A strategy to find novel candidate anti-Alzheimer's disease drugs by constructing interaction networks between drug targets and natural compounds in medical plants

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Background Alzheimer' disease (AD) is an ultimately fatal degenerative brain disorder that has an increasingly large burden on health and social care systems. There are only five drugs for AD on the market, and no new effective medicines have been discovered for many years. Chinese medicinal plants have been used to treat diseases for thousands of years, and screening herbal remedies is a way to develop new drugs.

Methods We used molecular docking to screen 30,438 compounds from Traditional Chinese Medicine (TCM) against a comprehensive list of AD target proteins. TCM compounds in the top 0.5% of binding affinity scores for each target protein were selected as our research objects. Structural similarities between existing drugs from DrugBank database and selected TCM compounds as well as the druggability of our candidate compounds were studied. Finally, we searched the CNKI database to obtain studies on anti-AD Chinese plants from 2007 to 2017, and only clinical studies were included.

Results A total of 1476 compounds (top 0.5%) were selected as drug candidates. Most of these compounds are abundantly found in plants used for treating AD in China, especially the plants from two genera Panax and Morus. We classified the compounds by single target and multiple targets and analyzed the interactions between target proteins and compounds. Analysis of structural similarity revealed that 17 candidate anti-AD compounds were structurally identical to 14 existing approved drugs. Most of them have been reported to have a positive effect in AD. After filtering for compound druggability, we identified 11 anti-AD compounds with favorable properties, 7 of which are found in anti-AD Chinese plants. Of 11 anti-AD compounds, 4 compounds 5862, 5863, 5868, 5869 have anti-inflammatory activity. The compound 28814 mainly has immunoregulatory activity. The other 6 compounds have not yet been reported for any biology activity at present.

Discussion Natural compounds from TCM provide a broad prospect for the screening of anti-AD drugs. In this work, we established networks to systematically study the connections among natural compounds, approved drugs, TCM plants and AD target proteins with the goal of identifying promising drug candidates. We hope that our study will facilitate in-depth research for the treatment of AD in Chinese medicine.

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ABSTRACT

Background

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Methods

We used molecular docking to screen 30,438 compounds from Traditional Chinese Medicine (TCM) against a comprehensive list of AD target proteins. TCM compounds in the top 0.5% of binding affinity scores for each target protein were selected as our research objects. Structural similarities between existing drugs from DrugBank database and selected TCM compounds as well as the druggability of our candidate compounds were studied. Finally, we searched the CNKI database to obtain studies on anti-AD Chinese plants from 2007 to 2017, and only clinical studies were included.

Results

A total of 1476 compounds (top 0.5%) were selected as drug candidates. Most of these compounds are abundantly found in plants used for treating AD in China, especially the plants from two genera *Panax* and *Morus*. We classified the compounds by single target and multiple targets and analyzed the interactions between target proteins and compounds. Analysis of structural similarity revealed that 17 candidate anti-AD compounds were structurally identical to 14 existing approved drugs. Most of them have been reported to have a positive effect in AD. After filtering for compound druggability, we identified 11 anti-AD compounds with favorable properties, 7 of which are found in anti-AD Chinese plants. Of 11 anti-AD compounds, 4 compounds 5862, 5863, 5868, 5869 have anti-inflammatory activity. The compound 28814 mainly has immunoregulatory activity. The other 6 compounds have not yet been reported for any biology activity at present.

47 **Discussion**

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50 compounds, approved drugs, TCM plants and AD target proteins with the goal of identifying

51 promising drug candidates. We hope that our study will facilitate in-depth research for the

52 treatment of AD in Chinese medicine.

INTRODUCTION

Alzheimer's disease (AD), a progressive and ultimately fatal degenerative brain disorder, is thought to be one of the main causes of dementia in senior citizens (Fan & Chiu 2014; Song et al. 2015) (Fan & Chiu 2014; Song et al. 2015). The psychiatric symptoms observed in AD patients, include irritability, changes in mood or personality, paranoid delusions and hallucinations (Coyle et al. 1983). The pathological features of AD include senile plaques and neurofibrillary degeneration (Dickson 1997). Degeneration, caused by neurofibrillary tangles (intracellular fibrous aggregations of tau protein), mainly occurs in brain regions involved in learning, memory, and emotional behaviors, such as the hippocampus, basal forebrain, entorhinal cortex and amygdala (Mattson 2004). The various hypotheses regarding AD pathogenesis suggest the involvement of many pathways and target proteins, such as the amyloid (Goedert & Spillantini 2006), cholinergic (Craig et al. 2011), oxidative stress (Pratico 2008), glutamatergic (Bezprozvanny & Mattson 2008), inflammatory (Trepanier & Milgram 2010) and metal hypotheses (Bonda et al. 2011). However, the causes of AD remain unclear due to the complexity of this multifactorial disease (Armstrong 2013). To date, five symptom-relieving drugs are available to AD patients in a clinical setting, including four cholinesterase inhibitors and one N-methyl-D-aspartate (NMDA) -receptor antagonist. However, there is currently no method for reversing or curing AD (Cummings et al. 2014; Peng et al. 2016), and Tacrine has been discontinued in the United States market. Thus, the discovery of new drugs for treating AD patients remains a challenge.

Traditional Chinese Medicines (TCMs) have been used in therapy and for treating various diseases for several thousand years of Chinese history, and some natural ingredients in TCMs have been successfully developed into drug, such as artemisinin. Screening natural ingredients or compounds from herbal remedies and TCMs may be an effective way to develop new drugs (Normile 2003; Sanderson 2011; Sucher 2013). For example, interactions between some ingredients from anti-AD herbs and corresponding anti-AD target proteins (Sun et al. 2013) as well as between 12 ginger components and 13 anti-AD target proteins have been reported (Azam

81   et al. 2014). Many AD target proteins have previously been validated, including AchE

82   (Yiannopoulou & Papageorgiou 2013), BchE (Darvesh 2016; Mushtaq et al. 2014), RAGE (Cai

83   et al. 2016; Deane 2012), TNF-alpha (Leszek et al. 2016; Wyss-Coray & Rogers 2012), PLA2

84   (Gentile et al. 2012; Lee et al. 2011) and others. These proteins are involved in a variety of AD-

85   associated pathways. Because we wanted to study a comprehensive range of AD target proteins,

86   we selected 30 target proteins with protein crystal structures (including ligand present in the

87   crystal structure) from all of the validated AD therapeutic target proteins provided by Thomson

88   Reuters Integrity database as our research objects. To explore the interactions between the 30

89   validated AD therapeutic target proteins, which represent a variety of hypotheses regarding AD

90   pathogenesis, and compounds in TCM plants, we established interaction networks among the

91   target proteins, compounds, approved drugs and TCMs. Finally, we identified 11 structurally

92   novel candidate anti-AD compounds with favorable druggability properties, 7 of which are found

93   in anti-AD Chinese plants. The 11 compounds identified in this study may be valuable in future

94   anti-AD drug development, though further experiments are needed to prove their usefulness as

95   drugs. The results suggest that the strategy of interaction network-based drug discovery may be

96   very helpful for drug development.

97   **MATERIALS AND METHODS**

98   **Data collection and preprocessing**

99   More than 60,000 natural compounds from 8,529 different plants were from the TCM

100   Database@Taiwan (http://tcm.cmu.edu.tw/). This web-based database is the most comprehensive

101   non-commercial database of TCM(Chen 2011). We obtained the 3D structures of the compounds

102   from the database as mol2 files and converted them to the pdbqt format and SMILES string using

103   Open Babel toolbox v2.3.1(O'Boyle et al. 2011).

104   The validated therapeutic AD target proteins and pathways were provided by Thomson

105   Reuters Integrity database (https://integrity.thomson-pharma.com/integrity/). Studies on the

106   target proteins were obtained using Pubmed and PMC (https://www.ncbi.nlm.nih.gov/pubmed).

107   The target proteins structures were obtained from the Protein Data Bank (PDB) database

108   (http://www.rcsb.org/pdb/home/). We selected target proteins of interest by determining whether

109   studies relating them to AD and crystal protein structures (with ligand present) were available.

110   Inhibitors or agonists were used to confirm the ligands in the references we identified. We

111   ultimately selected 30 target proteins. Information regarding the 30 target proteins, such as the

112   Integrity name, corresponding name in the Uniprot database (http://www.uniprot.org/), Uniprot

113   ID, PDB ID, ligand ID and document IDs in Pubmed and PMC are available in Supplementary

114   Table 1. The 3D structures of the proteins are available as files in the pdb format. This format

115   was converted to the pdbqt format using AutoDock tools v1.5.6 (Morris et al. 2009), and the 3D

116   view was generated by Discovery Studio v3.1 (http://accelrys.com/products/collaborative-

117   science/biovia-discovery-studio/).

