Heliyon 10 (2024) e28852

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Network pharmacology-based approach to elucidate the pharmacologic mechanisms of natural compounds from *Dictyostelium discoideum* for Alzheimer's disease treatment

Nil Patil [a,b], Rupal Dhariwal [a,b], Arifullah Mohammed [c], Lee Seong Wei [c,*], Mukul Jain [a,b,**]

[a] *Cell & Developmental Biology Lab, Research & Development Cell, Parul University, Vadodara, Gujarat, 391760, India*
[b] *Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, Vadodara, Gujarat, 391760, India*
[c] *Department of Agriculture, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli, 17600, Malaysia*

ARTICLE INFO

Keywords:
Network pharmacology
Secondary metabolites
Alzheimer's disease
Molecular docking
COX2

ABSTRACT

Alzheimer's disease (AD) is increasingly becoming a major public health concern in our society. While many studies have explored the use of natural polyketides, alkaloids, and other chemical components in AD treatment, there is an urgent need to clarify the concept of multi-target treatment for AD. This study focuses on using network pharmacology approach to elucidate how secondary metabolites from *Dictyostelium discoideum* affect AD through multi-target or indirect mechanisms. The secondary metabolites produced by *D. discoideum* during their development were obtained from literature sources and PubChem. Disease targets were selected using GeneCards, DisGeNET, and CTD databases, while compound-based targets were identified through Swiss target prediction and Venn diagrams were used to find intersections between these targets. A network depicting the interplay among disease, drugs, active ingredients, and key target proteins (PPI network) was formed utilizing the STRING (Protein-Protein Interaction Networks Functional Enrichment Analysis) database. To anticipate the function and mechanism of the screened compounds, GO and KEGG enrichment analyses were conducted and visually presented using graphs and bubble charts. After the screening phase, the top interacting targets in the PPI network and the compound with the most active target were chosen for subsequent molecular docking and molecular dynamic simulation studies. This study identified nearly 50 potential targeting genes for each of the screened compounds and revealed multiple signaling pathways. Among these pathways, the inflammatory pathway stood out. COX-2, a receptor associated with neuroinflammation, showed differential expression in various stages of AD, particularly in pyramidal neurons during the early stages of the disease. This increase in COX-2 expression is likely induce by higher levels of IL-1, which is associated with neuritic plaques and microglial cells in AD. Molecular docking investigations demonstrated a strong binding interaction between the terpene compound PQA-11 and the neuroinflammatory receptor COX2, with a substantial binding affinity of −8.4 kcal/mol. Subsequently, a thorough analysis of the docked complex (COX2-PQA11) through Molecular Dynamics Simulation showed lower RMSD, minimal RMSF fluctuations, and a reduced total energy of −291.35 kJ/mol compared to the standard drug. These findings suggest that the therapeutic effect of PQA-11 operates through the inflammatory

* Corresponding author.
** Corresponding author. Cell & Developmental Biology Lab, Research & Development Cell, Parul University, Vadodara, Gujarat, 391760, India.
E-mail addresses: leeseong@umk.edu.my (L.S. Wei), jmukul801@gmail.com (M. Jain).

https://doi.org/10.1016/j.heliyon.2024.e28852
Received 27 October 2023; Received in revised form 23 March 2024; Accepted 26 March 2024
Available online 6 April 2024

pathway, laying the groundwork for further in-depth research into the role of secondary metabolites in AD treatment.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly, marked by a gradual deterioration in cognitive function and memory. In 2019, approximately 50 million individuals worldwide received a diagnosis of AD, with nearly 10 million new cases being reported annually [1]. With declining birth rates and extended life expectancy worldwide, AD and other forms of dementia have been recognized as one of the top cause of disability among individuals aged 60 and above at global level. Early detection and intervention in cognitive dysfunction can potentially delay or even prevent the onset and advancement of dementia, enabling individuals to reduce its impact on their cognitive health and improve overall quality of life [2]. The Alzheimer's disease pathogenesis involves the development of extracellular amyloid-β plaques, the abnormal accumulation of phosphorylated tau within neurons, synaptic impairment, and neuronal cell damage [3]. Among the Aβ variants, Aβ40 and Aβ42 have been identified as the primary pathogenic species responsible for the production of amyloid plaques in the AD-affected brain, with Aβ40 being the more abundant isoform within these plaques. The aggregation process involves formation of insoluble aggregates that later convert into plaques [4]. In the domain of existing clinical medications, four conventional therapeutic approaches are employed dealing with Alzheimer's disease (AD). These approaches involves the enhancement of cognitive function, by activating the α-secretase activity, inhibiting the β-secretase and γ-secretase activity, and the mitigating the aberrant Tau phosphorylation [5]. At present, therapeutic interventions for Alzheimer's disease primarily focuses on the administration of cholinesterase inhibitors, including Tacrine, Donepezil, Rivastigmine, and Galantamine, in conjunction with the non-competitive antagonist of the N-Methyl-D-aspartic acid (NMDA) receptor, Memantine [6]. On the other hand, many of the natural compounds from plants and fungi may became new hope as therapeutic of AD and more detail is given in Table 1.

Among the Acetylcholinesterase inhibitors (AChE inhibitors) with clinical significance, Galantamine stands as the solitary naturally occurring compound belonging to the *Amaryllidaceae* family [19]. Moreover, secondary metabolites obtained from plants, including alkaloids, terpenoids, and flavonoids, have demonstrated their efficacy in the treatment of various medical conditions such as cancer, neurodegenerative diseases, skin disorders, and a range of cardiovascular ailments. Flavonoids, categorized as plant secondary compounds, possess antioxidant, *anti*-amyloidogenic, and anti-inflammatory properties and regulate cell-signaling pathways. Their demonstrated acetylcholinesterase inhibitory activity makes them a hopeful candidate for AD treatment [20]. In addition, terpenoids have many biological activities, and researchers have focused their attention on its anti-cholinesterase activity. Numerous herbal plants like *Curcuma Longa*, and other organisms, are also employed in diverse therapeutic approaches, serving as memory enhancers and displaying anticholinesterase properties [21]. As all studies indicate that the natural modulator or natural compound can make a significant impact on reduction of AD progression, there is need for more natural compounds that can suppress AD. The *Dictyostelium*

Table 1
Depiction of various models for AD therapy using natural, unrefined, or partially purified plant and fungus extracts.

Sr.	Source	Compound	*In-vitro*/*In – vivo*/human trail	Mechanism	Refs.
1	*Allium sativum* . (plant)	AGE (Aged Garlic Extract)	Aβ (25–35)-induced PC12 cells	Reduced levels of ROS, caspase-3 activity, and PARP cleavage	[7]
2	*Ginkgo biloba* (plant)	GBE (Ginkgo biloba extract)	AD Rat model	Anti-inflammatory properties, ameliorating the cognitive and memory impairment	[8]
3	*Angelica gigas* (plant)	Ethanolic extract INM-176	Scopolamine- or Aβ (1–42)-induced mice	Increase AChE inhibiting activity	[9]
4	*Crocus sativus* (plant)	Root extract	AD mice model Human trail (20 patients)	Increase anti-inflammatory activity Enhanced cognition and memory in AD patient	[10]
5	*Camellia sinensis* (plant)	Epigallocatechin-3- gallate (EGCG)	TgCRND8 (Tg) mice	Enhanced brain health and slowed AD progression.	[11]
			Aβ (1–42)-injected mouse, PS2 mutant AD mice	Increased α-secretase, reduced β- and γ-secretase, and decreased Aβ.	[12]
6	*Melissa officinalis* (plant)	Leaf extract	Human trail (40 patients)	Increased cognitive function and reduced agitation in AD patient	[13]
7	*Ptychopetalum olacoides* (plant)	Ethanolic extract	Aβ (1–42)-induced mice	Reduced Aβ toxicity, diminished Aβ deposits, and alleviated astrogliosis.	[14]
8	*Valeriana amurensis* (plant)	50% ethanol extracts of macroporous resin group from roots and rhizomes	Aβ (1–40)-induced rat cortical and hippocampus neurons	Decreased β-APP, reduced Aβ (1-40), and diminished Caspase-3	[15]
9	*Malus domestica* (plant)	Fruit Juice	Transgenic mice	Reduce γ secretase expression	[16]
10	*Zataria multiflora* (plant)	Oil extract	Aβ (25–35)-induced rat hippocampus	Increase Anti-inflammatory	[17]
11	*Sargassum serratifolium* (Algae)	Sargaquinoic acid extracted (Meroterpenoids)	Aβ (1–42)-induced mice	Reduced Aβ and diminished ROS.	[18]

discoideum, a cellular slime mold, has also served as a model organism for investigating eukaryotic cell functions due to its samll genome and manageable nature. It also releases diverse array of secondary metabolites during its different developmental stages through biosynthetic pathway to engulf soil bacteria and survive. The stages during which these metabolites are released includes pre stalk, pre spore stage. Of these secondary metabolites [22] some are polyketide, polyphenolic, alkaloids and terpenes and show different biological activities like anti-bacterial, anti-fungal, anti-cancer, and anti-APP activity [23]. The current *in silico* approach of network pharmacology could aid in predicting and identifying the target proteins associated with these secondary metabolites and also explore how these secondary metabolites could act as potent drugs and exert their effect on the AD condition. Additionally, it could aid in discovering fresh drug candidates and targets and repurpose the existing drug compounds for diverse therapeutic purposes by exploring potential target areas. The main aim of this study is to investigate and explore the working fashion of secondary metabolites derived from *D. discoideum* for the therapeutics development in AD through computational approaches using network pharmacology.

2. Materials and methods

By employing receptor mining and using various online tools, we successfully identified the receptors responsible for secondary metabolites within *Dictyostelium discoideum*, along with the genes associated with Alzheimer's disease (AD). The selection of potential anti-AD genes was achieved through the intersection of these datasets, namely DisGeNET, CTD, and Gene Cards. Subsequently, we conducted an in-depth analysis of protein-protein interactions (PPI), KEGG pathways, and Gene Ontology (GO) to elucidate potential targets implicated in AD pathology. The identified key genes from the revious analysis were further investigated for their expression across distinct brain regions using AlzData. Following the screening of five proteins, a set of seven refined ligands underwent rigorous computational analysis, involving molecular docking and molecular dynamics simulations.

2.1. Data collection & processing

The composition and molecular target data of Alzheimer's disease were obtained from DisGeNET (https://www.disgenet.org/) [24], GeneCards (https://www.genecards.org/) [25], and CTD (https://ctdbase.org/) [26]. Swiss Target Prediction (http://www.swisstargetprediction.ch/) [27] made the prediction of compound-based targets. Similarity between the compound-based targets and database-based targets were used for further network pharmacological analysis and validation through AutoDock [20].

2.2. BBB permeability and drug-likeness screening

The BBB is a distinctive biological barrier comprising microvascular endothelial cells within the CNS. These cells serve as gatekeepers, regulating the passage of substances into and out of the brain. The primary function of the BBB is to maintain CNS homeostasis by restricting the transport of harmful substances while facilitating the removal of metabolic byproducts from the brain [28]. For many contemporary drugs, the BBB poses as a strong obstacle. In our study, we performed *in silico* assessments of the potential of secondary metabolites from *D. discoideum* to traverse the blood-brain barrier using the online server pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) [29]. Afterward, a comprehensive examination was carried out of all structures to ensure their compliance with the ADMET criteria and Lipinski's Rule of Five. This evaluation was performed using online tools like Swiss ADME. Structures that fulfilled Lipinski's Rule of Five, Veber's Law, and the specified ADME criteria were chosen for further investigations involving docking studies. Key information obtained from SwissADME includes data on molecular weight, MlogP, the quantity of hydrogen bond donors and acceptors, the number of rotatable bonds, topological surface area, and a bioavailability score [30].

