDA receptors. Among these proteins, muscarinic acetylcholine receptor M1 (ACM1) probably is not an ideal drug target for AD treatment because 7 of 8 drugs targeting ACM1

are discontinued or withdrawn due to undesirable adverse effects,[28] and only one is in phase II of clinical trial. In addition, it is well-known that cleavage of amyloid precursor protein (APP) by β-secretase (BACE1) is the rate-limiting step in beta-amyloid production, which suggests a potential target for drug development of AD. Currently, most of BACE1 inhibitors are still in phase I and II clinical trials. MK-8931 developed by Merck is the only BACE1 inhibitor that is currently in phase III clinical trial. However, the research and development pace on BACE1 as the major AD therapeutic target has been slow. Several concerns have been raised about the potential side effects of BACE1-targeted inhibitors,[29] because BACE1 also has important roles in myelination, retinal homeostasis, brain circuitry, and synaptic function.[30] Therefore, inhibition of the

Figure 4. Overview of the application of the TargetHunter program for AD target prediction of small molecules. (A) Input interface; (B) backend server; (C) output predicted results; and (D) the Bioassay GeoMap function can be used to find potential collaborators for targets validation experimentally.
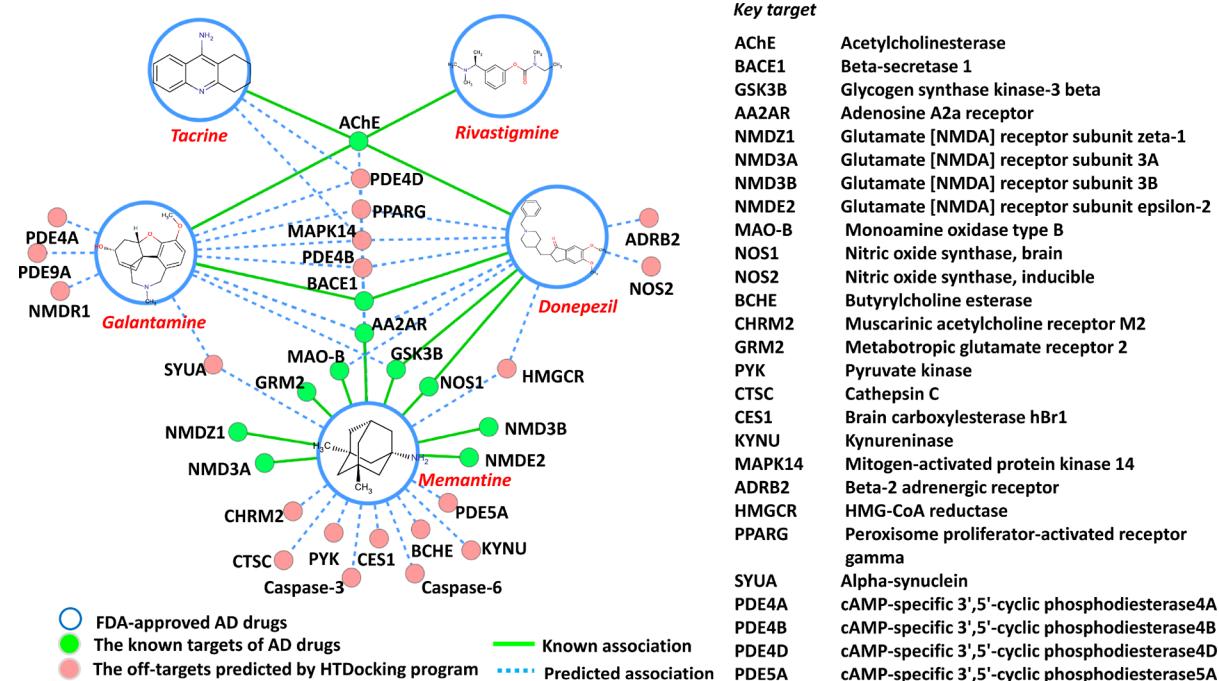
BACE1 enzyme could have toxic consequences. PEN2 could be a good target because it has a gradient number of drugs (9, 2, and 1, respectively) in phases I, II, and III.