118   **Molecular docking between natural compounds and AD target proteins**

119   Docking is tantamount to position the ligand in different orientations and conformations

120   within the binding site to calculate optimal binding geometries and energies. Interactions

121   between natural compounds and the AD target proteins were previously predicted using

122   AutoDock Vina 1.1.2 (Trott & Olson 2010). The docking binding site center for each target

123   protein is the structural binding center of the ligand present in the crystal structure. The ligands

124   were confirmed by the studies we identified. The coordinates of the docking center, ligand ID in

125   PDB database and supporting documents are shown in Supplementary Table1. To allow free

126   rotation of the compounds, the search space was set to $25 \times 25 \times 25$ Å in each axis. The default

127   settings were used for all of the other docking parameters. Each docking was performed by a

128   command that contained the space size and three-dimensional coordinate of the docking center.

129   For each compound, the binding pose with the lowest energy for each docking test was

130   considered the best binding mode. A lower energy score indicated a stronger binding affinity

131   between the ligand and receptor. The compounds with the top 0.5% docking score were chosen

132   as the candidate ligands for each target protein.

133   **Validation of the docking results**

134   To validate the docking results, three methods were used. First, we manually checked the

135   docking results and visualized the interaction between the compound and receptor to verify that

136   the compound was in the binding pocket. Second, the original ligand in the crystal structure was

137   set as the reference. The docking energy of candidate anti-AD compounds should be better than

138   or comparable to that of original ligand. Third, we check whether the top 0.5% TCM compounds

139   are similar to the existing drugs that have been reported in anti-AD research.

140   **Interactions among the target proteins, compounds and plants**

141   The interaction between each target protein and its best-binding TCM compound was

142   displayed using the PyMOL (PyMOL Molecular Graphics System, version 1.7) program

143   (https://pymol.org). The pharmacophore was displayed using Discovery Studio v3.1.

144   The target-compound and target-plant networks were constructed using Cytoscape v3.4.0

145   (Shannon et al. 2003). In these networks, the target protein and compound were considered to be

146   connected if the compound successfully docked to the target protein, and the target protein and

147   the plant were considered to be connected if the plant with the compound was able to interact

148   with the target protein. The strength of the links is represented by the line' thickness, which

149   indicates the number of compounds shared between the target protein and plant.

150   **Collection of anti-AD plants from Chinese medicine prescription**

151   The term "senile dementia" was searched in the subject column of the CNKI database

152   (http://www.cnki.net/) to retrieve Chinese medicine prescriptions for anti-AD from the relevant

153   Chinese articles. Articles from clinical studies between 2007 and 2017 were selected. Chinese

154   medicine prescriptions and the usage frequency were also obtained from these articles. The

155   common anti-AD plants in traditional Chinese clinical medicines were identified from the

156   prescriptions. For reference, Supplementary Table 2 shows information regarding the Chinese

157   version of the raw prescription data with corresponding English, the Latin name of the anti-AD

158   plants in each prescription, the patient number (male and female if available), the article title of

159   the study, published data (years) and the article format.

160   **Similarity between candidate compounds and existing drugs**

161   The Tanimoto coefficient (Tc) and Pybel(O'Boyle et al. 2008) Python package were used to

162 measure the structural similarities between compounds. The fingerprint FP2 implemented in

163 Pybel was generated for each structure and used to calculate Tc. Tc is defined as Tc = C(i, j)/U(i,

164 j), where C(i, j) is the number of common features in the fingerprints of molecules i and j and U(i,

165 j) is the number of all of the features in the union of the fingerprints of molecules i and j. If the

166 fingerprints of two compounds are Tc = 1, even if they differ among themselves by isolated

167 instances of C, N or O atoms, they will be considered structurally identical.

168 Cytoscape v3.4.0 was used to construct a network linking the candidate compounds, their

169 target proteins and structurally identical drugs. A natural compound and an existing drug in the

170 DrugBank database (Wishart et al. 2006) were considered to be connected, if their Tc score was

171 1. The natural compounds and their target proteins were also connected in this network.

172 Clusters of potential candidate compounds for AD

173 One-thousand-four-hundred-seventy-six compounds located in the top 0.5% of all

174 compounds that docked with 30 target proteins were regarded as potential candidate compounds

175 for AD. The cluster ligands protocol in BOVIA Pipeline Pilot V8.5

176 (http://accelrys.com/products/collaborative-science/biovia-pipeline-pilot/) was used to cluster the

177 compounds. During clustering, a set of compounds was assigned to different clusters based on

178 the similarity of their properties. Clustering was based on the root-mean-square (RMS)

179 difference of the descriptor properties or the Tanimoto distance for fingerprints. In our study, we

180 clustered the compounds based on the Tanimoto distance using the fingerprint FP2. Cluster

181 ligands were performed by the number of size or the number of molecules per cluster. The

182 default parameter in Pipeline Pilot V8.5 was set to fixed number of 10 clusters. For simplicity,

183 we used default parameters to cluster our compounds. The molecule with the lowest total

184 distance to all other members of the cluster was considered the cluster center.

185 ADMET and logP properties for candidate compounds for AD

186 The ADMET and logP properties of candidate compounds for AD were estimated using

187 Discovery Studio. ADMET refers to absorption, distribution, metabolism, excretion and toxicity

188 and logP refers to the logarithm of the partition coefficient. The ADMET properties, including

189 aqueous solubility, blood brain barrier penetration (BBB), human intestinal absorption (HIA),

190 plasma protein binding (PPB) and hepatotoxicity, as well as logP were used to filter the

191 compounds. The values of these properties were set as the controlled parameters, which were

192 3~4 (3: good; 4: optimal) for aqueous solubility, 1~2 (1: high; 2: medium) for BBB, 0 (0: good)

193 for HIA, FALSE for both PPB and hepatotoxicity and logP < 5.

194 **RESULTS**

195 **Molecular docking of natural compounds and embedded ligands to the 30 AD target**

196 **proteins**

197 Of the 60,000 compounds in the TCM Database, 30,438 contain plant information, and these

198 compounds were docked with the 30 selected AD target proteins. The original ligands of each

199 target protein were also docked to their corresponding target proteins. The docking results are

200 shown in Table 1. The docking scores of ligands embedded in the protein crystal structure ranged

201 from −3.31 to −12.65 (kcal/mol). The lowest docking energy scores for the 30 target proteins

202 ranged from −8.44 to −14.5 (kcal/mol). Some target proteins, such as Caspase-3, QC, IDO and

203 GLP-1R, were able to bind over 20,000 natural compounds with docking scores superior to those

204 of their embedded ligands. However, the docking scores of the target RAR with natural

205 compounds were inferior to those of its embedded ligand.

206 Because many TCM compounds can bind to AD target proteins, for each target protein, only

207 the top 0.5% of compounds in terms of docking scores (a total of 1476 compounds) were

208 selected as candidate compounds for AD. The original study of AutoDockVina showed that the

209 success rate of Vina is 80% (RMSD <2) for an independent validation dataset. Furthermore,

210 Vina achieves a low standard error of 2.85 kcal/mol compared with the experimental free

211 energies. There is a highly positive correlation between the predicted and experimental free

212 energies of binding. In our study, almost all of the docking energies of the top 0.5% of

213 compounds bound to target proteins were superior to those of their embedded ligands (Fig. 1).

214 Thus, the 1,476 compounds are likely candidate compounds for AD.

215 **Analysis of the interactions between target proteins and ligands including TCM**

216 **compounds, original ligands and approved AD drugs**

217   The docking pose interactions between the target proteins and ligands including their best-

218 binding TCM compounds and original ligands are shown in Fig. 2A and Supplementary Fig. 1 to

219 29. The figures show side-by-side-comparisons of best TCM ligands and original ligands. The

220 best TCM ligands and original ligands are located in the same binding pocket for each target

221 protein and they all have some common residues. Taking AchE as an example: the best-binding

222 TCM compound for AchE is 24829, the original ligand is Huperzine A, and their common

223 residues are TYR-124, PHE-297, PHE-338, TYR-337, ASP-74. Furthermore, we compared the

224 structures of the best-binding TCM compounds and previously known ligands (Supplementary

225 Table 3) and found the structures to be different. The Tc scores are between 0.06 and 0.48.

226 Therefore, most of the best-binding TCM compounds are novel scaffolds.

227   We analyzed the pharmacophore of SIRT1 using the top 10 TCM compounds binding the

228 target protein (Fig. 2B). The pharmacophore model consists of one hydrogen bond acceptor

229 (HBA, green) and five hydrophobic centers (blue); therefore, we think that compounds that have

230 this model may easily bind with SIRT1.

231   We also compared the docking energy scores between the approved AD drugs and the

232 candidate compounds for the protein AchE (Supplementary Table 4). The approved AD drugs

233 that we analyzed were Donepezil, Galantamine and Rivastigmine. All the three drugs were AchE

234 inhibitors. The average docking energy score of the candidate compounds was superior to that of

235 the approved AD drugs for the protein AchE. The docking pose interaction of the three drugs and

236 the best-binding TCM compound with the protein AchE is shown in Supplementary Fig. 30. The

237 three drugs and best-binding TCM compound were located in the same binding pocket, and their

238 docking poses were different because their structures were different.

