Original Article

Thai Journal of Pharmaceutical Sciences

Anthraquinones extracted from *Rubia cordifolia* Linn as potential ligands to treat Alzheimer's disease

Pottella Srinivasulu[1,2], Janga Ramesh Babu[3], T. N. V. Ganesh Kumar[4], Padarthi Pavan Kumar[2], S. Vidyadhara[3]

[1]Research Scholar, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India, [2]Department of Physiology and Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Andhra Pradesh, India, [3]Department of Pharmaceutics, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Andhra Pradesh, India, [4]Department of Pharmaceutical Chemistry, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Andhra Pradesh, India

Corresponding author:
Pottella Srinivasulu, Department of Physiology and Pharmacology, Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Andhra Pradesh, India.
E-mail: vasusri38cology@gmail.com

Received: : Jun 08, 2020
Accepted: Nov 23, 2020
Published: Mar 18, 2021

ABSTRACT

Objective: The present investigation aims to predict the pharmacokinetic aspects and assessing the anti-Alzheimer's activity using both molecular docking platform and *ex vivo* pharmacological studies on muscle contraction. **Materials and Methods:** 2-Methyl Anthraquinone (MAQ) and Quinizarine (QNZ) were isolated from *Rubia cordifolia* L., and evaluated for absorption, distribution, metabolism, excretion, and toxicity. Molecular docking was performed with the help of Mcule software on five drug targets acetylcholinesterase enzyme (AChE), cyclin-dependent kinase5 (CDK5), glycogen synthase kinase 3β (GSK3β), monoamine oxidase (MAO-B), and β-secretase enzyme (BACE). The pharmacological activity was screened by recording the muscle contractions of chick ileum. **Results:** The selected compounds were following Lipinski rule of five and the *in silico* studies have shown that the compounds possess drug likeliness, appreciated absorption, distribution, admired bioavailability, acceptable toxicity profile, and ability to cross the blood-brain barrier. The multi-target screening of AChE, CDK5, MAOB, GSK3β, and BACE for MAQ and QNZ showed greater binding energy of −9.9, −10, −10.5, −9.4, and 8.2 kcal/mol and −9.6, −9.1, −9.9, −8.9, and 7.8 kcal/mol, respectively. The *ex vivo* studies have shown to possess synergistic effect in increasing the muscle contractility response of MAQ and QNZ with acetylcholine. **Conclusion:** The *in silico* parameters have shown good pharmacokinetic profile of the isolated compounds. The binding energy and affinity of amino acids with ligands have similar behavior in the *ex vivo* studies. It is suggested that the isolated compounds have promising anti-Alzheimer's activity. Further studies are required to confirm the possibility of anti-Alzheimer's activity of the selected compounds.

Keywords: *2*-Methyl Anthraquinone, acetylcholinesterase enzyme, chick ileum, cyclin-dependent kinase5, glycogen synthase kinase 3β, monoamine oxidase, quinizarine, *Rubia cordifolia*, β-secretase enzyme 1

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder that damages cholinergic neurons in cerebral cortex resulting in loss of memory.[1] Presence of intracellular neurofibrillary tangles and extracellular amyloid plaques is characteristic features of AD.[2] Several reported reasons include oxidative stress, mitochondrial damage, protein aggregation, and release of inflammatory mediators, thus affecting brain functions. The recent data show that nearly 50 million people were suffering from dementia and the number is increasing rapidly to several million.[3] At present, FDA approved drugs to treat AD comes under acetylcholinesterase inhibitors (donepezil [DPZ], rivastigmine, and galantamine) and N-Methyl D-Aspartate receptor antagonists (memantine). Till date, around 112 compounds are under clinical trials, in which 26, 63, and

23 compounds are in Phase-III, II, and I, respectively.[4] Few hypotheses explain the cause of AD include cholinergic hypothesis, amyloid hypothesis, and tau hypothesis.[5,6] In cholinergic pathway, biosynthesis and release of acetylcholine (ACh) were decreased and the metabolism was increased by acetylcholinesterase enzyme (AChE), whereas, amyloid hypothesis, β-secretase enzyme (BACE) responsible for the production of extracellular Aβ plaques (insoluble) during the metabolism of amyloid precursor protein (APP). As per the Tau hypothesis, neurofibrillary tangles were caused by hyperphosphorylation of tau protein.