The statistics of AD targets and their drugs were plotted according to the pathways the targets involved (Figure 2E). It is not a surprise that Alzheimer's disease pathway (KEGGID: hsa05010) is among the top of the pathway list. We also notice that pathways related with drug addiction, such as amphetamine addiction (KEGGID: hsa05031), cocaine addiction (KEGGID: hsa05030), and alcoholism (KEGGID: hsa05034), are also enriched on the top list, which could imply that AD shares some common pathways with drug addictions.[31] Furthermore, our data show that cannabinoid receptors, the key drug abuse related proteins, are among the top list, which is congruent with the reports that cannabinoid receptors are important in the pathology of AD, and cannabinoids succeed in preventing the neurodegenerative process in AD.[32]

AlzPlatform also features an integrated cloud computing service with intrinsic scalability and convenient features for further expansion. It provides powerful computing and sourcing services for both computational queried data storage and reretrieval, which can facilitate AD drug research and development. In the next section, three case studies demonstrate the usage of these computational tools on data-mining of the chemogenomics database for AD drug research.

Case Study 1: Prediction and Experimental Validation of Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) as the Neuroprotective Target of a Natural Product Methyl Sandaracopimarate (MS). Target identification of small chemicals is essential for unraveling the underlying mechanisms of their bioactivities. Often, natural products exhibit significant efficacy, yet their molecular mechanisms remain elusive. We used a natural product to illustrate how the AlzPlatform and high-throughput docking (HTDocking, https://www.cbligand.org/AD/) can be used to identify potential targets and explore the mechanism of action for natural products in which there are often multiple chemical components.

Key target

AChE	Acetylcholinesterase
BACE1	Beta-secretase 1
GSK3B	Glycogen synthase kinase-3 beta
AA2AR	Adenosine A2a receptor
NMDZ1	Glutamate [NMDA] receptor subunit zeta-1
NMD3A	Glutamate [NMDA] receptor subunit 3A
NMD3B	Glutamate [NMDA] receptor subunit 3B
NMDE2	Glutamate [NMDA] receptor subunit epsilon-2
MAO-B	Monoamine oxidase type B
NOS1	Nitric oxide synthase, brain
NOS2	Nitric oxide synthase, inducible
BCHE	Butyrylcholine esterase
CHRM2	Muscarinic acetylcholine receptor M2
GRM2	Metabotropic glutamate receptor 2
PYK	Pyruvate kinase
CTSC	Cathepsin C
CES1	Brain carboxylesterase hBr1
KYNU	Kynureninase
MAPK14	Mitogen-activated protein kinase 14
ADRB2	Beta-2 adrenergic receptor
HMGCR	HMG-CoA reductase
PPARG	Peroxisome proliferator-activated receptor gamma
SYUA	Alpha-synuclein
PDE4A	cAMP-specific 3',5'-cyclic phosphodiesterase4A
PDE4B	cAMP-specific 3',5'-cyclic phosphodiesterase4B
PDE4D	cAMP-specific 3',5'-cyclic phosphodiesterase4D
PDE5A	cAMP-specific 3',5'-cyclic phosphodiesterase5A

Figure 5. Illustration of HTDocking server (https://www.cbligand.org/AD/) for polypharmacology analysis of 5 approved AD drugs. The large circles (cyan) represent FDA-approved AD drugs (tacrine, donepezil, rivastigmine, galantamine, and memantine). Each drug is linked to its predicted targets. Among them, the green nodes and edges denote the known targets of drugs. Others pink nodes represent new potential off-targets and their interactions are linked by cyan dotted edges.

In our previous study, the extract of seeds of *Platycladus orientalis* can significantly extend lifespan of *C. elegans* and protect against Aβ toxicity in transgenic *C. elegans* expressing human Aβ.[33] In addition, methyl sandaracopimarate (MS), a known diterpenoid compound, was isolated and identified from the active extract. In order to evaluate the protective effect of the compound against Aβ-induced toxicity, the paralysis assay was conducted in the transgenic *C. elegans*.CL4176 strain, which expresses human $A\beta_{1-42}$. As shown in Figure 3A, MS significantly protected against Aβ-induced rapid paralysis at 300 μM in comparison with the untreated control ($p < 0.05$). Moreover, the mean survival rate of the treated groups was increased by 7.2%.