239 **Analysis of single-target and multi-target compounds**

240   Among the 1,476 candidate compounds for AD, there were 850 compounds with a single

241 target and 626 compounds with multiple targets (see Supplementary Fig. 31 and 32). The single-

242 target compounds corresponding to each target varied widely in number. For example, in single-

243   target networks, target SIRT1 corresponds to 30 compounds, whereas target lyn just corresponds

244   to 4 compounds. The multi-target compounds were classified into 18 networks based on their

245   corresponding target numbers, which ranged from 2 to 24. As the number of targets per

246   compound increased, the number of compounds in that category decreased. For example, the

247   two-target network contained 260 compounds, whereas the three-target and four-target networks

248   contained 90 and 77 compounds, respectively. Finally, we observed that compound 24508 could

249   bind to 25 AD target proteins. The structure and network of compound 24508 are shown in

250   Supplementary Fig. 32.

251   **Candidate AD compounds and their enrichment plants**

252   We mapped 1476 candidate AD compounds (corresponding to 30 AD target proteins) to the

253   334 plants. The plant numbers for each target protein ranged from 42 to 71, whereas the

254   compound numbers for each target protein ranged from 62 to 132 (Fig. 3 and Supplementary Fig.

255   33).

256   We selected 101 clinically related studies out of over 10,000 senile dementia-related articles

257   and identified 141 anti-AD traditional Chinese plants from the clinical prescriptions in these

258   articles. The 141 traditional Chinese anti-AD plants were classified based on their functional

259   properties in the TCM database. Most of the 141 anti-AD plants were in the 'Tonifying,

260   Replenishing' category, and the plants in this category accounted for 28.45% of all anti-AD

261   plants (Supplementary Fig. 34).

262   The best-associated plant for each target protein contained the greatest number of

263   compounds capable of docking with the target protein (Table 2 and Fig. 4). The number of

264   compounds in Table 2 was based on the top 0.5% of compounds successfully docking to each

265   target protein. Thus, 30 target proteins corresponded to 16 best-associated plants, of which the

266   top 5 plants were anti-AD traditional Chinese plants, including *Panax* and *Morus*, which

267   corresponded to 7 and 5 target proteins, respectively, as well as *Salvia, Rheum* and *Paeonia*.

268   **Similarities between candidate compounds and existing drugs**

269   A structural comparison between all existing approved drugs recorded in the DrugBank and

270   the top 0.5% of the natural compounds tested demonstrated that some compounds were identical

271   to existing drugs (Tc=1). The connection network among candidate compounds, existing drugs

272   and AD target proteins was established, and the chemical structures of the compounds are also

273   shown in Fig. 5. There were 17 candidate compounds, 14 existing drugs and 25 AD-associated

274   target proteins in the network. The 14 drugs included Lutein (DB00137), Vitamin A (DB00162),

275   Vitamin E (DB00163), Azelaic Acid (DB00548), Ergotamine (DB00696), Estradiol (DB00783),

276   Menthol (DB00825), Drostanolone (DB00858), Glyburide (DB01016), Tubocurarine (DB01199),

277   Metocurine (DB01336), Yohimbine (DB01392), Lactose (DB04465), Artemether (DB06697).

278   Most of the drugs listed above have been reported to have a positive effect in AD. Some

279   studies have shown that Lutein is involved in preventing cognitive decline and decreasing the

280   risk of AD; thus, Lutein may contribute to the treatment of AD (Kiko et al. 2012; Min & Min

281   2014; Xu & Lin 2015). Similarly, Vitamin A, Vitamin E, Estradiol, Menthol, Glyburide and

282   Yohimbine are also considered useful in the prevention and treatment of AD (Bhadania et al.

283   2012; Dysken et al. 2014; Lamkanfi et al. 2009; Lan et al. 2016; Mohamd et al. 2011; Ono &

284   Yamada 2012; Peskind et al. 1995; Takasaki et al. 2011). Therefore, compounds with structures

285   similar to the existing drugs may also have anti-AD function by interacting with similar target

286   proteins.

287   Of the 17 candidate compounds, 11 can only bind with one target protein, whereas the rest,

288   which are similar to the 14 drugs discussed above, can interact with more than one target protein.

289   For example, compound 18491 which is similar to Menthol can only interact with the target

290   Ftase and compounds 19476 and 19477 are similar to Tubocurarine and Metocurine, respectively,

291   whereas compound 18582 which is similar to Ergotamine can bind with 17 target proteins.

292   **Structure clusters of candidate AD compounds**

293   To compare the structural features of the candidate AD compounds, the 1,476 candidate

294   compounds were assigned to 10 clusters. The structures of the cluster center compounds and

295   maximal common substructure of each cluster are shown in Fig. 6. The Generate Maximal

296   Common Substructure component must contain the proportion of the cluster molecules. The

297 proportion was set to 0.5 to find the largest maximal common substructure that was contained in

298 at least 50% of the cluster molecules. All of the compounds in the center of the cluster contained

299 the carbocyclic structure, similar to the five approved drugs for AD. The cluster sizes varied,

300 with the largest containing 464 compounds and the smallest containing only 6 compounds. Every

301 cluster had a primary target protein that could better combine with the compounds in the cluster.

302 The binding of the best member of the cluster to the primary target protein and their structures

303 are also shown in Fig. 6.

304   To see if there were similar or different scaffolds able to bind each target protein, clusters in

305 the sets of ligands that bind to each individual protein were shown in Supplementary Table 5 to

306 34. Each set of ligands was assigned to 10 clusters. The compounds in the center of the cluster do

307 not have the same scaffolds for each individual protein.

11 candidate compounds for AD with favorable ADMET and logP properties

309   Most orally approved drugs have favorable druggability properties. After ADMET and logP

310 filtering, 11 compounds from 1476 candidate anti-AD compounds possessed favorable properties

311 (Table 3). The 11 compounds all have good HIA and don't have PPB and hepatotoxicity.

312 Compound 28468 has optimal aqueous solubility. And compound 16167 has high BBB. Of the

313 11 compounds, 8 were single-target compounds, and the remaining 3 were multi-target

314 compounds. For example, compounds 5868, 9593, 10639, 28814 and 30713 could only bind with

315 one target protein (GABA(B), MAOB, PDE4, RAR and MAOA, respectively), whereas

316 compounds 5862, 5869 and 16167 shared one common target (AchE). Compound 5863 was able

317 to bind with three target proteins (AchE, GABA(B) and MGLUR), and compound 26629 and

318 28468 were able to interact with five target proteins. These 11 compound structures and their

319 corresponding plants are shown in Fig. 7. The plants corresponding to 7 of these compounds are

320 regarded as anti-AD plants in TCM, including *Curcuma kwangsiensis, Poria cocos, Lindera*

321 *aggregate, Ophiopogon japonicus (L. f.) Ker-Gawl.* and *Glycyrrhiza glabra*. 11 compounds

322 belong to different organic compound classes. Compounds 5862, 5863, 5868, 5869 belong to

323 linear diarylheptanoids. Compound 10639, 16167, 26629, 28468, 28814, 30713 belongs to

324 guanidines, pyridines, hydrophenanthrenes, angular pyranocoumarins, naphthyridines, 8-

325 prenylated isoflavanones, respectively. We also checked the 11 comopund structures and

326 existing research in pubchem database. Compounds 5862, 5863, 5868, 5869 have anti-

327 inflammatory activity and compound 28814 mainly has immunoregulatory activity. The other 6

328 compounds have not yet been reported for any biology activity at present.

329 **DISCUSSION**

330   Candidate compounds from traditional Chinese plants provide a broad prospect for screening

331 anti-AD drugs. We established a network between compounds in traditional Chinese plants and a

332 comprehensive list of anti-AD target proteins involved in various hypotheses. This network,

333 which links compounds, TCM plants and target proteins, may be very helpful for anti-AD drug

334 design.

335   During our manual assessment of the binding pockets and modes of compounds, we

336 discovered that some receptor had a large binding pocket and some had their binding pocket

337 exposed on the surface. Therefore, the number of successfully docked compounds for these

338 receptors is more than that of other receptors. This result suggests that for these receptors, there

339 may be many false positive compounds that do not bind to the receptor. Thus, we choose TCM

340 compounds with top 0.5% docking scores as our objects to avoid false positive compounds that

341 do not bind to the receptor.

342   ADMET is an important index in drug development. After filtering the compounds

343 according to five ADMET properties, 11 candidate anti-AD compounds with novel structures

344 remained. Among the 11 compounds, 8 were single-target compounds and the remaining 3 had

345 more than two target proteins. The structures and target proteins of these compounds are known,

346 so they can be easily studied in future drug development, because these compounds have

347 favorable druggability properties, they may become the promising candidate drugs for AD. Of

348 course, further experiments are necessary to establish their viability as real candidate drugs.