2.3. Determination of Alzheimer's disease-related targets of the active compounds

After screening through ADMET and BBB permeability, compounds were further used to find out targets. Screened compounds, SMILES files were generated through ChemDraw [31]. After conversion from SDF structure to SMILE, file structures were imported into the Swiss Target Prediction database (http://swisstargetprediction.ch/) to obtain ligand-based receptors [27]. All targets were normalized according to the UniProt database (https://www.uniprot.org). A network diagram representing the interaction between drugs and their respective targets was generated using Cytoscape version 8.0.0 [32]. Subsequently, an assessment of the active compounds in *D. discoideum* was conducted utilizing the Network Analyzer tool.

2.4. Construction of PPI network and selection of hub genes

We identified tightly interconnected regions within molecular complexes in the acquired Protein-Protein Interaction (PPI) network, characterizing them as topological modules based on network properties. Additionally, we defined pharmacological modules as collections of nodes sharing similarities and functional relationships within the same pharmacological category. The shared potential target genes were incorporated into the STRING 11.0 database (https://string-db.org/) to form the protein-protein interaction (PPI) network, specifically focusing on the *Homo sapiens* organism. Only interactions exceeding the minimum required interaction score threshold of >0.4 were considered statistically significant. The PPI network comprises nodes representing target proteins and edges denoting protein-protein interactions. Node degree indicates the number of direct connections a node possesses, with higher degrees signifying greater importance. To strengthen the reliability of our results, we implemented a stringent interaction score threshold of ≥ 0.9 within the STRING database. Core target identification was accomplished through network analysis using Cytoscape software,

along with its Network Analyzer plugin [33]. By utilizing CytoHubba, we constructed a functionally relevant protein network specifically related to the screened compounds within the context of Alzheimer's disease (AD). This was achieved using the topological analysis method known as Maximal Clique Centrality (MCC). Scores reflecting the strength of associations between nodes and edges were calculated through the MCC algorithm, where higher scores and darker colorations indicated a more pronounced correlation of the genes with AD [34]. Subsequently, we pinpointed the top 15 target genes/proteins with the highest scores for each active ingredient. Furthermore, target proteins underwent classification using the Panther classification system (http://pantherdb.org/) [35]. Subsequently, we utilized the Cytoscape plugin MCODE to dissect the corresponding network, culminating in the identification of the core Protein-Protein Interaction network cluster.

2.5. GO and KEGG enrichment analyses

Our analysis involved performing GO biological process and KEGG pathway enrichment analyses via the Metascape platform, accessible at https://metascape.org/gp. Significance criteria for enriched terms were established as having a p-value less than 0.01, a minimum count of 3, and an enrichment factor exceeding 1.5 [36]. Terms meeting these criteria were considered statistically significant. Subsequently, we carried out the analysis and visualization of these enriched terms using the SRplot tool and generated an enrichment bubble graph for both GO and pathway enrichment analyses. We utilized symbols or gene names as input for these analyses. The GO analysis encompassed the assessment of molecular function (MF), biological process (BP), and cellular component (CC). Additionally, for pathway analysis, we further evaluated the highest enrichment scores represented as *-log10 p-values* [37].

2.6. Validation of key compound–hub target gene interaction through molecular docking & molecular simulation

We acquired crystal structures of all proteins from the Protein Data Bank (PDB) database (Table 2; https://www.rcsb.org/) to facilitate docking experiments. The 3D structures of the small molecules, namely PQA-11, Discoidol, Dictyosterol, Pt-4, Dictyoquinone, PQA-18, and Discodiene, were obtained from the PubChem database. For molecules not available in PubChem, we manually drew their structures using ChemDraw. Subsequently, energy minimization was conducted using the MMFF94s force field within the Avogadro Software. In this study, molecular docking analyses were carried out using AutoDock Vina 1.1.2 software (https://vina.scripps.edu/). Before initiating the docking procedure, we subjected all receptor proteins to preprocessing using PyMol 2.5, which can be accessed at https://pymol.org/2/. In the preprocessing stage, water molecules, salt ions, and small molecules were removed from the protein structures. The resulting structures were exported in. pdbqt format. To establish the dimensions of the docking box, we utilized the PyMol plugin 'center of mass. py,' aligning the box's center with the crystal ligand's position. The box's side length was configured to be 40 Å [38]. Furthermore, Open Babel was employed to convert all processed small molecules and receptor proteins into the PDBQT format, a necessary prerequisite for AutoDock Vina 1.1.2 docking Table 2. Throughout the docking process, we configured the global search exhaustiveness to 32, while maintaining the default values for the remaining parameters. The resultant docked conformation with the highest score was designated as the binding conformation. Subsequent docking results were visualized and subjected to analysis using Biovia Discovery Studio.

After initial screening for binding affinity and hydrogen bonds, chosen drug-receptor complexes underwent Molecular Dynamics Studies (MDS) with GROMACS v2016.16 and CHARMM 27 force field. Ligand topologies came from SwissParam (https://www.swissparam.ch/), and simulations followed a standard protocol. This involved creating a triclinic water box, solvating it with 'spc216 - Simple Point Charge water,' adding counter ions, and minimizing the system. Protein-ligand coupling was followed by a two-step equilibration, NVT and NPT, each for 10 ns, using the V-rescale modified Berendsen thermostat [39]. A 100 ns Molecular Dynamics (MD) run was performed with GPU acceleration using CUDA Toolkit 12.2 (https://developer.nvidia.com/cuda-downloads) under periodic boundary conditions. Results were analyzed with VMD and Chimera, focusing on RMSD, RMSF, Rg, interaction energy, hydrogen bonds, and their distances. GRACE was used to create graphical representations.

3. Results

3.1. Evaluation of secondary metabolites from D. discoideum

The potential metabolites derived from *Dictyostelium discoideum* were subjected to a comprehensive analysis of their pharmacological and molecular characteristics. This examination encompassed various properties, such as molecular weight, oral bioavailability, drug-likeness, CaCO-2 permeability, blood-brain barrier penetration, fractional negative accessible surface area, log P

Table 2
Proteins name with PDB ID & Active site dimension used for docking.

Sr.	Protein Name	PDB ID	Chain	Active Site
1.	PTGS2/COX2	5IKR	A	37.757, 21.234, 73.009
2.	CYP19A1	3S79	A	88.208, 49.487, 51.124
3.	HSP90AB1	1UYM	A	0.401, 14.877, 20.821
4.	MTOR	3JBZ	A	18.656, −64.458, −9.520
5.	MAPK8	3PZE	A	15.178, 18.841, 27.393

(lipophilicity), hydrogen bond donor and acceptor count, the number of rotatable bonds, and topological polar surface area. All of these data were obtained from the pkCSM server. Supplementary Table 1 shows the details of the ADMET properties analysis through pkCSM analysis of 61 metabolites. Compounds Discoidol, Dictyoquinone, Pt-4, PQA-11, PQA-18, Dictyosterol, and Discodiene were significantly absorbed, while compound 1–43, 46, 48, 49, 52–57, 59 and 60 were found to be poorly absorbed through the Blood-brain barrier with a log BBB >0.3. After selection with Lipinski rule, selection is done by BBB permeability, and only Discoidol, Dictyoquinone, Pt-4, PQA-11, and Discodiene show a log P value < 5 for good oral and intestinal absorption. The intestinal absorption values of these five compounds were greater than 30% making them a better absorbing compound from the intestine after oral administration, which indicates that all the natural compounds could be remarkably absorbed from the intestine of humans. The analysis of CYP (1A2, 2C9, 2C19, 2D6, and 3A4) parameters conducted through ADMET pertains to phase-1 drug bioinformatics and its role in drug metabolism. Of particular significance in this study is CYP3A4, where the ligand PQA-11 was found to inhibit CYP3A4 activity. In contrast, the remaining four compounds, namely Discoidol, Dictyoquinone, Pt-4, and Discodiene, can undergo metabolism by CYP3A4 in the liver. The active compounds underwent additional assessment through ProTox-II, focusing on toxicity and lethal dose (LD50) values categorized into classes: class 1 and 2 (fatal), class 3 (toxic), class 4 and 5 (less harmful), and class 6 (non-toxic). All Compounds proved to be non-toxic for human consumption with their toxicity class being 4 and 5 Table 3.

3.2. Screening of targets of secondary metabolites against AD-associated receptors

After searching for candidate targets for the selected seven secondary metabolites from Swiss Target Prediction databases, deduplication occurred following UniProt standardization. After the removal of duplicate data, 100 targets for each compound were identified. Subsequently, we employed Cytoscape 3.6.0 software to construct an interaction network depicting the relationship between metabolites and targets. Venn analysis was conducted, utilizing the 100 targets associated with each secondary metabolite, and approximately 2520 Alzheimer's disease-related target genes for each of the seven metabolites. The target genes were gathered from the DisGeNET, Gene card, and CTD databases. On average, 58 common targets were obtained for each metabolite that was common from ligand-based and disease-based resources Fig. 1 (**A: PQA18, B: Dictyoquinone, C: Discodiene, D: Dictyosterol, E: PQA11, F: Pt4, G: Discoidol)**. Further, the interaction of all the common gene targets with all 7 metabolites was obtained for analyzing the possible interactions that the receptors show with the metabolites as shown in Fig. 2.

According the PPI network, the top 15 core nodes were identified, ranked by MCC (Maximal Clique Centrality) in the 'cytoHubba' plugin. Nodes with higher MCC scores are more central in the network, indicating that they are part of larger cliques (groups of highly interconnected nodes) and have a more influential role in connecting various proteins within the network. Thus, 15 genes were identified as hub genes in respect to all seven compounds, as shown in Fig. 3. (A. PQA11, B. Dictyosterol, C. Pt4, D. Discodiene, E. Discoidol, F. PQA18, and G. Dictyoquinone)

These hub genes are considered to be highly important in the context of the network, as they have a significant number of interactions with other genes. Their identification will be helpful in understanding the biological significance and roles in cellular processes or pathways. Top-ranked nodes, which are dark in color, were further analyzed through MCODE, as these nodes were not only highly central in the global network but also crucial components of localized functional modules or complexes. For MCODE analysis, genes above 0.2 cutoff a threshold that helps to determine the minimum density or connectivity required for a set of genes to be considered a cluster will be selected. The results depict that despite the presence of multiple clusters identified by MCODE, the analysis or focus selected the first cluster of those 15 genes from cytoHubba of each compound that meets the criteria for being a high-value cluster as shown in Fig. 4 (A. Pt4, B. PQA18, C. PQA11, D. Dictyoquinone, E. Discoidol, F. Discodiene, and G. Dictyosterol).