Rubia cordifolia L. was found to possess anthraquinones (AQ) that have anthracene-9, 10-dione. Literature reported having an inhibiting capacity of AQ for tau aggregation, β-secretase, and acetylchoinesterase[5, 6]. Thus, in the current study, we planned to observe the pharmacological activity of two AQ's (2-Methyl AQ [MAQ], Quinizarine [QNZ]) was isolated from petroleum ether extract of *R. cordifolia* L. As a part of drug likeliness, the compounds are investigated to predict ADMET for assessing pharmacokinetic behavior and their toxicity, before studies on animals. The compounds are assessed for drug binding affinity on key markers for the AD pathology. Acetylcholine act on the muscarinic receptor, which is found on the chick ileum, thereby causing muscle contraction through αq mediated response of Guanine-protein coupled receptor for assessing the anti-Alzheimer's activity. Thus, the current study aimed to predict the ADMET properties and *in silico* anti-Alzheimer's activity through molecular docking studies. The *ex vivo* studies were performed to correlate the *in silico* outcome.

MATERIALS AND METHODS

Chemicals and Tissue

Calcium chloride ($CaCl_2$), D-Glucose, magnesium sulfate ($MgSO_4$), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), and acetylcholine were procured from National Scientific Products, Guntur, A.P. India. MAQ and QNZ were obtained from Sigma-Aldrich, Bengaluru, India. The chick ileum was obtained from a local slaughterhouse, Guntur, A. P. India.

Ligand Preparation

The MAQ, QNZ, and standard drugs 3D structures were obtained from PubChem drug database.[7] The ligand and standard geometries were optimized by energy minimization using MMFF94 force field and Gasteiger-Marsili charges for the atoms, till a gradient of 0.001 kcal/mol/A° was reached.

Determination of ADMET properties

The pharmacokinetic parameters were predicted using predesigned software's pkCSM[8] and PreADMET.[9]

Protein Preparation

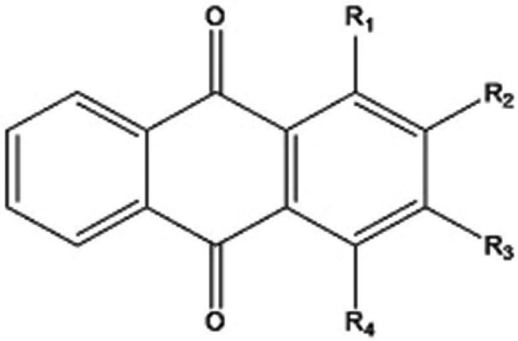
The proteins AChE, cyclin-dependent kinase5 (CDK5), glycogen synthase kinase 3β (GSK-3β) BACEI, monoamine oxidase (MAO-B), and their protein codes are 4PQI, 1UNH, 3L1S, 3K5D, and 2V5Z, respectively, were obtained from protein data bank.[10] The chosen protein is validated using the Ramachandran plot analysis.[11]

Determination of Binding Site

Binding and active sites of proteins are often associated with structural pockets and cavities. The catalytic sites of AChE[12] (4PQE; X: −25.5827, Y: 15.5693, Z: −5.9425), CDK5[13] (1UNH; X: 39.2941, Y: 16.3967, Z: 31.5158), MAO-B[14] (2V5Z; X: 54.7022, Y: 147.33, Z: 20.6817), GSK3B[15] (3L1S; X: 38.0409, Y: 34.5497, Z: 53.495), and BACE1[16] (3K5D; X: −1.33006, Y: 5.59003, Z: 34.1211) were obtained from the literature. The interaction between the ligand and protein was studied in determining the catalytic residue. The suitable confirmation was obtained by protein minimization. The structural and sequence of amino acids were monitored for their virtual binding sites.