349   Many compounds that bind to AD-associated target proteins were observed in plants that

350 have not been used to treat AD in traditional Chinese clinical prescription. Thus, some

351 previously non-anti-AD plants may become the anti-AD plants, which will provide more natural

352 compound resources for AD drug discovery and be will also be beneficial for the development of

353 TCMs.

CONCLUSION

355 In summary, this study offers one strategy to find novel candidate anti-AD drugs from

356 traditional Chinese plants by constructing interaction networks between AD target proteins and

357 natural compounds in TCM plants. We got a total of 1476 drug candidates (top 0.5% docked

358 compounds for each target) using this strategy. Of 1476 drug candidates, 17 candidate anti-AD

359 compounds were structurally identical to 14 existing approved drugs. In addition, 11 anti-AD

360 candidate compounds with favorable ADMET and logP properties were identified. Of 11

361 identified compounds, 4 compounds have anti-inflammatory activity, including compounds 5862,

362 5863, 5868, 5869 and compound 28814 mainly has immunoregulatory activity. Other 6

363 compounds have not bioassay research in pubchem database at present. Further experiments are

364 needed to verify our drug candidates. This strategy and identified drug candidates may be helpful

365 for anti-AD drug discovery.

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REFERENCES

Armstrong RA. 2013. What causes Alzheimer's disease? *Folia Neuropathologica* 51:169-188.
10.5114/fn.2013.37702

Azam F, Amer AM, Abulifa AR, and Elzwawi MM. 2014. Ginger components as new leads for
the design and development of novel multi-targeted anti-Alzheimer's drugs: a
computational investigationle. *Drug Design Development and Therapy* 8:2045-2059.

Bezprozvanny I, and Mattson MP. 2008. Neuronal calcium mishandling and the pathogenesis of
Alzheimer's disease. *Trends Neurosci* 31:454-463. 10.1016/j.tins.2008.06.005

Bhadania M, Joshi H, Patel P, and Kulkarni VH. 2012. Protective effect of menthol on beta-
amyloid peptide induced cognitive deficits in mice. *European Journal of Pharmacology*
681:50-54. 10.1016/j.ejphar.2012.01.035

Bonda DJ, Lee HG, Blair JA, Zhu X, Perry G, and Smith MA. 2011. Role of metal
dyshomeostasis in Alzheimer's disease. *Metallomics* 3:267-270. 10.1039/c0mt00074d

Cai Z, Liu N, Wang C, Qin B, Zhou Y, Xiao M, Chang L, Yan LJ, and Zhao B. 2016. Role of
RAGE in Alzheimer's Disease. *Cell Mol Neurobiol* 36:483-495. 10.1007/s10571-015-
0233-3

Chen CY. 2011. TCM Database@Taiwan: the world's largest traditional Chinese medicine
database for drug screening in silico. *PLoS One* 6:e15939. 10.1371/journal.pone.0015939

Coyle JT, Price DL, and DeLong MR. 1983. Alzheimer's disease: a disorder of cortical
cholinergic innervation. *Science* 219:1184-1190.

Craig LA, Hong NS, and McDonald RJ. 2011. Revisiting the cholinergic hypothesis in the
development of Alzheimer's disease. *Neurosci Biobehav Rev* 35:1397-1409.
10.1016/j.neubiorev.2011.03.001

Cummings JL, Morstorf T, and Zhong K. 2014. Alzheimer's disease drug-development pipeline:
few candidates, frequent failures. *Alzheimers Res Ther* 6:37. 10.1186/alzrt269

Darvesh S. 2016. Butyrylcholinesterase as a Diagnostic and Therapeutic Target for Alzheimer's
Disease. *Current Alzheimer Research* 13:1173-1177.
10.2174/1567205013666160404120542

Deane RJ. 2012. Is RAGE still a therapeutic target for Alzheimer's disease? *Future Medicinal
Chemistry* 4:915-925. 10.4155/Fmc.12.51

Dickson DW. 1997. Neuropathological diagnosis of Alzheimer's disease: a perspective from
longitudinal clinicopathological studies. *Neurobiol Aging* 18:S21-26.

Dysken MW, Sano M, Asthana S, Vertrees JE, Pallaki M, Llorente M, Love S, Schellenberg GD,
McCarten JR, Malphurs J, Prieto S, Chen P, Loreck DJ, Trapp G, Bakshi RS, Mintzer JE,
Heidebrink JL, Vidal-Cardona A, Arroyo LM, Cruz AR, Zachariah S, Kowall NW,
Chopra MP, Craft S, Thielke S, Turvey CL, Woodman C, Monnell KA, Gordon K,
Tomaska J, Segal Y, Peduzzi PN, and Guarino PD. 2014. Effect of vitamin E and
memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative
randomized trial. *JAMA* 311:33-44. 10.1001/jama.2013.282834

Fan LY, and Chiu MJ. 2014. Combotherapy and current concepts as well as future strategies for
the treatment of Alzheimer's disease. *Neuropsychiatric Disease and Treatment* 10:439-

411   451. 10.2147/Ndt.S45143

412   Gentile MT, Reccia MG, Sorrentino PP, Vitale E, Sorrentino G, Puca AA, and Colucci-D'Amato

413   L. 2012. Role of cytosolic calcium-dependent phospholipase A2 in Alzheimer's disease

414   pathogenesis. *Mol Neurobiol* 45:596-604. 10.1007/s12035-012-8279-4

415   Goedert M, and Spillantini MG. 2006. A century of Alzheimer's disease. *Science* 314:777-781.

416   10.1126/science.1132814

417   Kiko T, Nakagawa K, Tsuduki T, Suzuki T, Arai H, and Miyazawa T. 2012. Significance of

418   lutein in red blood cells of Alzheimer's disease patients. *J Alzheimers Dis* 28:593-600.

419   10.3233/JAD-2011-111493

420   Lamkanfi M, Mueller JL, Vitari AC, Misaghi S, Fedorova A, Deshayes K, Lee WP, Hoffman

421   HM, and Dixit VM. 2009. Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. *J Cell*

422   *Biol* 187:61-70. 10.1083/jcb.200903124

423   Lan YL, Zou S, Zhang C, Li J, Xu Y, and Li S. 2016. Update on the effect of estradiol in

424   postmenopause women with Alzheimer's disease: a systematic review. *Acta Neurol Belg*

425   116:249-257. 10.1007/s13760-015-0593-y

426   Lee JC, Simonyi A, Sun AY, and Sun GY. 2011. Phospholipases A2 and neural membrane

427   dynamics: implications for Alzheimer's disease. *J Neurochem* 116:813-819.

428   10.1111/j.1471-4159.2010.07033.x

429   Leszek J, Barreto GE, Gasiorowski K, Koutsouraki E, Avila-Rodrigues M, and Aliev G. 2016.

430   Inflammatory Mechanisms and Oxidative Stress as Key Factors Responsible for

431   Progression of Neurodegeneration: Role of Brain Innate Immune System. *Cns &*

432   *Neurological Disorders-Drug Targets* 15:329-336.

433   10.2174/1871527315666160202125914

434   Mattson MP. 2004. Pathways towards and away from Alzheimer's disease (vol 430, pg 631,

435   2004). *Nature* 431:107-107. 10.1038/nature02940

436   Min JY, and Min KB. 2014. Serum lycopene, lutein and zeaxanthin, and the risk of Alzheimer's

437   disease mortality in older adults. *Dement Geriatr Cogn Disord* 37:246-256.

438   10.1159/000356486

439   Mohamd EM, Ahmed HH, Estefan SF, Farrag AERH, and Salah RS. 2011. Windows into

440   estradiol effects in Alzheimer's disease therapy. *European Review for Medical and*

441   *Pharmacological Sciences* 15:1131-1140.

442   Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, and Olson AJ. 2009.

443   AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility.

444   *J Comput Chem* 30:2785-2791. 10.1002/jcc.21256

445   Mushtaq G, Greig NH, Khan JA, and Kamal MA. 2014. Status of acetylcholinesterase and

446   butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus. *CNS Neurol*

447   *Disord Drug Targets* 13:1432-1439.