3.3. Gene Ontology enrichment and Kyoto Encyclopedia of genes and Genomes analysis

The compounds hub target genes–pathways network was utilized to filter the major hub genes and key compounds of *D. discoideum* to treat AD. Additionally, the key genes were further analyzed through GO & KEGG to validate their biological functions Fig. 5 (A. Pt4, B. PQA18, C. PQA11, D. Discoidol, E. Discodiene, F. Dictyosterol, and G. Dictyoquinone). The GO provides a way to describe the functions and attributes of genes by annotating specific GO terms that describe the genes' known or predicted functions in biological processes, molecular functions, and cellular components. Here, the Cellular Components (CC) group mainly included the 'vesicle lumen', 'Membrane raft', membrane micro domain, spanning component of the plasma membrane, and ficolin-1-rich granule lumen. This implicates the role of genes and their associated proteins i.e. cellular components in various pathways and mechanisms such as synaptic dysfunctioning and loss, abnormalities generation in vesicle trafficking and neurotransmission; generating disruptions in

Table 3
Drug-likeness properties of selected secondary metabolites.

Compound	Molecular weight	Log BBB	Log P	Toxicity Class	LD50 (mg/kg)
Discoidol	222.372	0.589	3.92	5	2830
Dictyoquinone	208.257	0.424	2.4769	4	2000
Pt-4	278.348	0.491	3.7286	4	2000
PQA-11	357.45	0.655	4.7913	4	600
Discodiene	162.276	0.76	3.699	4	1680
PQA-18	407.554	0.411	6.8783	4	600
Dictyosterol	414.718	0.813	7.8807	4	667

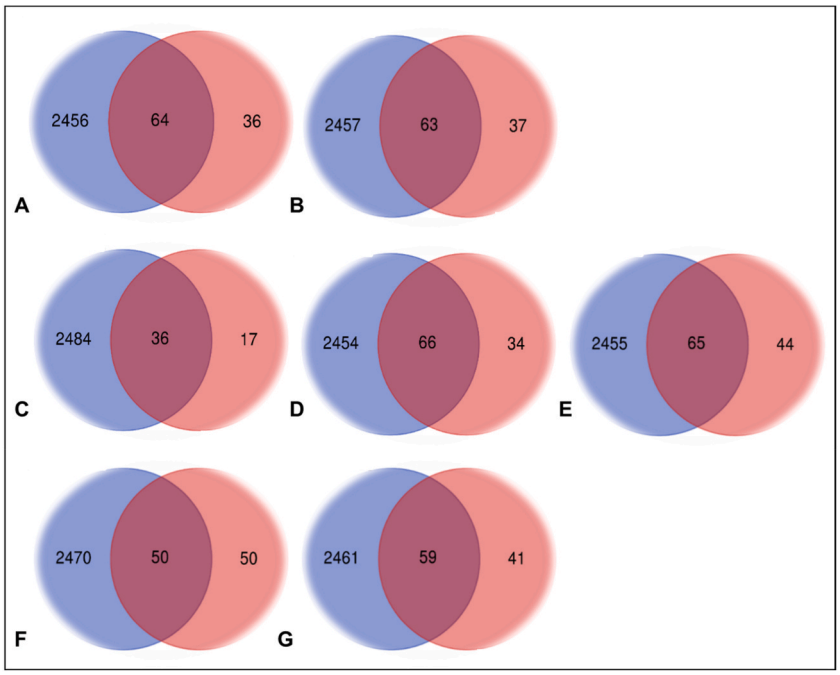

Fig. 1. Venn graph representation of AD databases & screened seven secondary metabolites for common targets finding related to Alzheimer's disease. (A: PQA18, B: Dictyoquinone, C: Discodiene, D: Dictyosterol, E: PQA11, F: Pt4, G: Discoidol).

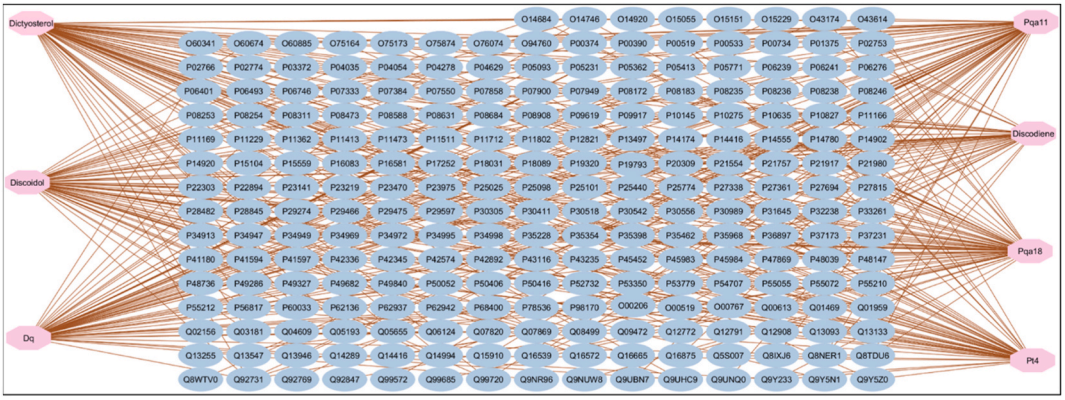

Fig. 2. Screened seven compounds - target network for AD. Pink nodes represent compounds & Blue nodes represents shared targets. *(*__Dq__ is Dictyoquinone)*. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

membrane lipid composition and lipid rafts, and promoting aggregation and accumulation of amyloid-beta (Aβ) peptides.

In the molecular function (MF) group, the GO term involved protein serine/threonine kinase activity, MAPK activity, steroid binding, phosphatase binding, nuclear receptor activity, and collagen binding. This implicates the functions of the selected genes as protein kinase regulators that have a crucial role in various cellular processes, including signal transduction pathway; MAPK protein kinase regulators that help in maintaining neuronal plasticity, memory, and learning; steroidal hormones level modulator that have a major impact on neuronal functioning; homeostasis maintaining unit between kinases and phosphatases. Lastly, the Biological Processes (BP) group highlighted the significance of these targeted genes in multiple pathways. These pathways include peptidyl-serine phosphorylation to prevent abnormal tau phosphorylation, regulation of DNA-binding transcription factors for memory and synaptic plasticity maintenance, immune response activation in response to lipopolysaccharides, mammary gland development, the mechanism for nicotine response, regulation of cholesterol storage for proper neuronal function through cholesterol homeostasis, and the maintenance of collagen composition and integrity, crucial for vascular function. Additionally, KEGG enrichment analysis showed in Fig. 5 that the screened genes of each compound were potentially targeted to several critical pathways associated with AD such as IL-17 signaling pathway, TNF signaling pathway, Prolactin signaling pathway, and PPAR signaling pathway. The genes may potentially affect these pathways as they regulate IL-17 (Interleukin-17) and TNF, pro-inflammatory cytokines associated with inflammatory

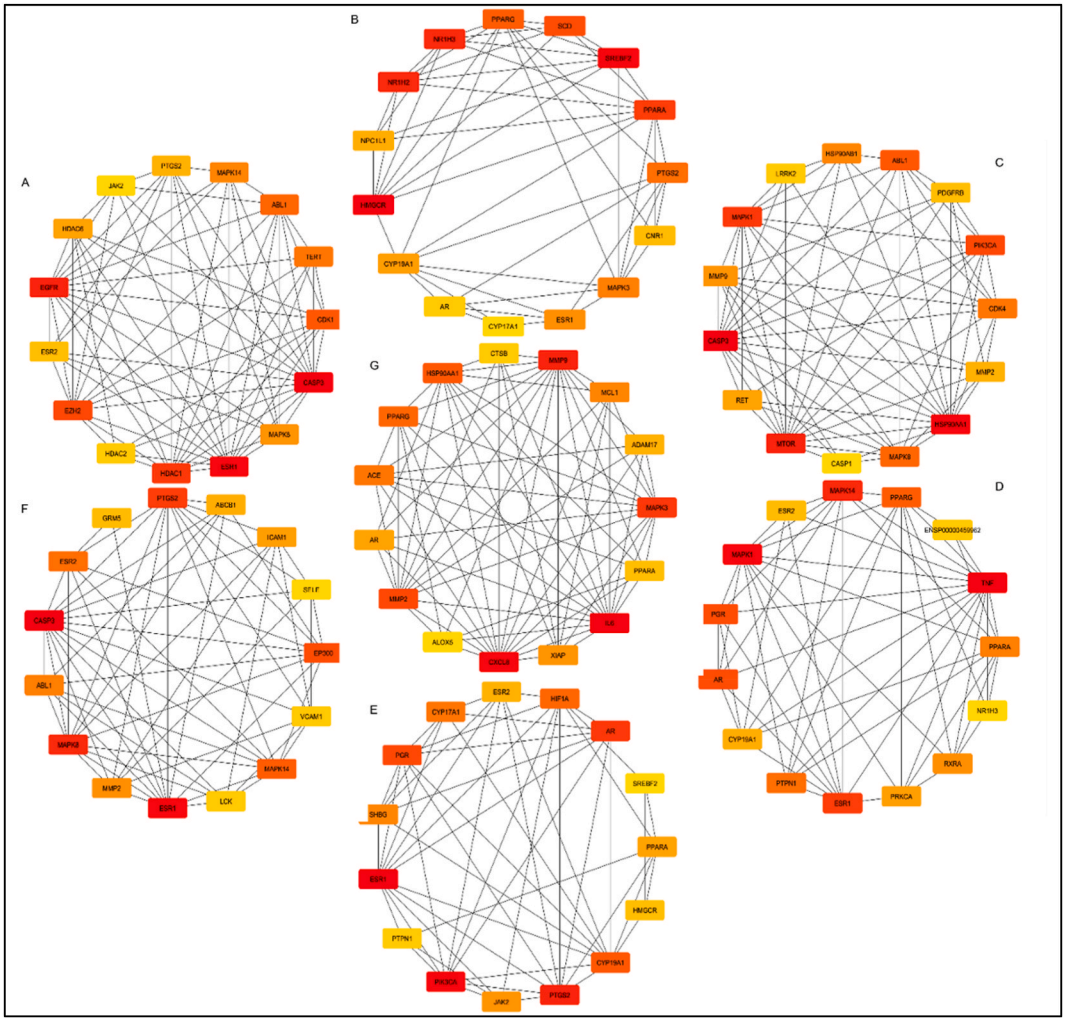

Fig. 3. CytoHubba plug-in of Cytoscape was used to select the molecular complexes and core targets for all seven screened compounds (A. PQA11, B. Dictyosterol, C. Pt4, D. Discodiene, E. Discoidol, F. PQA18, and G. Dictyoquinone).

responses generated through the IL-17 signaling pathway and TNF Signaling Pathway, respectively. They also play a role in managing prolactin hormone, which may have neuroprotective effects and contribute to neuronal repair and regeneration.

Additionally, they modulate PPAR (nuclear receptors) that influence lipid metabolism through the PPAR Signaling Pathway. The top neuroinflammatory pathway with the highly associated gene PTGS2 (COX2) was demonstrated in Fig. 6.

3.4. Molecular docking analysis

From the main potential compound targets - AD targets network Fig. 2, the most significant 22 genes (Degree = ≤ 40) were consider for the docking study. Additionally, based on the outcomes of the KEGG pathway enrichment analysis, we focused on the inflammatory pathway within Alzheimer's disease complications. This pathway exhibited the highest percentage of genes participating in various biological functions and signaling pathways among the total number of intersecting genes. Subsequently, we subjected this pathway to further analysis. Further upregulation receptors were screened through AlzData (http://www.alzdata.org/), the listed receptors CYP19A1, ESR1, MAPK1, TNF, PTPN1, MAPK14, SELE, ESR2, VCAM1, MMP2, MAPK8, EP300, CASP3, PTGS2, MTOR, HSP90AA1, HSP90AB1, CASP1, CDK4, IL6, CTSB and MMP9 were upregulated in Alzheimer's disease. These 22 targets were dock with seven screened compounds and after comparison with standard drugs as depicted in Fig. 7.