To explore further the underlying mechanisms of neuroprotection for MS, the AlzPlatform and HTDocking program were used to predict the possible targets. The result shows that the compound is predicted to interact with the peroxisome proliferator-activated receptor gamma (PPARγ), acetylcholinesterase and cGMP-specific 3', 5'-cyclic phosphodiesterase (PDE5A) (Figure 3B). Among them, PPARγ is listed as one of the top targets with docking score 6.5, suggesting that PPARγ is likely to be a key target for MS. Furthermore, the predicted interactions between the compound and the ligand binding domain of PPARγ were shown in Figure 3C. Residues Phe264, Ser342, Ile341, Phe287, Cys285, Arg288, Gly284, and His266 form a hydrophobic interaction cleft around MS, and the carbonyl group of the compound exhibits the hydrogen-bonding interactions with the backbone of His266 and Lys265, which are vital residues modulating the activation of the receptor,[34] suggesting that MS might be a potential PPARγ agonist.

The prediction was further validated by in vitro PPARγ responsive luciferase assay that allows the quantification of the ligand activated PPARγ on the basis of its specific binding to PPRE sequences.[35] The result indicated that the MS activates PPARγ in a concentration dependent manner with EC_{50} value of 15 μM (Figure 4D). According to accumulating evidence, PPARγ is involved in the regulation of β-secretase1 and neuroinflammatory responses.[13,36] PPARγ overexpression decreases β-secretase1 gene transcription and reduces the intracellular and plaque Aβ generation in vivo.[37,38] Moreover, neurotoxic activities under inflammatory conditions of microglia and astrocytes are reduced by PPARγ agonists.[39] On the basis of our study, enhancing PPARγ expression may be one of the mechanism by which MS protected against Aβ-induced toxicity.

Case Study 2: Prediction of Endoplasmic Reticulum-Associated Amyloid Beta-Peptide-Binding Protein (ERAB) and Cyclooxygenase-2 (COX-2) as Novel Targets for Acteoside and Search of Potential Collaborators for Experimental Validation by TargetHunter. In addition to the protein-based HTDocking program, we have established the ligand-based TargetHunter tool (http://cbligand.org/TargetHunter) which is an online program for targets identification and drug repurposing. We also used natural product to illustrate the application of TargetHunter for target prediction. Acteoside, isolated from *Orobanche minor*, can significantly inhibit the aggregation of amyloid-β with IC_{50} value of 8.9 μM[40] and can attenuate the Aβ induced toxicity.[41] However, the neuroprotective mechanisms are still not exactly known. To identify further the underlying targets, the structure of acteoside was submitted as a query to the TargetHunter program. As shown in Figure 4A−C, two related compounds (ChEMBL510539 and 455827, with scores of 0.82 and 0.75, respectively) were retrieved. The first compound, ChEMBL510539, targets the endoplasmic reticulum-associated amyloid beta-peptide-binding protein (ERAB, tested in the PubChem bioassay AID: 886 with potency value of 0.1 μM). The protein is an

Table 1. Comparison of the Experimental pK_i and the Predicted pK_d Values for the FDA-Approved AD Drugs

drug	target	experimental K_i (nM)	experimental ($-$pK_i)	HTDocking score predicted ($-$pK_d)
tacrine	acetylcholinesterase	225[a]	6.65	6.11
galantamine	acetylcholinesterase	62[b]	7.21	7.18
rivastigmine	acetylcholinesterase	920[c]	6.04	6.08
donepezil	acetylcholinesterase	23[d]	7.64	7.25
	glutamate [NMDA] receptor subunit 3a	700[e]	6.15	7.17
	glutamate [NMDA] receptor subunit 3b	540[f]	6.27	6.33
memantine	glutamate [NMDA] receptor subunit zeta-1	1200[g]	5.92	6.82
	glutamate [NMDA] receptor subunit epsilon 2	1020[h]	6.00	6.33