448   Normile D. 2003. Asian medicine. The new face of traditional Chinese medicine. *Science*

449   299:188-190. 10.1126/science.299.5604.188

450   O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, and Hutchison GR. 2011.

451   Open Babel: An open chemical toolbox. *J Cheminform* 3:33. 10.1186/1758-2946-3-33

452 O'Boyle NM, Morley C, and Hutchison GR. 2008. Pybel: a Python wrapper for the OpenBabel
453   cheminformatics toolkit. *Chem Cent J* 2:5. 10.1186/1752-153X-2-5
454 Ono K, and Yamada M. 2012. Vitamin A and Alzheimer's disease. *Geriatr Gerontol Int* 12:180-
455   188. 10.1111/j.1447-0594.2011.00786.x
456 Peng X, Xing P, Li X, Qian Y, Song F, Bai Z, Han G, and Lei H. 2016. Towards Personalized
457   Intervention for Alzheimer's Disease. *Genomics Proteomics Bioinformatics* 14:289-297.
458   10.1016/j.gpb.2016.01.006
459 Peskind ER, Wingerson D, Murray S, Pascualy M, Dobie DJ, Le Corre P, Le Verge R, Veith RC,
460   and Raskind MA. 1995. Effects of Alzheimer's disease and normal aging on
461   cerebrospinal fluid norepinephrine responses to yohimbine and clonidine. *Arch Gen*
462   *Psychiatry* 52:774-782.
463 Pratico D. 2008. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends in*
464   *Pharmacological Sciences* 29:609-615. 10.1016/j.tips.2008.09.001
465 Sanderson K. 2011. Databases aim to bridge the East-West divide of drug discovery. *Nat Med*
466   17:1531. 10.1038/nm1211-1531a
467 Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, and
468   Ideker T. 2003. Cytoscape: a software environment for integrated models of biomolecular
469   interaction networks. *Genome Res* 13:2498-2504. 10.1101/gr.1239303
470 Shen J, and Wu J. 2015. Nicotinic Cholinergic Mechanisms in Alzheimer's Disease. *Int Rev*
471   *Neurobiol* 124:275-292. 10.1016/bs.irn.2015.08.002
472 Song F, Han G, Bai Z, Peng X, Wang J, and Lei H. 2015. Alzheimer's Disease: Genomics and
473   Beyond. *Int Rev Neurobiol* 121:1-24. 10.1016/bs.irn.2015.05.001
474 Sucher NJ. 2013. The application of Chinese medicine to novel drug discovery. *Expert Opin*
475   *Drug Discov* 8:21-34. 10.1517/17460441.2013.739602
476 Sun Y, Zhu RX, Ye H, Tang KL, Zhao J, Chen YJ, Liu Q, and Cao ZW. 2013. Towards a
477   bioinformatics analysis of anti-Alzheimer's herbal medicines from a target network
478   perspective. *Briefings in Bioinformatics* 14:327-343. 10.1093/bib/bbs025
479 Takasaki J, Ono K, Yoshiike Y, Hirohata M, Ikeda T, Morinaga A, Takashima A, and Yamada
480   M. 2011. Vitamin A has Anti-Oligomerization Effects on Amyloid-beta In Vitro. *Journal*
481   *of Alzheimers Disease* 27:271-280. 10.3233/Jad-2011-110455
482 Trepanier CH, and Milgram NW. 2010. Neuroinflammation in Alzheimer's disease: are NSAIDs
483   and selective COX-2 inhibitors the next line of therapy? *J Alzheimers Dis* 21:1089-1099.
484 Trott O, and Olson AJ. 2010. AutoDock Vina: improving the speed and accuracy of docking
485   with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*
486   31:455-461. 10.1002/jcc.21334
487 Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, Chang Z, and Woolsey J.
488   2006. DrugBank: a comprehensive resource for in silico drug discovery and exploration.
489   *Nucleic Acids Res* 34:D668-672. 10.1093/nar/gkj067
490 Wyss-Coray T, and Rogers J. 2012. Inflammation in Alzheimer disease-a brief review of the
491   basic science and clinical literature. *Cold Spring Harb Perspect Med* 2:a006346.
492   10.1101/cshperspect.a006346

493   Xu X, and Lin X. 2015. [Advances in the researches of lutein and alzheimer's disease].
494   *Zhonghua Yu Fang Yi Xue Za Zhi* 49:456-460.
495   Yiannopoulou KG, and Papageorgiou SG. 2013. Current and future treatments for Alzheimer's
496   disease. *Ther Adv Neurol Disord* 6:19-33. 10.1177/1756285612461679
497

Details of the docking results of 30 anti-AD targets with the number of successfully docked TCM compounds

1   **Table 1.** Details of the docking results of 30 anti-AD targets with the number of successfully
2   docked TCM compounds

RCSB ID	Protein Name	Original Ligands ID	Binding Energy of Original Ligand[a]	Lowest Docking Energy	Compound Number[b]
1DB4	PLA2(Phospholipase A2, membrane associated)	8IN	-7.31	-11.55	5290
1DQA	HMG-COA(3-hydroxy-3-methylglutaryl-coenzyme A reductase)	NAP	-7.42	-9.78	437
1NME	Caspase-3	159	-4.57	-10.24	21028
1OJA	MAOB(Amine oxidase [flavin-containing] B)	ISN	-6.58	-12.2	11173
1TB7	PDE4(cAMP-specific 3',5'-cyclic phosphodiesterase 4D)	AMP	-6.47	-14.5	17375
1TN6	Ftase(Protein farnesyltransferase subunit beta)	FII	-6.59	-11.9	14437
2AFW	QC(Glutaminyl-peptide cyclotransferase)	AHN	-4.48	-11.11	23635
2AZ5	TNF(Tumor necrosis factor)	307	-5.66	-9.53	9261
2D0T	IDO(Indoleamine 2,3-dioxygenase 1)	PIM	-5.71	-12.4	20739
2DQ7	Fyn(Tyrosine-protein kinase Fyn)	STU	-10.28	-12.41	63
2VQM	HDAC(Histone deacetylase 4)	HA3	-7.11	-11.33	5356
2Z5Y	MAOA(Amine oxidase [flavin-containing] A)	HRM	-7.96	-12.8	5299
3A4O	lyn(Tyrosine-protein kinase Lyn)	STU	-9.4	-12.53	431
3G9N	JNK(Mitogen-activated protein kinase 10)	J88	-7.19	-10.36	1606
3IKA	EGFR(Epidermal growth factor receptor)	0UN	-7.64	-11.45	6324
3KMR	RAR(Retinoic acid receptor alpha)	EQN	-12.65	-11.4	0
3O3U	RAGE(Advanced glycosylation end product-specific receptor)	MLR	-7.76	-14.08	13309
4DJU	BACE-1(Beta-secretase 1)	0KK	-7.12	-12.2	14161
4EY5	AchE(Acetylcholinesterase)	HUP	-8.5	-10.6	329
4MS4	GABA(B)(Gamma-aminobutyric acid type B receptor subunit 1)	2C0	-5.73	-10.6	13107
4OC7	RXR(Retinoic acid receptor RXR-alpha)	2QO	-8.48	-11.3	708
4XAR	MGLUR(Metabotropic glutamate receptor 3)	4OF	-4.98	-8.5	9244
4YLK	DYRK1A(Dual specificity tyrosine-phosphorylation-regulated kinase 1A)	4E2	-8.13	-12.54	7167
4ZGM	GLP-1R(Glucagon-like peptide 1 receptor)	32M	-3.31	-9.06	24782
4ZZJ	SIRT1(NAD-dependent protein	4TQ	-6.89	-8.86	108

	deacetylase sirtuin-1)				
5A46	FGFR1(Fibroblast growth factor receptor 1)	38O	-8.54	-12.8	699
5AFH	α7NACHR(Neuronal acetylcholine receptor subunit alpha-7)	L0B	-6.02	-9.64	6934
5H8S	AMPA(Glutamate receptor 2)	5YC	-5.3	-8.44	8926
5HK1	SIG-1R(Sigma non-opioid intracellular receptor 1)	61W	-9.29	-12.8	1281
5IH5	CKI-δ(Casein kinase I isoform delta)	AUE	-7.62	-12.5	5998

3   a. 'Binding Energy of Original Ligand' indicates the docking energy of the ligand embedded in

4   the crystal structure.

5   b. The number of compounds with better docking scores than that of the original ligand

6   embedded in the crystal structure.

AD targets and their best-associated plant with the most compounds docking with the target

1   **Table 2. AD** targets and their best-associated plant with the most compounds docking with the
2   target

Target	Top1 Plant	Target	Top1 Plant	Target	Top1 Plant
PLA2	Bletilla(5)[a]	HMG-COA	Morus(9)	Caspase-3	Paeonia(4)
MAOB	Corydalis(16)	PDE4	Isatis(4)	Ftase	Panax(8)
QC	Panax(4)	TNF	Panax(10)	IDO	Morus(7)
Fyn	Papaver(11)	HDAC	Bletilla(5)	MAOA	Corydalis(11)
lyn	Claviceps(5)	JNK	Morus(8)	EGFR	Artemisia(7)
RAR[b]	Rauwolfia(8)	RAGE	Fritillaria(7)	BACE-1	Lonicera(6)
AchE	Piper(6)	SIRT1	Panax(18)	GABA(B)	Morus(11)
RXR	Salvia(10)	MGLUR	Morus(4)	DYRK1A	Strychnos(6)
GLP-1R	Panax(9)	FGFR1	Rheum(6)	α7NACHR	Panax(8)
AMPA	Panax(7)	SIG-1R	Corydalis(7)	CKI-δ	Salvia(11)

3   a. The numbers in this table are compound numbers which the best-associated plant for each
4   target protein contains.
5   b. The docking energy of TCM compounds is higher than that of the original ligand for RAR
6   protein.