Following their notable binding affinity (in Kcal/mol), as indicated in Table 4. Five receptors underwent additional screening. These receptors are CYP19A1, MAPK8, HSP90AB1, MTOR, and COX2. They were screened with their respective interacting ligands and standard drugs. In the molecular docking analysis, comparisons between metabolite-receptor and receptor-drug interactions revealed predominantly non-significant alterations in binding affinity. Metabolites predominantly interacted with receptors through polar and hydrophobic amino acid interactions. This hydrophobic effect facilitated the convergence of ligand and receptor

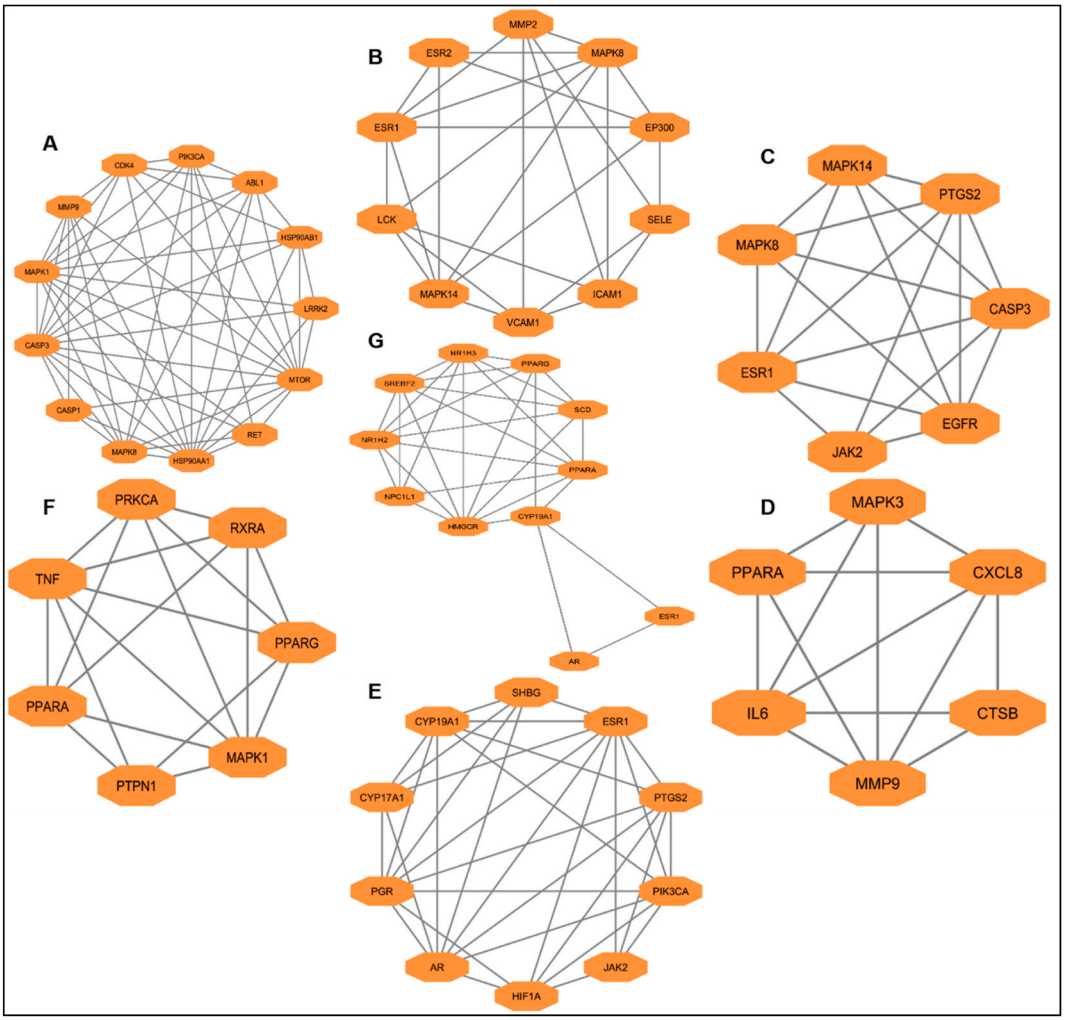

Fig. 4. MCODE plug-in of Cytoscape was used to select the highly prominent core targets for all seven screened compounds (A. Pt4, B. PQA18, C. PQA11, D. Dictyoquinone, E. Discoidol, F. Discodiene, and G. Dictyosterol).

hydrophobic regions, excluding water molecules and minimizing the destabilizing impact on the water structure surrounding hydrophobic regions. Consequently, this phenomenon led to the formation of a more stable complex. Furthermore, it is noteworthy that a statistically significant positive binding affinity was observed upon analyzing the interaction between the COX-2 receptor and the metabolites PQA11 and Discoidol, as well as between CYP19A1 and the metabolite Dictyosterol, when compared to the standard drugs Rofecoxib and Letrozole, respectively. One notable finding derived from the molecular docking analysis was the interaction between the metabolite Pt4 and the mTOR receptor, demonstrating comparable efficacy to its standard drug counterpart, Sirolimus. This observation suggests the potential effectiveness of the natural metabolite or compound in comparison to the synthetic drug. As evident from Fig. 7, the compound Pt4 interacts with the receptor mTOR majorly through polar amino acids such as SER1584, ALA1429, ILE1417, TYR1587, indicating higher bonding efficiency and H bond numbers, making it a potential candidate similar to Sirolimus drug. However, later on, the probable roles of the two selected receptors were discovered. As mTOR is involved in regulating synaptic plasticity, which is essential for learning and memory. Therefore, inhibiting mTOR could potentially disrupt synaptic plasticity, which may worsen cognitive function in AD. Another target, CYP19A1 has complex, multifaceted interaction that involves multiple mechanisms and less literature suggests removal of it and same as for HSP90AB1. At the end, modulating MAPK8 (Mitogen-Activated Protein Kinase 8) can lead to various systemic effects, as this kinase is active in many different tissues. This led to finalizing the use of COX2 having a key role as an amyloid beta accumulator, tau hyper phosphorylation, ROS generation via oxidative stress, etc. Therefore, only the COX2 (PTGS2) receptor and its respective two ligands (PQA11 & Discoidol) were further evaluated through molecular dynamic simulation-GROMACS.

Later, in Fig. 8, the lig plot interactions showed the interactive amino acids forming bonds between the molecules such as COX-2 is engaged in interactions with its ligands primarily via polar amino acids, including GLN, SER, ASN, and TYR, HIS, LYS, and ASP. These interactions contribute to ligand binding specificity and stability. Through the formation of hydrogen bonds, these polar residues

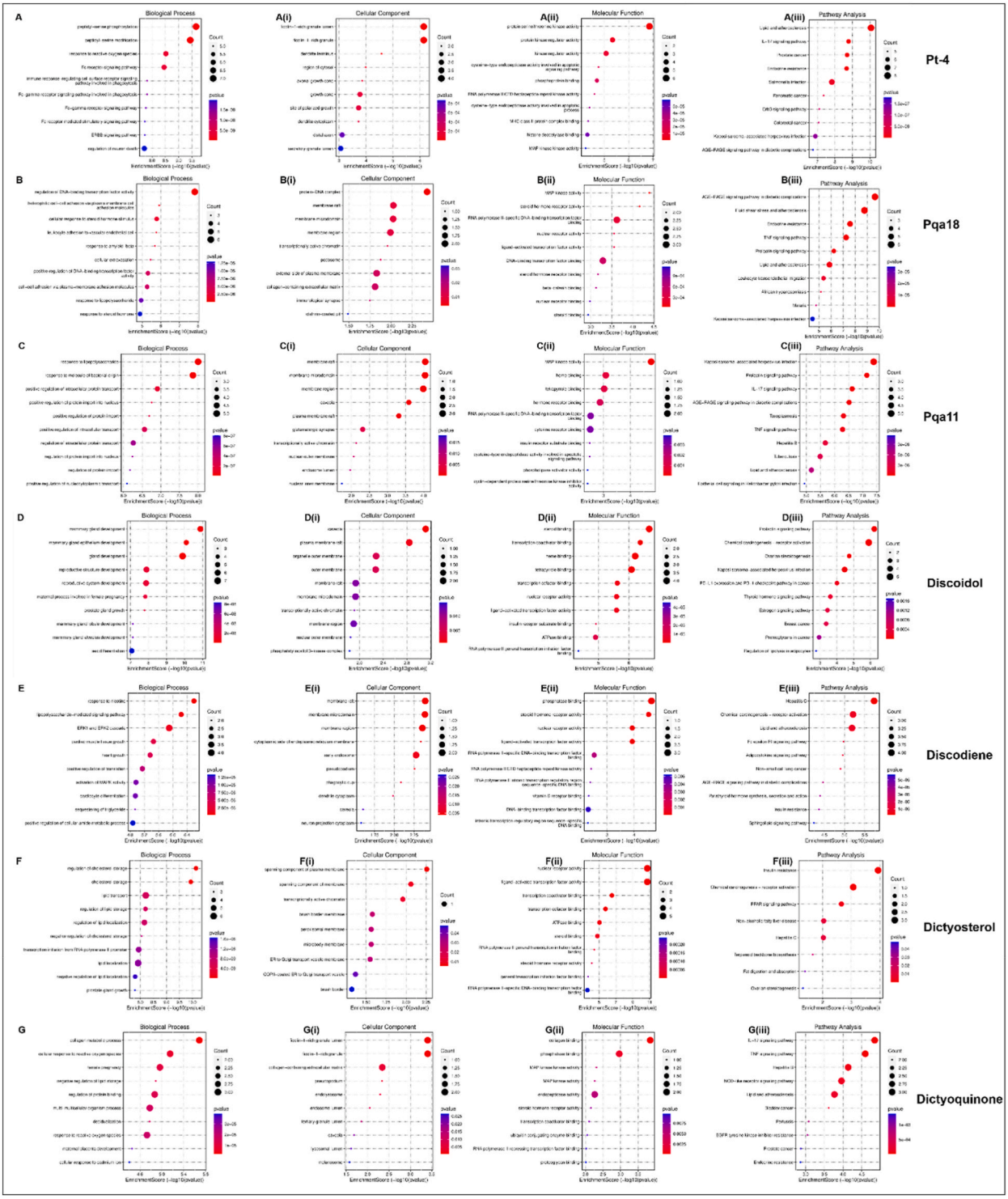

Fig. 5. GO function analysis of seven screened compounds in treatment of AD, The GO function analysis, including biological process (BP), cellular component (CC), and molecular function (MF). (A. Pt4, B. PQA18, C. PQA11, D. Discoidol, E. Discodiene, F. Dictyosterol, and G. Dictyoquinone).

prevent easy dissociation of the ligand from the protein, thereby prolonging the residence time of the complex. This phenomenon maximizes the number of hydrogen bonds formed with the metabolites, enhancing the overall stability and specificity of the COX-2-ligand interactions.