[a]Experimental data from ref 66. [b]Experimental data from ref 67. [c]Experimental data from ref 68. [d]Experimental data from ref 69. [e]Experimental data from ref 70. [f]Experimental data from ref 71. [g]Experimental data from ref 72. [h]Experimental data from ref 73.

intracellular Aβ-binding protein that contributes to the pathogenesis of AD. The toxic effect of Aβ on neuroblastoma cells is prevented by blocking ERAB and is enhanced by over-expression of ERAB.[42,43] Therefore, the inhibition of ERAB is likely to the cause of the protection against Aβ induced toxicity by acteoside. In addition, the Cyclooxygenase-2 (COX-2) targeted by another compound (ChEMBL455827) is an important target associated with inflammatory regulation in the pathogenesis of AD.[44] To facilitate the further target validation, BioassayGeoMap (http://cbligand.org/TargetHunter/bioassaygeomap.php) is implemented in the AlzPlatform to locate the nearby potential collaborators who reported their established bioassay. As show in Figure 4D, two research laboratories near the University of Pittsburgh were found by the program, and they could be potential collaborators for further experimental validation of the predicted target COX-2.

Case Study 3: The Prediction of Polypharmacology of Known AD Drugs for Multitarget Drug Discovery. Prediction of polypharmacology of known drugs is highly useful for finding new system polypharmacotherapy. There has been increasing interest in identifying additional targets for known drugs and predicting drug–target associations by in silico and experimental methods.[45] Accordingly, we used established chemoinformatics tools to predict potential target and polypharmacology for five FDA-approved AD drugs. Among them, four AD drugs (tacrine, rivastigmine, galant-amine, and donepezil) are acetylcholinesterase (AChE) inhibitors and the other one (memantine) is an N-methyl-D-aspartate (NMDA) receptor antagonist.[46] The protein targets identified by HTDocking program for each known AD drug were tabulated in an output window and ranked by docking scores. The five drugs and their top candidate targets (docking score higher than 6.0, green and pink nodes) were compiled to build a polypharmacological interacting network with Cyto-scape 2.8 (Figure 5). Unsurprisingly, the result shows that the known acetylcholinesterase and NMDA receptors (green nodes) were targeted by four AChE inhibitors and memantine, respectively. Moreover, the comparison of the predicted and experimental pK_i values for known AD drugs was visualized in Table 1. The result illustrates that the predicted targets and the binding affinities are correlated with reported experimental data. Indeed, the additional predicted associations or drug/protein networks (green nodes and edges), such as beta-secretase1 (BACE1), glycogen synthase kinase-3 beta (GSK3B), and mono-amine oxidase type B (MAO-B), have already been reported in the literature[47−55] (Table 2), indicating the reliability of the HTDocking program. Also, the remaining predicted targets (pink nodes) could be the new targets for the known drugs that merit further validation by experiments.

Table 2. Verification of Other Predicted Targets by Experiments for FDA-Approved AD Drugs

drug	target	experimental potency	ref
galantamine	beta-secretase1 (BACE1)	44% decrease in BACE1 level/0.3 μM	74
donepezil	beta-secretase1 (BACE1)	IC$_{50}$ = 3.2 μM	75
donepezil	nitric oxide synthase, brain (NOS1)	increase expression of NOS1/5 mg/kg in vivo	76
donepezil	glycogen synthase kinase-3 beta (GSK3B)	decrease 77% in vivo/ (1 mg/kg)	77
memantine	monoamine oxidase type B (MAO-B)	inhibition of 64%/1 mM	78
memantine	Adenosine receptor A2a (AA2AR)	increase 43% in vivo (25 mg/kg)	79
memantine	nitric oxide synthase, brain (NOS1)	active in vivo (10 mg/kg)	80
memantine	metabotropic glutamate receptor 2 (GRM2)	active/100 μM	81
memantine	glycogen synthase kinase-3 beta (GSK3B)	inhibit GSK-3/100 μM	82