ADMET and logP properties of 11 candidate drugs

1   **Table 3.** ADMET and logP properties of 11 candidate drugs

Name(ID)	Solubility Level	BBB Level	Hepatotoxic Prediction	Absorption Level	PPB Prediction	logP	Targets
(3S)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol(5862)	3	2	FALSE	0	FALSE	4.578	AchE
(3S)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6E)-6-hepten-3-ol(5863)	3	2	FALSE	0	FALSE	4.134	AchE,GABA(B),MGLUR
(3R)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)heptan-3-ol(5868)	3	2	FALSE	0	FALSE	4.578	GABA(B)
(3R)-1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-(6E)-6-hepten-3-ol(5869)	3	2	FALSE	0	FALSE	4.134	AchE
pallidine(9593)	3	2	FALSE	0	FALSE	1.913	MAOB
4,5-di-o-caffeoyl,quinic,acid(10639)	3	2	FALSE	0	FALSE	3.477	PDE4
Anagyrine(16167)	3	1	FALSE	0	FALSE	2.053	AchE
Blestrin D(26629)	3	2	FALSE	0	FALSE	4.578	PLA2,QC,HDAC,JNK,GABA(B)
Dibothrioclinin II(28468)	4	2	FALSE	0	FALSE	1.222	Ftase,QC,HDAC,GLP-1R,AMPA
5,7-Dihydroxy-6,8-dimethyl-3-(4'-hydroxy-3'-methoxybenzyl)chroman-4-one(28814)	3	2	FALSE	0	FALSE	1.144	RAR
Glabroisoflavanone A(30713)	3	2	FALSE	0	FALSE	1.913	MAOA

Figure 1

The docking energy scores of the top 0.5% TCM compounds and original ligands for 30 targets

Red boxes represent the top 0.5% of compounds for each target protein. Blue points represent the target proteins' embedded ligands.

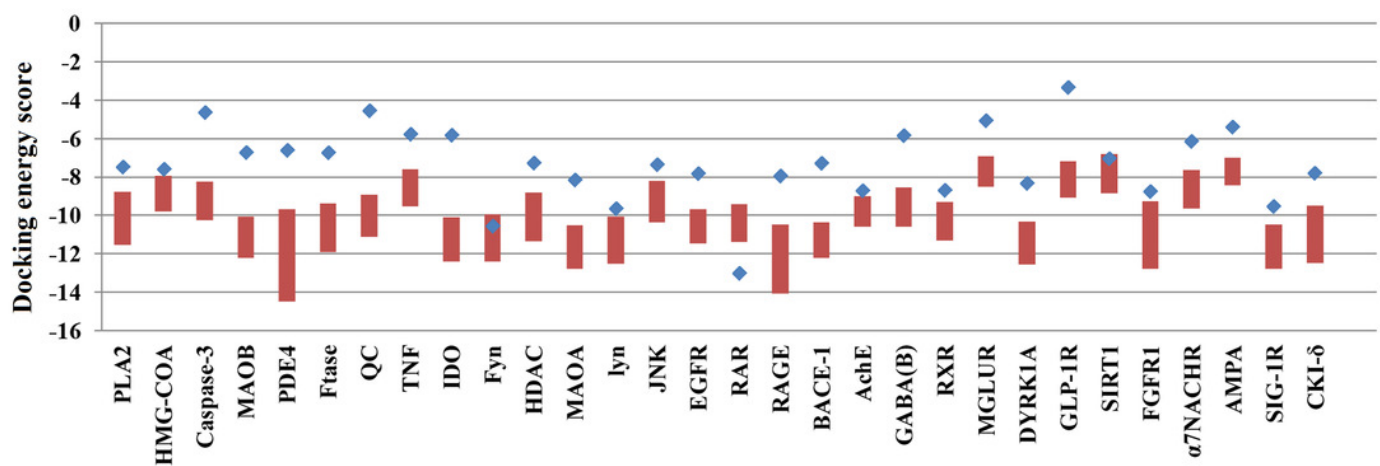



Figure 2

The docking pose interactions between the target proteins and ligands including their best-binding TCM compounds and original ligands and the pharmacophore of SIRT1

A. The 3D structures and binding model of ligands including best ligand and original ligand to the target protein AchE. The best ligand is green and the original ligand is magenta. The top panel shows the amino acid residues lying within 5 Å from the best ligand, and the bottom panel shows the amino acid residues lying within 5 Å from the original ligand.

B. The pharmacophore of SIRT1 using top 10 TCM compounds binding for the target protein. The hydrogen bond acceptor is in green and the hydrophobic centers are in blue.

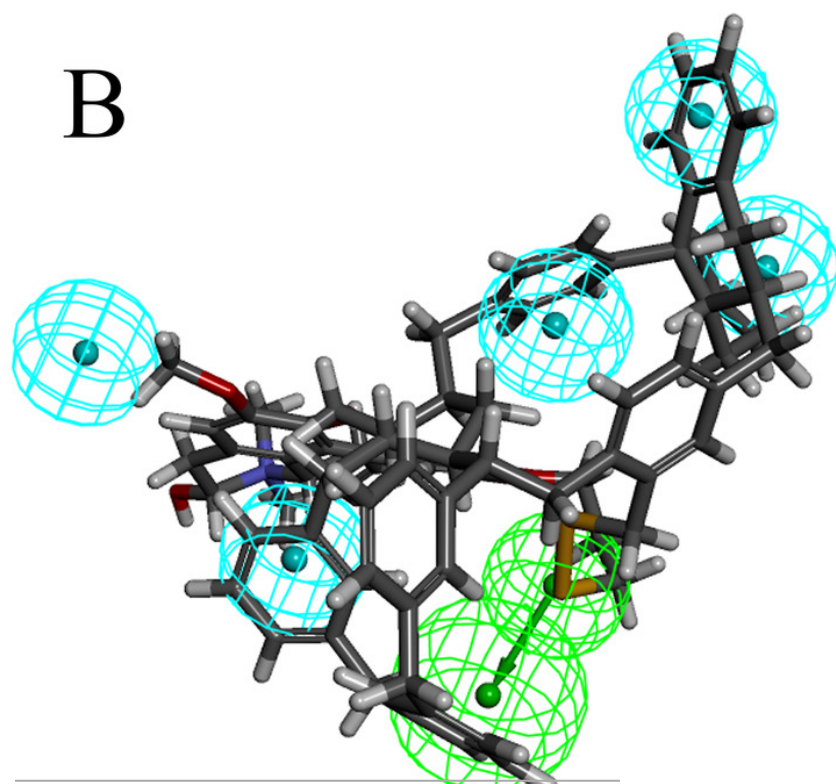
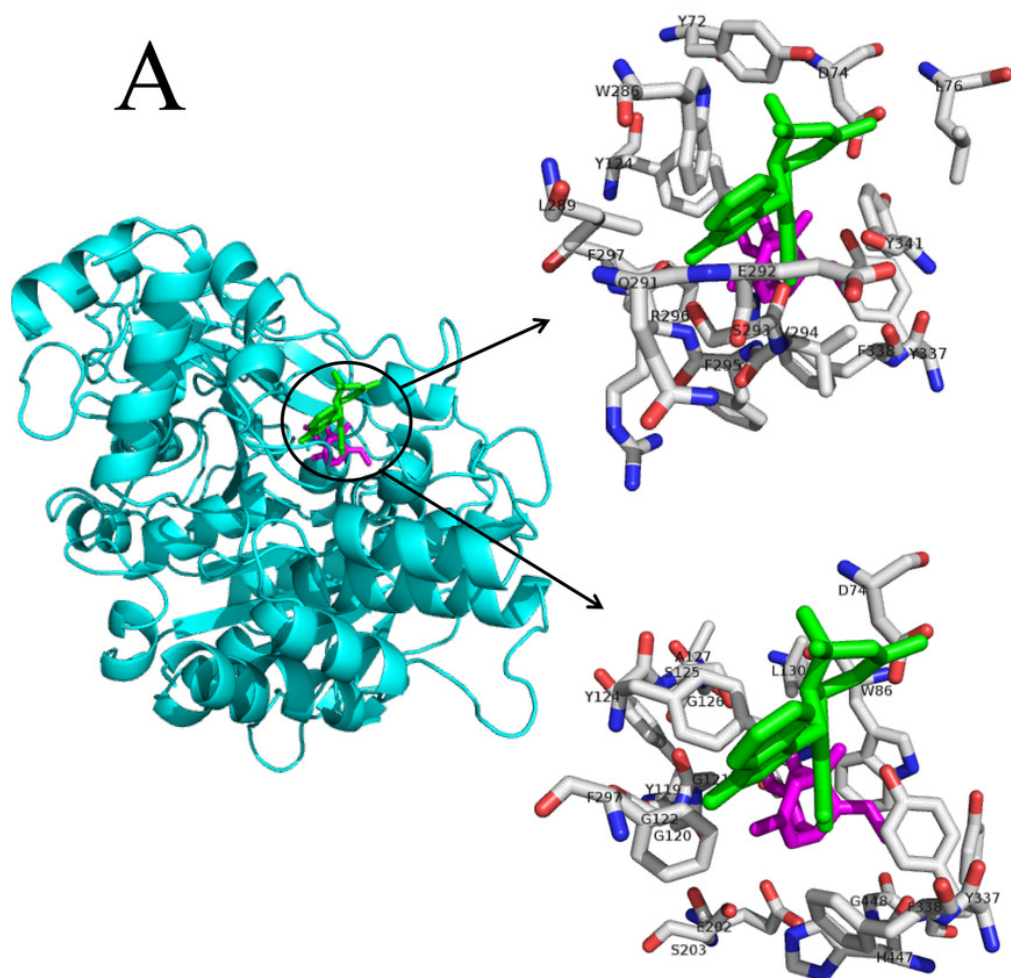



Figure 3

The exact number of candidate anti-AD compounds and their plants associated with each anti-AD target protein.