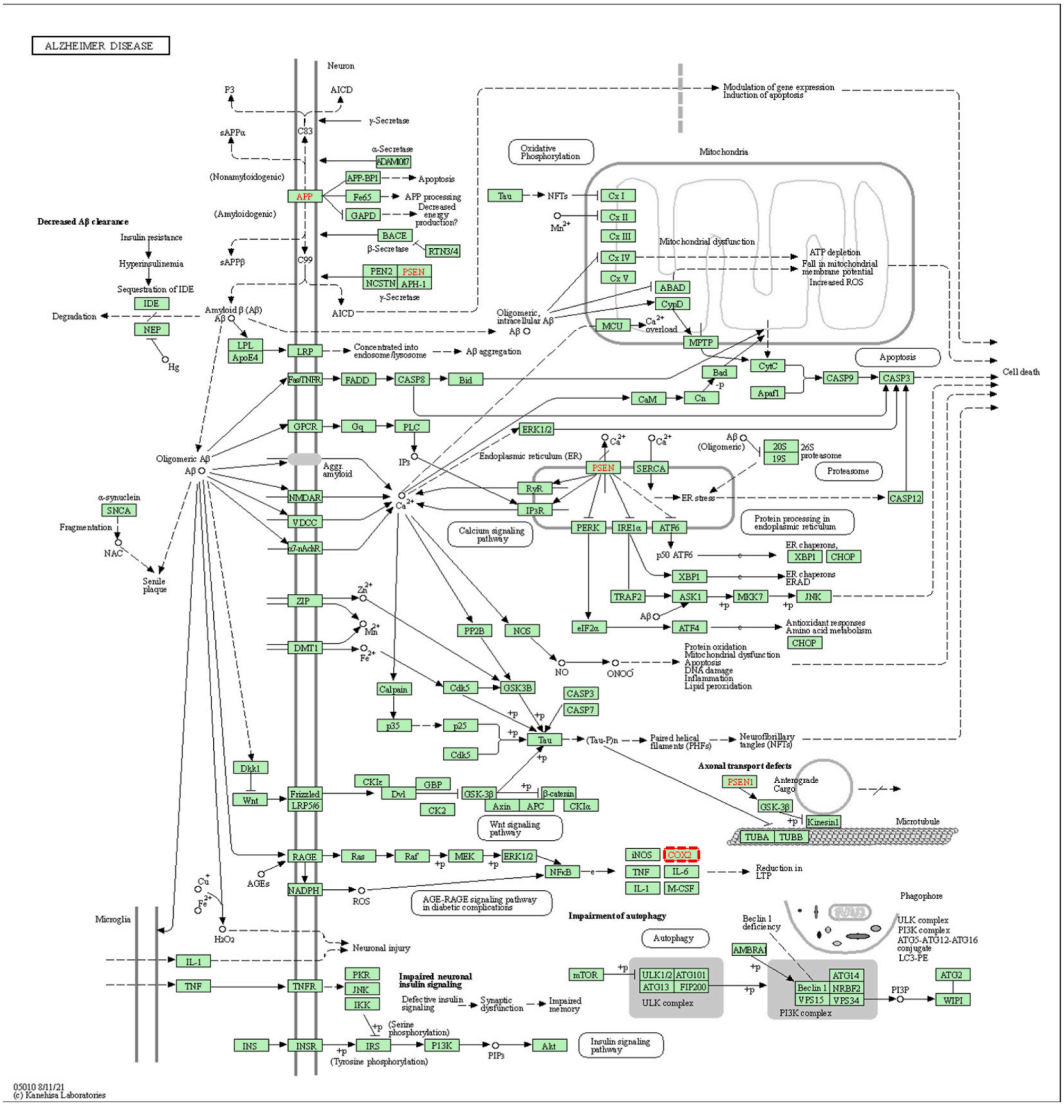

Fig. 6. KEGG pathway enrichment analyses of screened compounds in the treatment of AD. The neuroinflammatory pathway was highly involved and therefore COX2 was represent in red dotted box. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.5. Molecular dynamic simulations

MD modeling is tool for investigating protein-ligand complexes in cellular environment, flexibility of complex & stability of protein after binding with compound or ligand. Due to robust binding interactions, alignment with drug-like characteristics, and favorable ADMET properties, the COX2-Discoidol (Complex 1) and COX2-PQA-11 (Complex 2) complexes were chosen for a 100ns MDS. Additionally, a drug-control complex (Complex 3) was included for comparative analysis. I n the initial examination of the MD trajectories, alterations in the root-mean-square deviation (RMSD) values for protein atoms during the simulation were scrutinized. The plateau pattern observed in the RMSD analysis of the unbound COX2 protein suggested that the simulation duration was adequate for this protein under the specified conditions. The analysis was perform on steady system and their results are represented in Fig. 9 (A. RMSD plot, B. RMSF plot, C. SASA plot, D. H bond plot, E. Radius of Gyration, F. Total solvent energy plot). For the unbound protein, following an initial jump attributed to protein relaxation, the system achieved equilibrium after 5 ns. These observations not only validate the adequacy of the simulation duration but also suggest that there were no substantial alterations in the protein structure throughout the simulation. Later, on the RMSD were also calculated with the binding of the COX2 receptor with Discoidol and PQA-11. The results display that the binding of Discoidol in Complex 1 (COX2-Discoidol) fluctuated between 55 and 62ns and tended to be stabilized after 65ns. The complex reached the RMSD peak of 9 Å at 60ns and then continued to show stable RMSD change with a noticeable fluctuation until 65ns. On the other hand, the events in the RMSD changes of the COX2-PQA-11 (complex 2) showed that

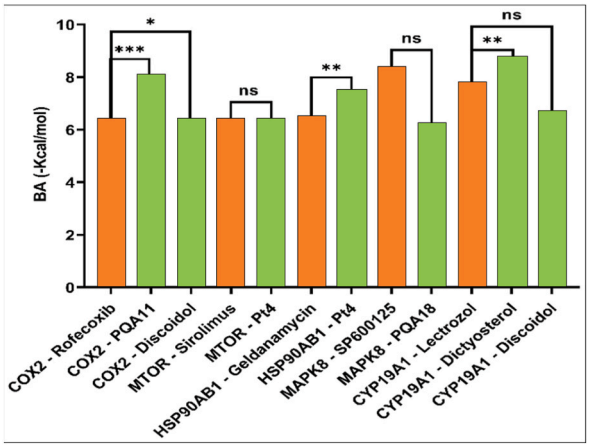

Fig. 7. Bar graph representation of screened compounds binding affinity in comparison to standard drugs (orange bar: drug & green bar: compounds). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Assessment of the binding energies of predominantly ranked secondary metabolites targeting five distinct Alzheimer's disease-related molecular targets and their corresponding interactive amino acid residues.

Protein	Compound	Binding Energy (-Kcal/mol)	No. of H bonds	Types of Bond present	Residues involved in H bond formation
PTGS2/ COX2	PQA-11	8.13 ± 0.163	7	Vander Waal, Conventional Hydrogen, Pi-Donor Hydrogen, Alkyl, Pi-Sigma, Pi-Alkyl	Arg120, Leu123, His90, Val89, Trp100,Ser119, Pro86
	Discoidol	6.13 ± 0.530	1	VanderWaal, Alkyl, Conventional Hydrogen	Gln451
CYP19A1	Dictyosterol	8.8 ± 0.0	4	Van Der Waals, Conventional Hydrogen, Carbon Hydrogen, Alkyl, Pi-Alkyl,	Tyr361, Pro429,Phe430, Gln428
	Discoidol	6.5 ± 0.0	1	Van Der Waals, Conventional Hydrogen, Carbon Hydrogen,	Met374
HSP90AB1	Pt-4	7.53 ± 0.048	6	Van Der Waals, Conventional Hydrogen, Pi-Donor Hydrogen, Alkyl, Pi-Sigma, Pi-Alkyl, Carbon–Hydrogen, Alkyl, Pi-Pi Stacked	Tyr129, Trp 162,Leu 107, Phe138, Val150, Asn51
MTOR	Pt-4	6.43 ± 0.108	4	Van Der Waals, Hydrogen, Alkyl, Pi-Sigma	Pro1426, His1454, Tyr1583, Ser1584
MAPK8	PQA-18	6.13 ± 0.227	5	Vander Waal, Carbon–Hydrogen, Alkyl, Pi-Sigma, Pi-Alkyl	Leu110, Gln37, Arg69, Ser34, Ser155

there is no significant change in protein structure during simulation and the PQA-11 bound with strong efficiency without any noticeable changes in the activation pattern or instability. Comparatively, the COX2-Rofecoxib drug complex showed an initial fluctuation at 5ns and then tended to stabilize with a noticeable Fluctuation until 100ns.

Root mean square fluctuation (RMSF) is a crucial metric for anticipating localized conformational alterations within both the protein chain and the ligands. Typically, lower RMSF values within structures affirm the existence of secondary structures and are generally consider superior in comparison to those with elevated RMSF values. The results show that the core protein COX2 maintains a relatively stable structure over time with maximum fluctuation between 0.05 and 0.3 nm wavelengths. The average RMSF of COX2-Discoidol (Complex 1) and (Complex 3) drug-control (COX2-Rofecoxib) was 0.1–0.3 nm, whereas COX2-PQA-11 (Complex 2) showed the most similar and stable configuration as the core protein range i.e. 0.05–0.3 nm indicating the stable nature of PQA-11-COX2 protein complexes during simulation. Of all the compounds, PQA-11 was considered the most appropriate as it showed minimum fluctuations and was the most stable with respect to other compounds used with COX2. The radius of gyration (Rg), which denote the compactness of the protein, expresses the rigidity of docked complexes and it's folding or unfolding. The radius of gyration of core protein was between 2.41 and 2.44 nm whereas in compound bound (Complex 1) COX2-Discoidol, COX2-PQA-11 (Complex 2), and COX2-Rofecoxib (Complex 3) fluctuated between 2.45 and 2.52 nm, 2.44–2.49 nm, and 2.45–2.51 nm, respectively. Of all the ligands, the binding of PQA-11 kept the protein stable, in well-folded form. Moreover, the analysis of the solvent-accessible surface area (SASA) was conducted for various protein-ligand complexes, as well as the unbound COX2 protein. As depicted in Fig. 9, the SASA value decreased during the simulation in all systems. This reduction can be attribute to the increased compactness of the protein structure and/or the closure of water inlet valves within the internal cavities, which restrict water diffusion into the protein's inner regions. These outcomes align with those of the Rg, collectively affirming that the protein experiences structural compression in an aqueous environment. The number of hydrogen bond were showing that, Rofecoxib-COX2 complex consist two H-bond for most of 100ns time period and Discoidol & PQA11 with COX2 showing one H-bond for most of the time period from 100ns. Apart from H-bonding analysis, Total solvent energy were calculated in kJ/mol suggests that, PQA11-COX2 complex possesses the least negative solvent energy