MOLECULAR DOCKING

Molecular docking was performed for the compounds (1–10) after ligand preparation using Mcule software[17] and discovery studio[18] and depicted in Figure 1. All the ligands were docked with all the prepared receptors. The binding energies were calculated and represented as the docking results. The bonding of ligands with amino acid residues and the inter hydrogen bonding were considered for the RMSD. The hydrophobic interaction and the Vander Waal interactions were measured.



Anthraquinones

Anthraquinones	R_1	R_2	R_3	R_4
MAQ	H	CH_3	H	H
MAR	H	H	OH	H
MAS	OH	H	CH_3	H
MAT	H	OH	H	H
MAU	H	H	H	CH_3
QNV	CH_3	H	H	H
QNW	H	OH	H	CH_3
QNX	CH_3	H	H	OH
QNY	OH	H	CH_3	H
QNZ	OH	H	H	OH

Figure 1: Anthraquinone derivatives

Preparation of Chick Ileum Muscle

The chick ileum was received from the near local slaughterhouse. The chick ileum was placed in the Petri dish containing Krebs' Physiological salt solution (KPSS) and O_2 supply for maintaining the tissue homeostasis. The ileum length of 2–3 cm was taken and through which KPSS was passed for cleaning the debris matter inside the lumen. The tissue was sutured with thread and mounted in the organ tube of single student organ bath with a mixture of 95% O_2 and 5% CO_2 and temperature 37°C. The tissue preparation was allowed for stabilization for 45 min under 0.5 g tension. One liter of KPSS was prepared with a composition of CaCl2 (1M, 2.52 mL), D-Glucose (2 g), MgSO4 (0.29 g), KCl (0.35 g), KH2PO4 (0.16 g), NaCl (6.9 g), and NaHCO3 (2.1 g).

Ex vivo Anti-Alzheimer Activity using Chick Ileum

The concentration-dependent responses of chick ileum[19-21] were recorded using a frontal lever on kymograph paper with the help of nib of sketch pen as a stylus by Sherrington's rotating drum. The experiment was carried out with following time cycle, that is, baseline for 30 s, contact period for 60 s, wash with Kreb's solution 3 times at 1 min intervals, and 5 min time cycle are kept for proper recording of responses. Primarily, the DRC of Acetylcholine as standard (100 µg/mL) was recorded, followed by QNZ (100 µg/mL) and MAQ (100 µg/mL) of four different doses (10, 2 0, 40, and 80 µg/mL). The acetylcholine responses were recorded with constant dose volume (0.1 mL) in the presence of QNZ and MAQ of four different doses at two different concentrations (20 and 40 µg/mL).

RESULTS AND DISCUSSION

Evaluation of ADMET Parameters

Molecules had good intestinal absorption but poor water solubility, due to their lipophilic nature can cross the blood-brain barrier, more amount of drug will be available in the brain; hence, it is used for treating neurodegenerative disorders. AQ have not shown any inhibition on CYP2C19, CYP2C9, CYP2D6, and CYP3A4 enzymes. The oral rat acute and sub-acute toxicity of MAQ and QNZ were 128.82, 60.95 and 134.27, 1.808 64.26 mg/kg body weight, respectively. Whereas, Ames test prediction AQ graded as a mutagen. The results are tabulated in Tables 1-3.

Docking Studies

The protein targets were minimized and were studied for their proper confirmation using Ramachandran plots and are shown in Table 4 and Figure 2.

AChE

The AChE is classified as serine-protease responsible for cleavage of acetylcholine into acetic acid and choline, degradation of acetylcholine levels in cholinergic neurons is responsible for the cause of AD. Therefore, AChE inhibitors are potential therapeutic targets for treating AD. Docked results stated that MAQ, QNZ found high binding affinity than DPZ and the amino acid residue interactions are shown in Table 5, Figures 3 and 4.