The number is tagged above each column, and the target proteins are displayed on the horizontal axis.

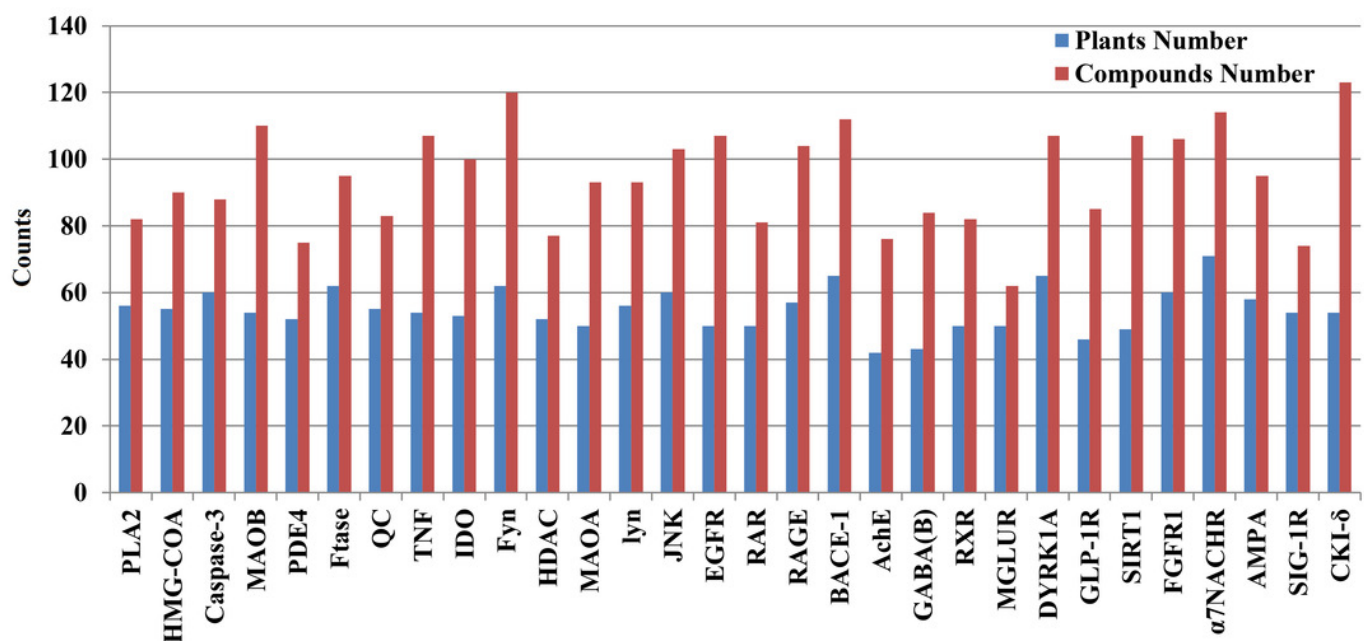



Figure 4

The network containing the target proteins and their best-associated plants

Pink boxes represent target proteins. Green boxes represent compounds.

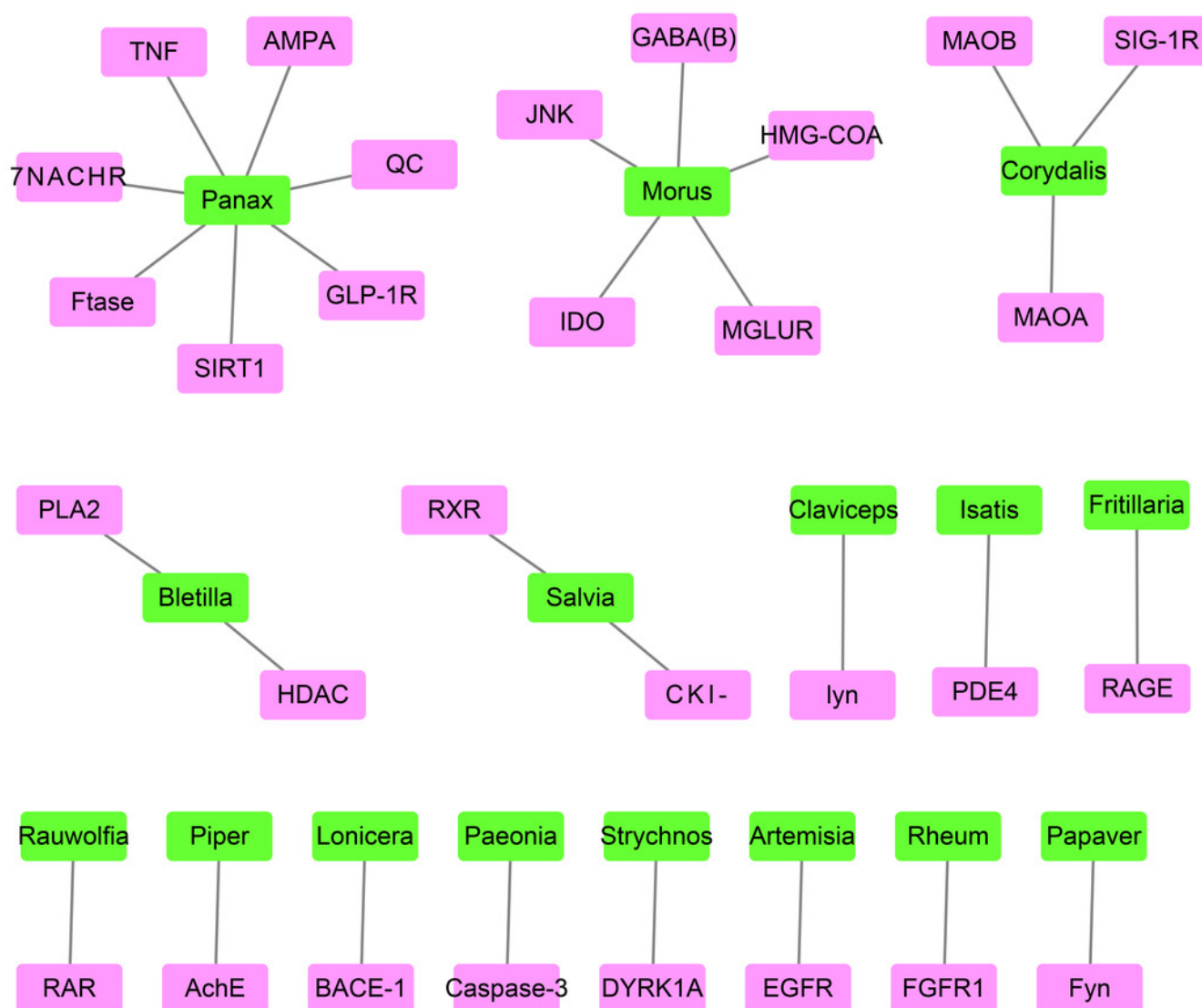



Figure 5

The network containing the anti-AD target proteins, TCM compounds and structurally identical drugs

Pink boxes represent targets. Yellow boxes represent compounds. Blue boxes represent drugs. The structures of TCM compounds are also shown.