Another finding in the network is the polypharmacological effects for two known drugs, galantamine and memantine (Figure 5). The network shows that besides binding to AChE, galantamine is predicted to interact with BACE1, mitogen-activated protein kinase 14 (MAPK14), and adenosine A2a receptor (AA2AR). Inhibitions of these proteins have effects on decreasing the Aβ production and Aβ-induced toxicity[47] and increasing the expression of nicotinic receptors.[56] Similarly, memantine is predicted to interact with GSK3B, BACE1, MAO-B, and nitric oxide synthase 1 (NOS1) besides binding to NMDA receptors. Inhibition of these proteins can prevent the accumulation of the misfolded proteins (Tau and Aβ) and enhance neuronal function.[51,55,57] Such in silico analysis of polypharmacological effects may explain why the combined use of memantine and galantamine can produce greater memory improvement than either treatment alone in clinical trials,[58] which will guide to design and discover new drug-like leads with the multitarget synergetic therapeutics for AD.

4. DISCUSSION

Alzheimer's disease is a complex multifactorial disorder.[1] With the extensive accumulation of molecular biological elucida-tions of AD signaling pathway at genes and proteins levels, several AD-related databases have been developed, such as AlzGene[6] and Alzpathway.[7] These databases together with other disease specific databases, such as HLungDB (human lung cancer database)[59] and CVDHD (Cardiovascular Disease Herbal Database),[60] provide alternative avenues to explore the molecular mechanisms and signaling pathways of diseases.

However, there is no comprehensive AD specific chemical genomics knowledgebase available for polypharmacology targets identification to facilitate novel AD drug discovery. Comparing with other general in silico docking platforms, such as DOCK Blaster,[61] our AlzPlatform also offers an AD domain-specific chemogenomics database with user-friendly query functions and polytarget identification algorithms implemented with ligand-based TargetHunter and protein structure-based HTDocking. AlzPlatform provides a promising alternative to bridge the knowledge gap between biology and chemistry related to AD, enhancing AD target research, polypharmacology analysis, and new drug discovery.

Our pilot studies demonstrated that the protein-based HTDocking program has been successfully used to identify the AD-related targets for small molecules, such as drugs, lead compounds, and natural product. HTDocking program provides a list of predicted targets and corresponding computational docking-based binding affinity (scores). The reliability of the HTDocking program has been confirmed by comparison of the predicted with the experimental pK_i values reported for known AD drugs, also by our in vitro experimental validation for an active natural product. Of course, HTDocking has certain limitations on availability of high-quality protein structures. As a complementary partner, the ligand-based TargetHunter tool is designed to predict the potential targets and off-targets of chemicals using our established chemogenomics database.[9] Our established programs also can be useful in the application of drug repurposing, and in the investigation of potential side effects related to AD drugs.[62] TargetHunter is a powerful cloud-computing tool with attractive features: usability, flexibility, and veracity. Furthermore, it embeds an important query function, i.e., the geographical bioassay locator can assist users to find nearby collaborators who have reported suitable bioassays in order to validate the target prediction, which will enhance the productivity of collaborative researchers and facilitate the chemogenomics data sharing and information communications.

In addition, our chemoinformatics tools can be used in mapping the drug−target network for polypharmacology investigation. Understanding drug−target associations can benefit the discovery of novel therapeutic applications and also reveal the possible side effects of drugs. It will transform the one-target-one-drug development process to a new multitarget−multidrug paradigm, thereby expanding the opportunity for system multitarget drug discovery.[63−65] By assembling many AD related drugs and small molecules with target annotations, AlzPlatform provides specific data and tools to help researchers conduct in-depth analysis for AD related targets and drugs and will also enable the chemists to design multitarget small molecules and to perform bioactivity test with the collaborators, which will boost the more effective system pharmacotherapy and drug design discovery.

5. CONCLUSION

AlzPlatform, a one-stop integrated cloud computing server, has been specifically developed as a public repository http://www.cbligand.org/AD/ for AD drug and targets research. The cloud computing server will augment our capacity to benefit the AD research community and will help break to the knowledge barrier, enhance the productivity of chemogenomics researchers, and accelerate advances in system biology computer-aided drug design by consolidating existing data and computational technology.

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Notes
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