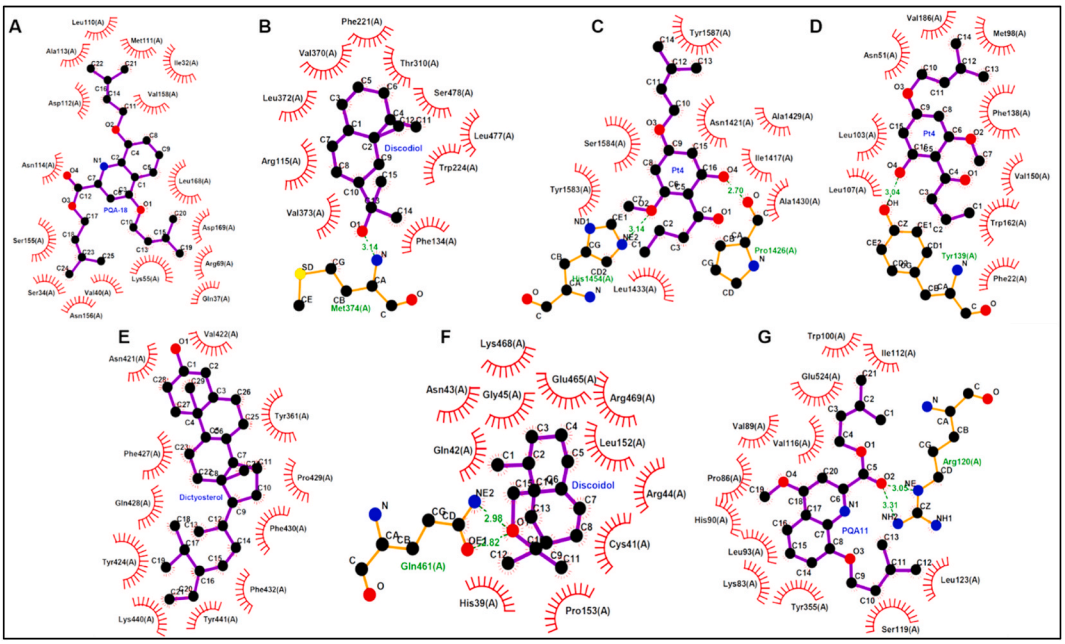

Fig. 8. Protein-Ligand Interactions showcasing major amino acids involved in bond formation between metabolites-receptor through LigPlot: A Comprehensive Structural Analysis Tool for Molecular Recognition studies (A: PQA18- MAPK8, B: Discoidol-CYP19A1, C:Pt4-MTOR, D: Pt4-HSP90AB1, E: Dictyosterol-CYP19A1, F: Discoidol-COX2, G: PQA11-COX2).

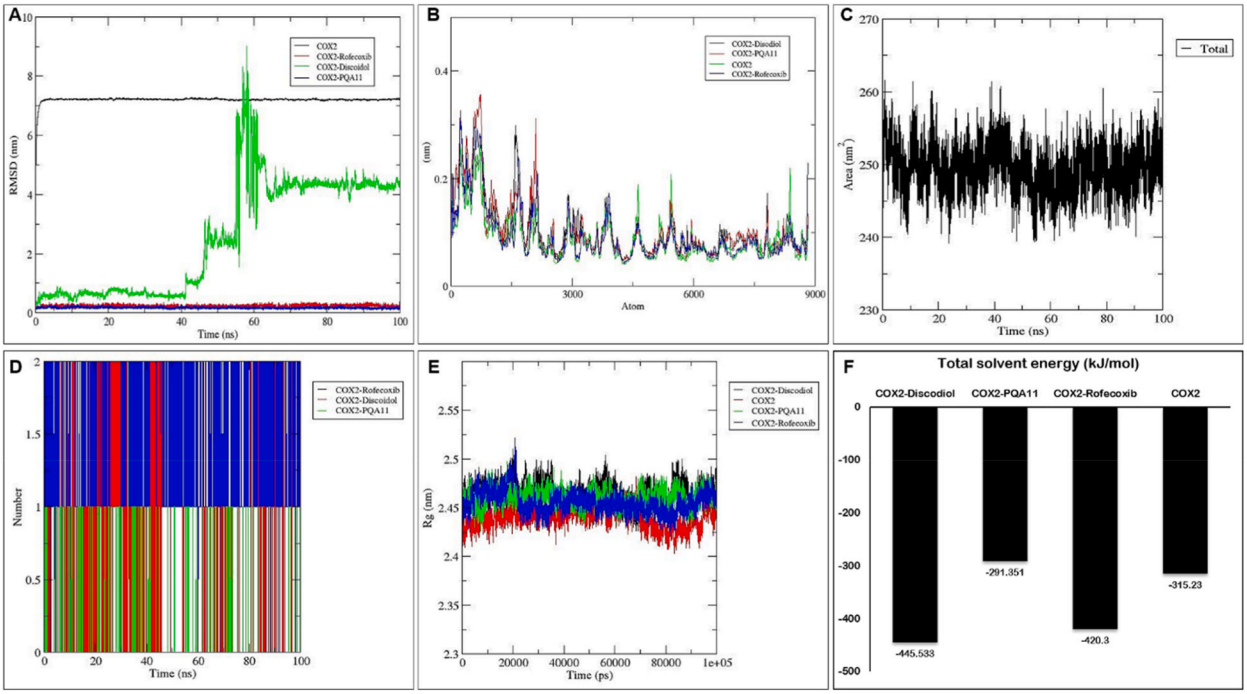

Fig. 9. Different measures of molecular dynamic simulation A. RMSD plot, B. RMSF plot, C. SASA plot, D. H bond plot, E. Radius of Gyration, F. Total solvent energy plot.

(−291.351), whereas COX2-Discoidol complex displayed portrayed highest total solvent energy (−445.535) (Fig. 9F). That strongly suggest COX2-PQA11 complex contribute highest solvent solubility in triclinic solvent box.

4. Discussion

In AD, it's believed that the primarily trigger for immune response in the brain is the presence of Abeta proteins, that activate microglial cells, a type of immune cell in the brain. Initially, these microglial cells become enlarged as they attempt to process the Abeta, but over time, they become less effective. Unfortunately, sustained immune system activation, caused by continuous exposure to Abeta, can actually worsen the progression of AD. This prolonged immune response sets off a vicious cycle, known as reactive microgliosis, leading to an accumulation of Abeta and the release of pro-inflammatory signaling molecules. This harmful cascade ultimately damages neurons, fuels neuroinflammation, and contributes to neurodegeneration [40]. It is critical to recognize that TNF-α is one of the most significant pro-inflammatory signaling molecules in AD. TNF- α elevates the expression of specific adhesion molecules on blood vessel walls, including VCAM-1, ICAM-1, and *E*-selectin. This facilitates the entry of white blood cells and other immune cells into the brain, worsening inflammation [41]. Furthermore, the enzyme COX-2 plays a significant role in mediating inflammation, particularly in peripheral tissues in the early stages of AD but less prevalent in later stages. While the exact metabolic processes involving COX-2 in AD are not fully understood, there is evidence to suggest its involvement [42]. Moreover, a study by Dudal & Sheri in 2003 found that microglia, immune cells in the brain, isolated from intracranial C6 tumors showed a significant increase in the levels of a molecule called PGE2 and the increase was linked to the activity of COX-2 [43]. Additionally, recent research has revealed that highly activated COX-2 and PGE2 can elevate the levels of another molecule, IL-1β mRNA, in primary rat microglia cells when they are exposed to lipopolysaccharide (LPS), a bacterial component that triggers immune responses [44]. Moreover, research from Wang et al., in 2015 suggests that high levels of IL-1β are associated with a greater risk of developing Alzheimer's disease. In AD-affected brains, activated glial cells (a type of immune cell in the brain) can further intensify immune responses and make Aβ, a hallmark protein of AD, more effective at activating these immune cells [45]. Additionally, previous studies, such as the one conducted by Buxbaum et al., in 1992, have shown that IL-1β not only influences the production and processing of amyloid precursor protein (APP) but also increases the expression of other molecules that are relevant to AD, such as IL-6 and TNF-α [46]. High levels of COX-2 can trigger specific signaling pathways within cells, like phosphatidylinositol-3-kinase/Protein Kinase B (PI3–K/AKT) and Protein kinase A/cAMP-response element binding protein (PKA/CREB) in a manner that depends on several other molecules, including HSP70, PGE2, and cAMP [47] (Fig. 10).

These pathways eventually lead to the activation of BACE-1, an enzyme involved in the production of Aβ, through the phosphorylation of a protein called NF-κB at specific sites. This complex series of events highlights the interplay between various molecules in the brain, ultimately connecting COX-2 to the production of Aβ and neuroinflammation. Furthermore, this cycle is not a one-way street, BACE-1, when activated, can also stimulate the production of COX-2 in glial cells when they are exposed to Aβ. This bidirectional relationship between inflammatory cells and neurons can significantly contribute to the development of AD [48]. To combat this cycle of inflammation and neuronal damage, researchers have explored the potential of natural compounds. These compounds have shown promise in reducing inflammation and potentially slowing down AD progression. For example, a study by Li XJ et al., in 2021 suggested that Frankincense oil, derived from the *Boswellia carterii* plant, contains a compound with the potential to inhibit COX-2 expression in an animal model of hind paw inflammation [49]. Similarly, research involving the fibrous root of the Chinese plant *Alangium chinense* revealed sesquiterpenes with anti-inflammatory properties, outperforming standard drugs in terms of their effectiveness [50]. These findings suggest that natural compounds may hold the key to managing inflammation and AD. Terpenes, which are naturally occurring compounds found in various plants, have also shown promise in reducing inflammation. A study by Hu et al., in 2017 highlighted the ability of β-Caryophyllene, a terpene, to inhibit inflammatory activity in microglial cells exposed to hypoxia and amyloid β (Aβ) peptide. Additionally, linalool, another terpene, has been found to reduce the expression of pro-inflammatory markers in the brains of mice with a genetic predisposition to AD [51]. These examples underscore the potential of natural compounds in addressing inflammation in AD. In this research, for further validation interaction between selected secondary metabolites and their target receptors, we conducted molecular docking and molecular dynamics simulations. These analyses revealed that the COX2 (PTGS2) receptors exhibited favorable interactions with two ligands, PQA-11 and Discoidol. These interactions displayed strong binding affinities, reinforcing the potential therapeutic relevance of these compounds. Furthermore, research suggests that targeting specific genes involved in inflammatory pathways could help prevent AD. PQA11 and Discoidol polyketides are compounds found to effectively inhibit the activation of inflammatory pathways. This approach, which is supported by a KEGG enrichment analysis, focuses on critical genes associated with AD and the RAGE-mediated NF-κB signaling pathway. This pathway is vital for the binding of Aβ to receptors, and blocking it can protect against AD [52]. Importantly, PTGS2, also known as COX-2, has been found to have increased expression in the neocortical region of AD-affected brains. This indicates that PTGS2 (COX-2) may be a potential target for treating AD by disrupting the inflammatory pathway. In summary, this approach, explores the potential role of the cyclooxygenase enzyme, particularly COX-2, in the development and progression of AD. It suggests that COX-2 could be a significant therapeutic target due to its involvement in neuronal functions and its increased presence in AD-affected brains. Currently, there are no FDA-approved medications that can target COX-2 for AD treatment. However, the findings from this research, combined with ongoing investigations, offer hope for the use of naturally occurring selective COX-2 inhibitors as a means of preventing and treating AD.

5. Conclusion

In conclusion, PQA11 and Discoidol have the capacity to interact with 65 and 59 targets associated with Alzheimer's disease (AD).