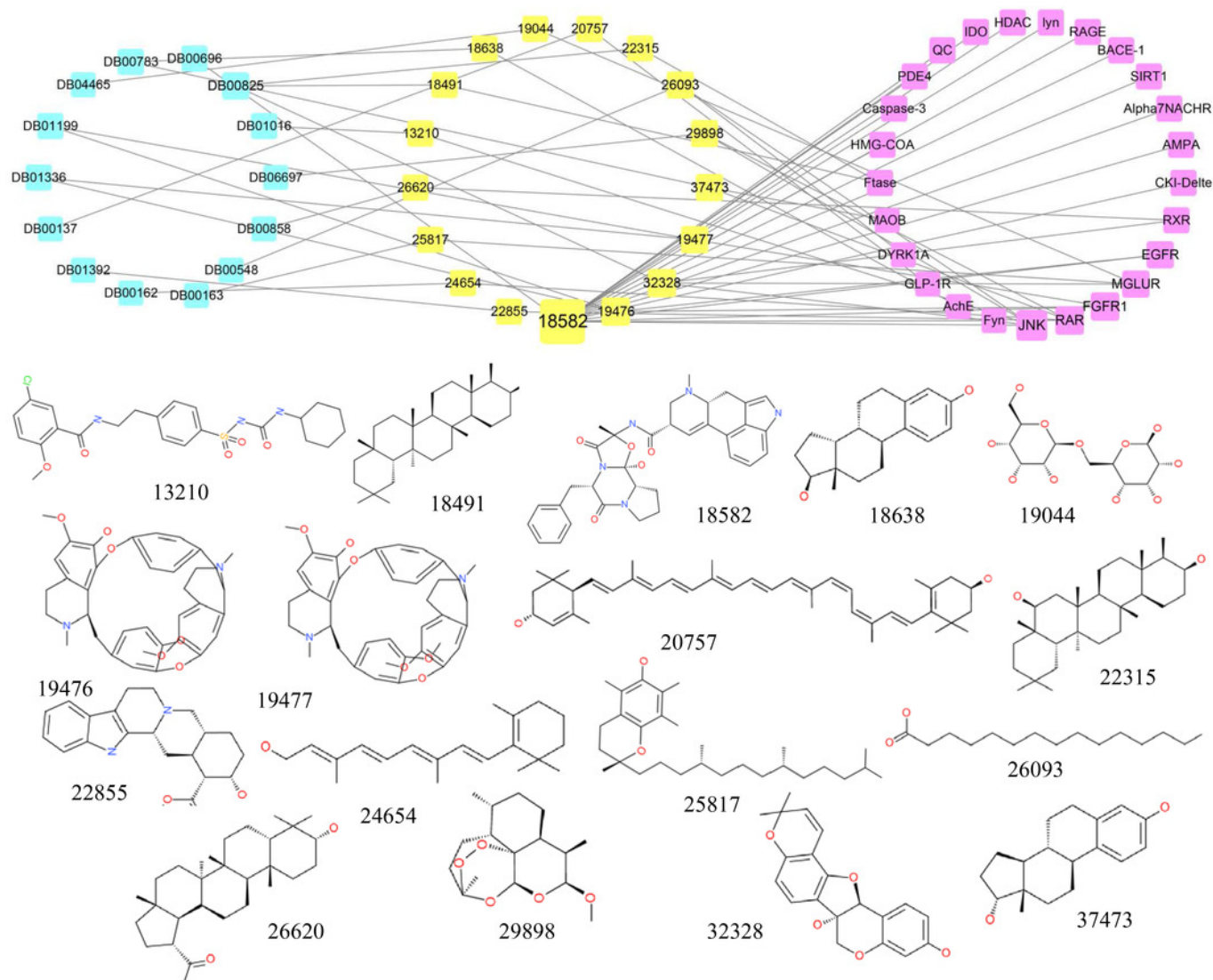



Figure 6

The 10 clusters of anti-AD TCM compounds and their primary targets

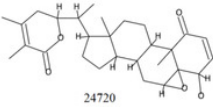
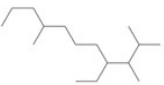
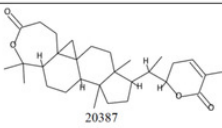
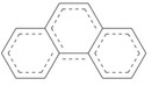
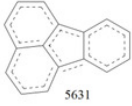
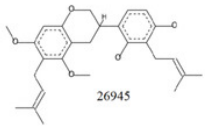
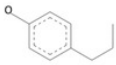
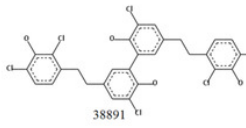
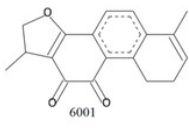
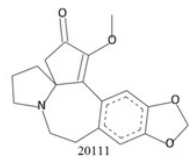
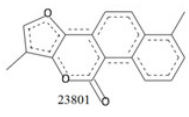
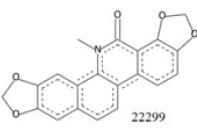
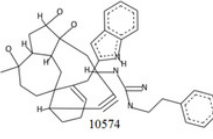
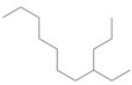
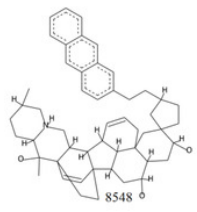
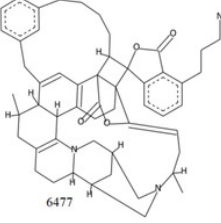
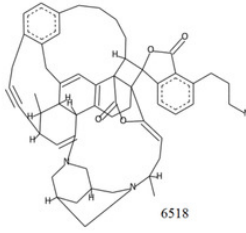
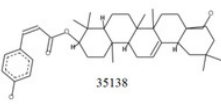
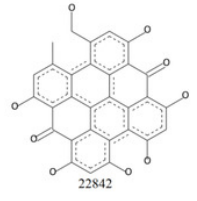
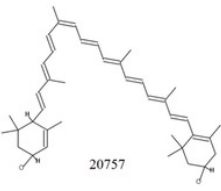
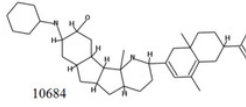
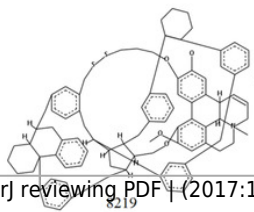
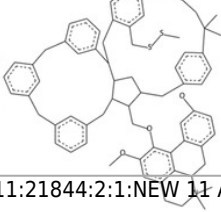
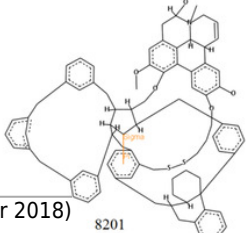
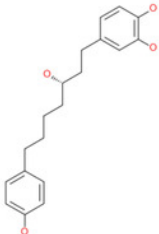
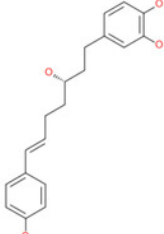
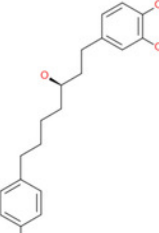
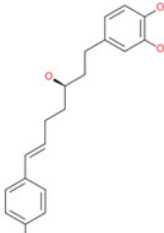
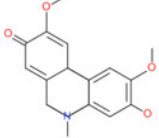
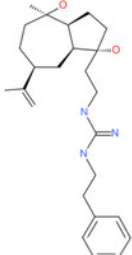
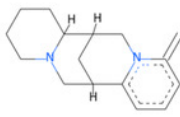
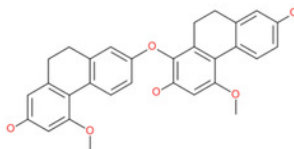
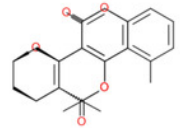
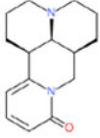
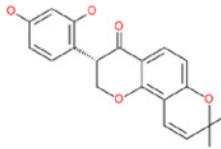
a. The Generate Maximal Common Substructure component must contain the proportion of the cluster molecules. The proportion was set to 0.5 to find the largest maximal common substructure contained in at least 50% of the cluster molecules.

Cluster	Cluster Center Structure	Maximal Common Substructure[a]	Best Member Structure	Cluster Size	Primary Targets
1	24720		20387	225	SIG-1R (33)
2	5625		5631	6	JNK(4), DYRK1A (4), CKI-δ(4)
3	26945		38891	277	JNK (35)
4	6001		20111	63	RAR (17)
5	23801		22299	57	CKI-δ(16)
6	10574		8548	130	FGFR1 (38)
7	6477		6518	140	SIRT1 (50)
8	35138		22842	464	Fyn (55)
9	20757		10684	73	QC (12), HDAC (12), RAGE (12)
10	8219		8201	41	SIRT1 (26)

Figure 7

2D structure and corresponding plants of 11 compounds with favorable ADMET
properties

Compound ID	Structure	Plant	Compound ID	Structure	Plant
5862		Curcuma kwangsiensis	5863		Curcuma kwangsiensis
5868		Curcuma kwangsiensis	5869		Curcuma kwangsiensis
9593		Lindera aggregate	10639		Taraxacum mongolicum
16167		Thermopsis lanceolata R. Br., Laburnum anagyroides, Sophora flavescens Alt., Sophora tonkinensis	26629		Bletilla striata
28468		Gerbera piloselloides Cass.	28814		Ophiopogon japonicus (L. f.) Ker-Gawl.
30713		Glycyrrhiza glabra			