Table 1: ADME profile of ligands

Parameters	ADME properties	2-Methyl Anthraquinone	Quinizarine
Absorption	Water solubility (log mol/L)	−3.779	−3.152
	Caco2 permeability (log Papp in 10–6 cm/s)	1.265	1.1
	Intestinal absorption (human) (% Absorbed)	99.436	95.18
	Skin Permeability (log Kp)	−2.145	−2.788
	P-glycoprotein substrate	Yes	Yes
	P-glycoprotein I inhibitor	No	No
	P-glycoprotein II inhibitor	No	No
Distribution	VDss (human) (log L/kg)	0.333	0.192
	Fraction unbound (human) (Fu)	0.12	0.154
	blood-brain barrier permeability (log BB)	0.398	0.085
Metabolism	CYP2D6 substrate	No	No
	CYP3A4 substrate	Yes	No
	CYP1A2 inhibitor	Yes	Yes
	CYP2C19 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	No	No
	CYP3A4 inhibitor	No	No
Excretion	Total clearance (log ml/min/kg)	0.211	−0.021
	Renal OCT2 substrate	No	No

MAQ interacted with the following amino acids of TRP286, TYR341, and TYR72. Meanwhile, QNZ and DPZ interacted with TYR124, TYR337, TRP286, TYR341, PHE297, and TYR337: OH, TRP286, TYR341, and PHE297 of amino acid residues, respectively.

CDK5

As stated in the tau protein hypothesis, hyperactivity of CDK5 triggers neurodegenerative disorders. Cdk5 inhibitors may be a potential target for treating AD. The docked results of MAQ, QNZ found good binding affinity than Roscovitine and the amino acid residue interactions shown in Table 5, Figures 2 and 3. The MAQ interacted with the following amino acids of ASN133: HD22, LEU122: CD2, LEU122: CD1, PHE69, and ALA132. Whereas, QNZ and Roscovitine interacted with ASN133, GLU70, ALA24, VAL53, ALA132, ILE9, VAL13, LEU122, PHE69, and ASN133: HD22, LYS26: HZ3, VAL13: CG2, GLN74: CA, ILE9: CG2, LEU122: CD2, and PHE69 of amino acid residues, respectively.

Table 2: Drug likeliness of ligands

S. No	Descriptor	2-Methyl anthraquinone	Quinizarine
1	Molecular Weight	222.243	240.214
2	LogP	2.77042	1.8732
3	H Acceptors	2	4
4	H Donors	0	2
5	Surface area	98.901	102.124

MAO-B

MAO-B, an enzyme-containing flavin located in the outer mitochondrial membrane responsible for the oxidative deamination of various neurotransmitters like those of dopamine and serotonin. In the brain of AD patients, elevated levels of MAO-B were likely due to transcriptional changes in MAO-B protein and which are predominate in plaque-associated astrocytes in neuropathologically verified AD brains. The docked results of MAQ, QNZ found great binding affinity than selegiline and the amino acid residue interaction shown in Table 5, Figures 3 and 4. The MAQ interacted with the following amino acids of TYR433: HH, TYR396, and TYR433. Whereas, QNZ and selegiline interacted with CYS170, LEU169, TYR396, TYR433, PHE341, and TYR396: HH, TYR433, TYR396, TYR433, TYR58, and PHE341 of amino acid residues, respectively.