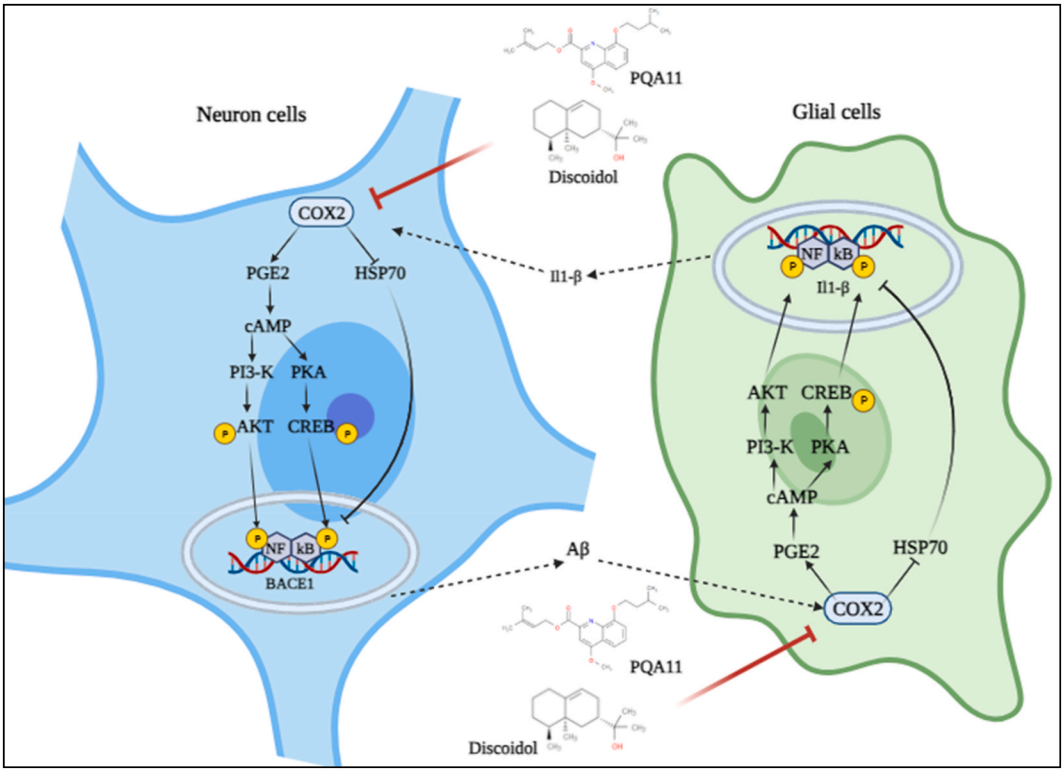

Fig. 10. The suggested sequence of signaling events in AD pathogenesis, involving COX-2-mediated interaction between IL-1b and Ab, is as follows: COX-2 triggers the release of IL-1β through a series of pathways in glial cells, including HSP70, PGE2, cAMP, PI3–K/AKT, PKA/CREB, and NF-kB. The elevated IL-1β, in turn, prompts the expression of COX-2 in neuron cells. Increased COX-2 levels activate the PI3–K/AKT and PKA/CREB pathways in a manner dependent on HSP70, PGE2, and cAMP, ultimately leading to the activation of BACE-1 by phosphorylating NF-kB. BACE-1 then reciprocally triggers COX-2 expression in glial cells in response to Aβ. These interactions between inflammatory and neuron cells may contribute to AD development. Importantly, the progression of this cascade can be inhibited by the compounds Discoidol and PQA11, potentially slowing AD progression.

These compounds have been identified as having potential in the treatment of AD through their interactions with multiple AD-related targets. The polyketidic nature of these compounds enables them to engage with seven targets related to inflammation, thereby inhibiting the expression of proinflammatory cytokines such as IL-1, IL-2, and TNF-α, as well as suppressing the formation of amyloid beta plaques in AD. This study is expected to expand the range of treatment options for AD and further underscores the feasibility of applying network pharmacology to analyze Traditional Chinese Medicine (TCM) prescriptions. However, further in vivo experiments are necessary to validate the effects of PQA11 on AD. Additionally, while only two compounds, PQA11 and Discoidol, have been identified as potential active ingredients of *D. discoideum* against AD, more experiments are required to screen for other compounds with anti-AD activity, particularly those interacting with seven or more targets.

Funding statement

This work received funding from Intramural project scheme by Research & Development Cell, Parul University (CR4D/IMSL/048).

Data availability statement

The data used to support the findings of this study are included within the article.

CRediT authorship contribution statement

Nil Patil: Visualization, Validation, Supervision, Software. **Rupal Dhariwal:** Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Arifullah Mohammed:** Software, Resources, Project administration, Methodology. **Lee Seong Wei:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision. **Mukul Jain:** Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lee Seong Wei, Associate Editor, Heliyon If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28852.

References

[1] Mukul Jain, Rupal Dhariwal, Nil Patil, Sandhya Ojha, Reshma Tendulkar, Mugdha Tendulkar, Parmdeep Singh Dhanda, Alpa Yadav, Prashant Kaushik, Unveiling the molecular Footprint: Proteome-based biomarkers for Alzheimer's disease, J. Proteome Res. 11 (4) (2023) 33, https://doi.org/10.3390/proteomes11040033.

[2] S.K. Roy, J.J. Wang, Y.M. Xu, 2023 Alzheimer's disease facts and figures, Alzheimers Dement 19 (2023) 1598–1695, https://doi.org/10.1002/alz.13016.

[3] Sneham Tiwari, Venkata Atluri, Ajeet Kaushik, Adriana Yndart, Madhavan Nair, Alzheimer's disease: pathogenesis, diagnostics, and therapeutics, Int. J. Nanomed. (2019) 5541–5554, https://doi.org/10.2147/IJN.S200490.

[4] Lei Gu, Zhefeng Guo, Alzheimer's Aβ42 and Aβ40 peptides form interlaced amyloid fibrils, J. Neurochem. 126 (3) (2013) 305–311, https://doi.org/10.1111/jnc.12202.

[5] Teresa Pardo-Moreno, Anabel González-Acedo, Antonio Rivas-Domínguez, Victoria García-Morales, Francisco Jose García-Cozar, Juan Jose Ramos-Rodríguez, Lucía Melguizo-Rodríguez, Therapeutic approach to Alzheimer's disease: current treatments and new perspectives, Pharmaceutics 14 (6) (2022) 1117, https://doi.org/10.3390/pharmaceutics14061117.

[6] George T. Grossberg, Cholinesterase inhibitors for the treatment of Alzheimer's disease: getting on and staying on, Curr. Ther. Res. 64 (4) (2003) 216–235, https://doi.org/10.1016/S0011-393X(03)00059-6.

[7] Ana L. Colín-González, Ricardo A. Santana, Carlos A. Silva-Islas, Maria E. Chánez-Cárdenas, Abel Santamaría, Perla D. Maldonado, The Antioxidant Mechanisms Underlying the Aged Garlic Extract-And S-Allylcysteine-Induced Protection, vol. 2012, Oxid. Med. Cell. Longev, 2012, https://doi.org/10.1155/2012/907162.

[8] Xiaopei Zou, Si Liu, Huihui Zou, Wanfei Zhou, Huaili Fu, Jiana Wei, Jiakang Zhang, et al., Inflammatory mechanisms of Ginkgo Biloba extract in improving memory functions through lncRNA-COX2/NF-κB pathway in mice with status epilepticus, CNS Neurosci. Ther. 29 (1) (2023) 471–482, https://doi.org/10.1111/cns.14019.

[9] Se Jin Park, Jun Man Jung, Hyung Eun Lee, Young Woo Lee, Dong Hyun Kim, Jong Min Kim, Jin Gyu Hong, et al., The memory ameliorating effects of INM-176, an ethanolic extract of Angelica gigas, against scopolamine-or Aβ1–42-induced cognitive dysfunction in mice, J. Ethnopharmacol. 143 (2) (2012) 611–620, https://doi.org/10.1016/j.jep.2012.07.019.

[10] Grazia D'Onofrio, Seyed Mohammad Nabavi, Daniele Sancarlo, Antonio Greco, Stefano Pieretti, Crocus sativus L.(Saffron) in Alzheimer's disease treatment: bioactive effects on cognitive impairment, Curr. Neuropharmacol. 19 (9) (2021) 1606, https://doi.org/10.2174/1570159X19666210113144703.

[11] Kavon Rezai-Zadeh, Doug Shytle, Nan Sun, Takashi Mori, Huayan Hou, Deborah Jeanniton, Jared Ehrhart, et al., Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice, J. Neurosci. 25 (38) (2005) 8807–8814, https://doi.org/10.1523/JNEUROSCI.1521-05.2005.

[12] Jae Woong Lee, Yong Kyoung Lee, Ok Ban Jung, Tae Youl Ha, Pyo Yun Yeo, Bae Han Sang, Ki Wan Oh, Tae Hong Jin, Green tea (-)-epigallocatechin-3-gallate inhibits β-amyloid-induced cognitive dysfunction through modification of secretase activity via inhibition of ERK and NF-κ B pathways in mice, J. Nutr. 139 (10) (2009) 1987–1993, https://doi.org/10.3945/jn.109.109785.

[13] Stephanie Andrade, Maria João Ramalho, Joana Angélica Loureiro, Maria do Carmo Pereira, Natural compounds for Alzheimer's disease therapy: a systematic review of preclinical and clinical studies, Int. J. Mol. Sci. 20 (9) (2019) 2313, https://doi.org/10.3390/ijms20092313.

[14] Adriana L. da Silva, LS Piato Ângelo, Simone Bardini, Carlos A. Netto, Domingos S. Nunes, Elaine Elisabetsky, Memory retrieval improvement by Ptychopetalum olacoides in young and aging mice, J. Ethnopharmacol. 95 (2–3) (2004) 199–203, https://doi.org/10.1016/j.jep.2004.07.019.

[15] Y.M. Zuo, Z.L. Zhang, Q.H. Wang, Ning Xie, H.X. Kuang, Effects of Valeriana amurensis on the expressions of beta-APP, Abeta (1-40) and caspase-3 in Alzheimer's disease model rat's brain, Zhong Yao Cai 33 (2) (2010) 233–236.

[16] Marissa J. Schafer, Melissa J. Alldred, Sang Han Lee, Michael E. Calhoun, Eva Petkova, Paul M. Mathews, Stephen D. Ginsberg, Reduction of β-amyloid and γ-secretase by calorie restriction in female Tg2576 mice, Neurobiol. Aging 36 (3) (2015) 1293–1302, https://doi.org/10.1016/j.neurobiolaging.2014.10.043.

[17] Mohabbat Ahmadi, Mahnaz Taherianfard, Tahora Shomali, Zataria multiflora could improve hippocampal tau protein and TNFα levels and cognitive behavior defects in a rat model of Alzheimer's disease, Avicenna J. Phytomedicine 9 (5) (2019) 465, https://doi.org/10.22038/ajp.2019.12839.

[18] Misung Kwon, Bonggi Lee, Sujin Lim, Hyeung-Rak Kim, Effects of sargaquinoic acid in sargassum serratifolium on inducing brown adipocyte-like phenotype in mouse adipocytes in vitro, Planta Med. 86 (1) (2020) 45–54, https://doi.org/10.1055/a-1023-7385.