GSK3β

The GSK3 is involved in hyperphosphorylation of tau proteins, amyloid-β formation, triggering inflammatory mediators leads to cause memory impairment, as well as which decreasing the acetylcholine formation in cholinergic neurons, these are all causing the AD together. The docked results of MAQ, QNZ found good binding affinity than tideglusib and the amino

Table 3: Toxicity parameters for ligands

S. No	Toxicity parameters	2-Methyl anthraquinone	Quinizarine
1	AMES toxicity	Yes	Yes
2	Human max. tolerated dose (log mg/kg/day)	0.122	0.615
3	hERG I inhibitor	No	No
4	hERG II inhibitor	No	No
5	Oral rat acute toxicity (LD50) (mol/kg)	2.11	1.785
6	Oral rat chronic toxicity (LOAEL) (log mg/kg_bw/day)	2.128	1.808
7	Hepatotoxicity	Yes	No
8	Skin Sensitization	No	No
9	*Tetrahymena pyriformis* toxicity (log ug/L)	1.302	0.693
10	Minnow toxicity (log mM)	0.86	1.928
11	Acute algae toxicity	0.06928	0.07065
12	2 years carcinogenicity bioassay in mouse	Positive	Positive
13	2 years carcinogenicity bioassay in rat	Negative	Positive
14	Acute daphnia toxicity	0.07553	0.11383
15	in vitro Human ether-a-go-go related gene channel inhibition	Medium risk	Medium risk
16	Acute fish toxicity (medaka)	0.00991	0.02344
17	Acute fish toxicity (minnow)	0.00636	0.01128
18	*In vitro* Ames test results in TA100 strain (Metabolic activation by rat liver homogenate)	Positive	Positive
19	*In vitro* Ames test results in TA100 strain (No metabolic activation)	Positive	Positive
20	*In vitro* Ames test results in TA1535 strain (Metabolic activation by rat liver homogenate)	Negative	Negative
21	*In vitro* Ames test results in TA1535 strain (No metabolic activation)	Negative	Negative

Table 4: Ramachandran plot analysis of 4PQE, 1UNH, 2V5Z, 3L1S, and 3K5D

Protein	Chain	Analyzed	Favored	Allowed	Outliers	Percentiles
4PQE (AChE)	A	522/543 (96%)	488 (94%)	29 (6%)	5 (1%)	49
1UNH (CDK5)	All	830/1000 (83%)	766 (92%)	55 (7%)	9 (1%)	16
2V5Z (MAOB)	All	989/1040 (95%)	968 (98%)	21(2%)	0	100
3L1S (GSK3B)	All	652/828 (79%)	630 (97%)	21(3%)	1 (0%)	81
3K5D (BACE1)	All	1114/1224 (91%)	1046 (94%)	65 (6%)	3 (0%)	75