[19] Thaiane Coelho dos Santos, Thaís Mota Gomes, Bruno Araújo Serra Pinto, Adriana Leandro Camara, Antonio Marcus De Andrade Paes, Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy, Front. Pharmacol. 9 (2018) 1192, https://doi.org/10.3389/fphar.2018.01192.

[20] Nil Patil, Vaishnavi Chandel, Aarzu Rana, Mukul Jain, Prashant Kaushik, Investigation of Cannabis sativa Phytochemicals as anti-Alzheimer's Agents: an in silico study, Plants 12 (3) (2023) 510, https://doi.org/10.3390/plants12030510.

[21] Shivkanya Fuloria, Jyoti Mehta, Aditi Chandel, Mahendran Sekar, Nur Najihah Izzati Mat Rani, M. Yasmin Begum, Vetriselvan Subramaniyan, et al., A comprehensive review on the therapeutic potential of Curcuma longa Linn. in relation to its major active constituent curcumin, Front. Pharmacol. 13 (2022) 820806, https://doi.org/10.3389/fphar.2022.820806.

[22] Yuzuru Kubohara, Haruhisa Kikuchi, Dictyostelium: an important source of structural and functional diversity in drug discovery, Cells 8 (1) (2018) 6, https://doi.org/10.3390/cells8010006.

[23] Michael A. Myre, Kevin Washicosky, Robert D. Moir, Giuseppina Tesco, Rudolph E. Tanzi, Wasco Wilma, Reduced amyloidogenic processing of the amyloid β-protein precursor by the small-molecule Differentiation Inducing Factor-1, Cell. Signal. 21 (4) (2009) 567–576, https://doi.org/10.1016/j.cellsig.2008.12.008.

[24] Janet Piñero, Josep Saüch, Ferran Sanz, Laura I. Furlong, The DisGeNET cytoscape app: exploring and visualizing disease genomics data, Comput. Struct. Biotechnol. J. 19 (2021) 2960–2967, https://doi.org/10.1016/j.csbj.2021.05.015.

[25] Michael Rebhan, Vered Chalifa-Caspi, Jaime Prilusky, Doron Lancet, GeneCards: a novel functional genomics compendium with automated data mining and query reformulation support, Bioinformatics 14 (8) (1998) 656–664, https://doi.org/10.1093/bioinformatics/14.8.656.

[26] Allan Peter Davis, Thomas C. Wiegers, Jolene Wiegers, Brent Wyatt, Robin J. Johnson, Daniela Sciaky, Fern Barkalow, Melissa Strong, Antonio Planchart, Carolyn J. Mattingly, CTD tetramers: a new online tool that computationally links curated chemicals, genes, phenotypes, and diseases to inform molecular mechanisms for environmental health, Toxicol. Sci. (2023) kfad069, https://doi.org/10.1093/toxsci/kfad069.

[27] David Gfeller, Aurélien Grosdidier, Matthias Wirth, Antoine Daina, Olivier Michielin, Zoete Vincent, SwissTargetPrediction: a web server for target prediction of bioactive small molecules, Nucleic Acids Res. 42 (W1) (2014) W32–W38, https://doi.org/10.1093/nar/gku293.
[28] Hossam Kadry, Behnam Noorani, Luca Cucullo, A blood–brain barrier overview on structure, function, impairment, and biomarkers of integrity, Fluids Barriers CNS 17 (1) (2020) 1–24, https://doi.org/10.1186/s12987-020-00230-3.
[29] Douglas EV. Pires, Tom L. Blundell, David B. Ascher, pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures, J. Med. Chem. 58 (9) (2015) 4066–4072, https://doi.org/10.1021/acs.jmedchem.5b00104.
[30] Antoine Daina, Olivier Michielin, Zoete Vincent, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (1) (2017) 42717, https://doi.org/10.1038/srep42717.
[31] Kimberley R. Cousins, ChemDraw Ultra 9.0. CambridgeSoft, 100 CambridgePark Drive, Cambridge, MA, 2005, pp. 4115–4116, https://doi.org/10.1021/ja0410237, 02140, www.cambridgesoft.com.SeeWebsiteforpricingoptions.
[32] Kai Feng Dong, Meng Qi Huo, Heng Ya Sun, Tian Ke Li, Dan Li, Mechanism of Astragalus membranaceus in the treatment of laryngeal cancer based on gene co-expression network and molecular docking, Sci. Rep. 10 (1) (2020) 11184, https://doi.org/10.1038/s41598-020-68093-0.
[33] Damian Szklarczyk, Annika L. Gable, David Lyon, Alexander Junge, Stefan Wyder, Jaime Huerta-Cepas, Milan Simonovic, et al., STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets, Nucleic Acids Res. 47 (D1) (2019) D607–D613, https://doi.org/10.1093/nar/gky1131.
[34] Chia-Hao Chin, Shu-Hwa Chen, Hsin-Hung Wu, Chin-Wen Ho, Ming-Tat Ko, Chung-Yen Lin, cytoHubba: identifying hub objects and sub-networks from complex interactome, BMC Syst. Biol. 8 (4) (2014) 1–7, https://doi.org/10.1186/1752-0509-8-S4-S11.
[35] Huaiyu Mi, Anushya Muruganujan, John T. Casagrande, D. Thomas Paul, Large-scale gene function analysis with the PANTHER classification system, Nat. Protoc. 8 (8) (2013) 1551–1566, https://doi.org/10.1038/nprot.2013.092.
[36] Yanan Chu, Fangcong Yu, Yakui Wu, Jinxiu Yang, Jiaran Shi, Tianxin Ye, Deheng Han, Xingxiang Wang, Identification of genes and key pathways underlying the pathophysiological association between nonalcoholic fatty liver disease and atrial fibrillation, BMC Med. Genom. 15 (1) (2022) 1–14, https://doi.org/10.1186/s12920-022-01300-1.
[37] Zhehao Dai, Haoyu Tang, Yue Pan, Junquan Chen, Yongping Li, Jun Zhu, Gene expression profiles and pathway enrichment analysis of human osteosarcoma cells exposed to sorafenib, FEBS Open Bio 8 (5) (2018) 860–867, https://doi.org/10.1002/2211-5463.12428.
[38] Manami Dhibar, Santanu Chakraborty, Souvik Basak, Paramita Pattanayak, Tanmay Chatterjee, Balaram Ghosh, Raafat Mohamed, Mohammed AS. Abourehab, Critical analysis and optimization of stoichiometric ratio of drug-coformer on cocrystal design: molecular docking, in vitro and in vivo assessment, Pharmaceuticals 16 (2) (2023) 284, https://doi.org/10.3390/ph16020284.
[39] Vincent Zoete, Michel A. Cuendet, Aurélien Grosdidier, Olivier Michielin, SwissParam: a fast force field generation tool for small organic molecules, J. Comput. Chem. 32 (11) (2011) 2359–2368, https://doi.org/10.1002/jcc.21816.
[40] Jefferson W. Kinney, Shane M. Bemiller, Andrew S. Murtishaw, Amanda M. Leisgang, Arnold M. Salazar, T. Lamb Bruce, Inflammation as a central mechanism in Alzheimer's disease, Alzheimer's Dementia: Transl. Res. Clin. 4 (2018) 575–590, https://doi.org/10.1016/j.trci.2018.06.014.
[41] Dan-in Jang, A-Hyeon Lee, Hye-Yoon Shin, Hyo-Ryeong Song, Jong-Hwi Park, Tae-Bong Kang, Sang-Ryong Lee, Seung-Hoon Yang, The role of tumor necrosis factor alpha (TNF-α) in autoimmune disease and current TNF-α inhibitors in therapeutics, Int. J. Mol. Sci. 22 (5) (2021) 2719, https://doi.org/10.3390/ijms22052719.
[42] Afshin Zarghi, Sara Arfaei, Selective COX-2 inhibitors: a review of their structure-activity relationships, J. Pharm. Res. 10 (4) (2011) 655, https://doi.org/10.22037/IJPR.2011.1047.
[43] Sherri Dudal, Influence of Mouse Genetic Background on hAPP Transgene-Induced Brain Amyloidosis and Inflammatory Response to Beta-Amyloid Protein, 2003.
[44] Molina HolgadoEduardo, Sergio Ortiz, Francisco Molina-Holgado, Carmen Guaza, Induction of COX-2 and PGE2 biosynthesis by IL-1β is mediated by PKC and mitogen-activated protein kinases in murine astrocytes, Br. J. Pharmacol. 131 (1) (2000) 152–159, https://doi.org/10.1038/sj.bjp.0703557.
[45] Wang Wen-Ying, Meng-Shan Tan, Jin-Tai Yu, Lan Tan, Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease, Ann. Transl. Med. 3 (10) (2015), https://doi.org/10.3978/j.issn.2305-5839.2015.03.49.
[46] Joseph D. Buxbaum, Masaki Oishi, Henry I. Chen, Ronit Pinkas-Kramarski, Eric A. Jaffe, Samuel E. Gandy, Greengard Paul, Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor, Proc. Natl. Acad. Sci. USA 89 (21) (1992) 10075–10078, https://doi.org/10.1073/pnas.89.21.10075.
[47] Jianjian Yang, Xue Wang, Yi Gao, Can Fang, Fan Ye, Bing Huang, Lequn Li, Inhibition of PI3K-AKT signaling blocks PGE2-induced COX-2 expression in lung adenocarcinoma, OncoTargets Ther. (2020) 8197–8208, https://doi.org/10.2147/OTT.S263977.
[48] Yaomin Chen, Xiumei Huang, Yun-wu Zhang, Edward Rockenstein, Guojun Bu, Todd E. Golde, Eliezer Masliah, Huaxi Xu, Alzheimer's β-secretase (BACE1) regulates the cAMP/PKA/CREB pathway independently of β-amyloid, J. Neurosci. 32 (33) (2012) 11390–11395, https://doi.org/10.1523/JNEUROSCI.0757-12.2012.
[49] Li Xiao-Jun, Yan-Jing Yang, Yu-Sang Li, Wei Kevin Zhang, He-Bin Tang. "α-Pinene, linalool, and 1-octanol contribute to the topical anti-inflammatory and analgesic activities of frankincense by inhibiting COX-2, J. Ethnopharmacol. 179 (2016) 22–26, https://doi.org/10.1016/j.jep.2015.12.039.
[50] Ting Xiao, Xingyan Cheng, Jiaoyan Huang, Zhenghong Guo, Ling Tao, Xiangchun Shen, Bioactive substances inhibiting COX-2 and cancer cells isolated from the fibrous roots of Alangium chinense (Lour.) Harms, RSC Adv. 13 (5) (2023) 3346–3363, https://doi.org/10.1039/d2ra06931h.
[51] Yawei Hu, Ziling Zeng, Baojie Wang, Shougang Guo, Trans-caryophyllene inhibits amyloid β (Aβ) oligomer-induced neuroinflammation in BV-2 microglial cells, Int. Immunopharm. 51 (2017) 91–98, https://doi.org/10.1016/j.intimp.2017.07.009.
[52] Shi Du Yan, Angelika Bierhaus, Peter P. Nawroth, David M. Stern, RAGE and Alzheimer's disease: a progression factor for amyloid-β-induced cellular perturbation? J. Alzheim. Dis. 16 (4) (2009) 833–843, https://doi.org/10.3233/JAD-2009-1030.