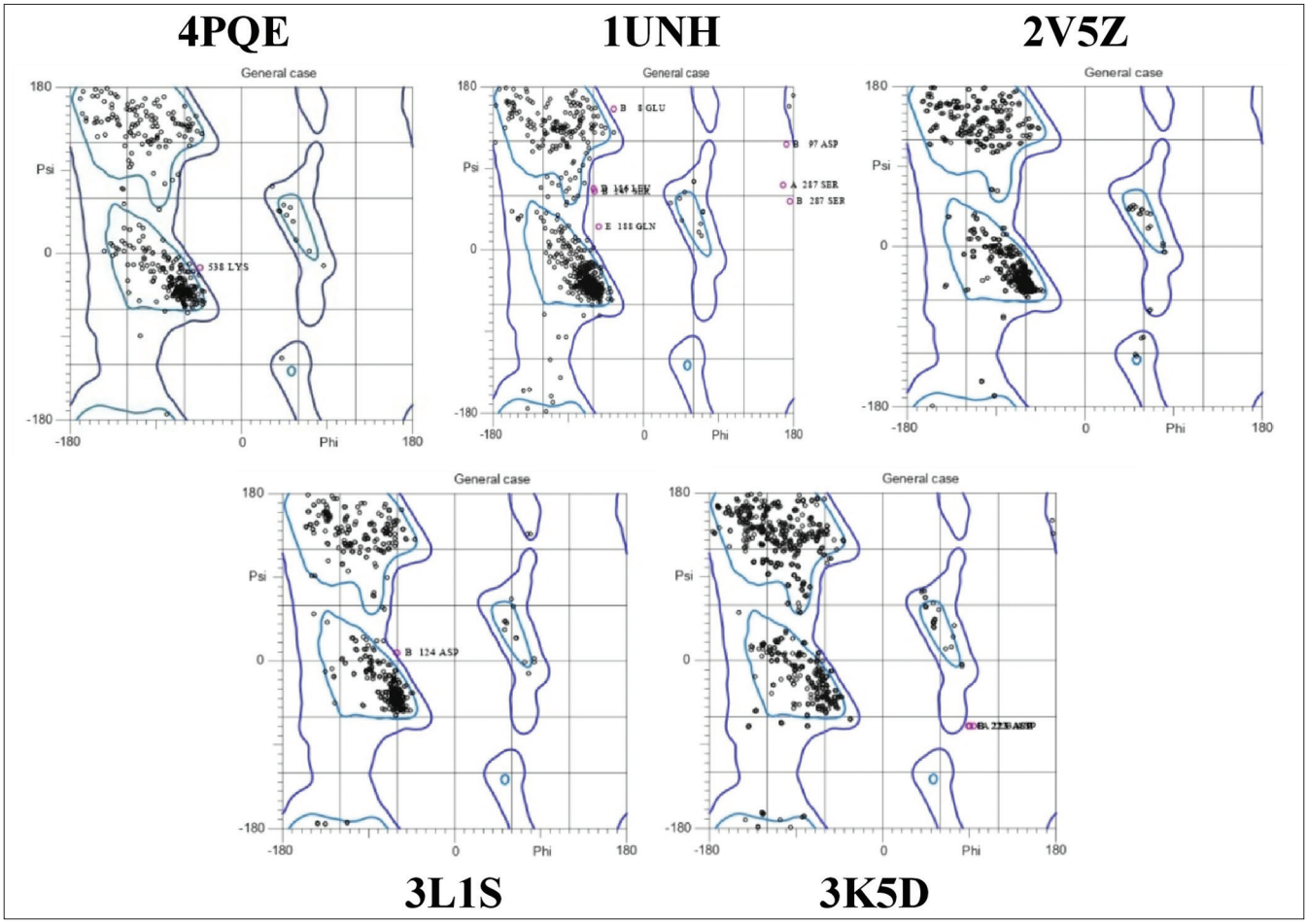


Figure 2: Ramachandran plot analysis of targeted proteins

acid residue interaction shown in Table 5, Figures 3 and 4. The MAQ has interacted with the following amino acids of VAL43: CG1, ILE35: CD1, LEU154: CD2, VAL43: CG2, PHE40, and CYS165: SG. Whereas, QNZ and Tideglusib interacted with ASP166, LYS58, ALA56, VAL43, LEU154, CYS165, and GLY36: CA, LEU154: CD2, VAL43: CG1, and CYS165: SG of amino acid residues, respectively.

BACE1

The APP is an integral membrane protein, due to proteolytic cleavage at β site of APP produce Aβ, which is insoluble and accumulated in the neurons and develop the AD. The docked results of MAQ, QNZ found good binding affinity than Elenbecestat and the amino acid residue interaction shown in Table 5, Figures 3 and 4. The MAQ interacted with the following amino acids of GLN76, ASP218, and TYR74. Whereas, QNZ and Elenbecestat interacted with THR221, GLN 76, TYR74and THR75, THR221, THR319, THR222, LYS214, THR75, TYR188, GLN76, ASP218, and ILE216 of amino acid residues, respectively.

The best score from the best pose for each compound was taken and compared to the scores of other compounds. The compounds which show the highest negative LeadIT score show the greater capability to bind strongly with the protein. In our study, MAQ showed greater free binding energy of −9.9, −10, −10.5, −9.4, and 8.2 kcal/mol on AChE, CDK5, MAOB, GSK3β, and BACE, respectively, compared with standards compounds. As well as, QNZ showed greater binding energy

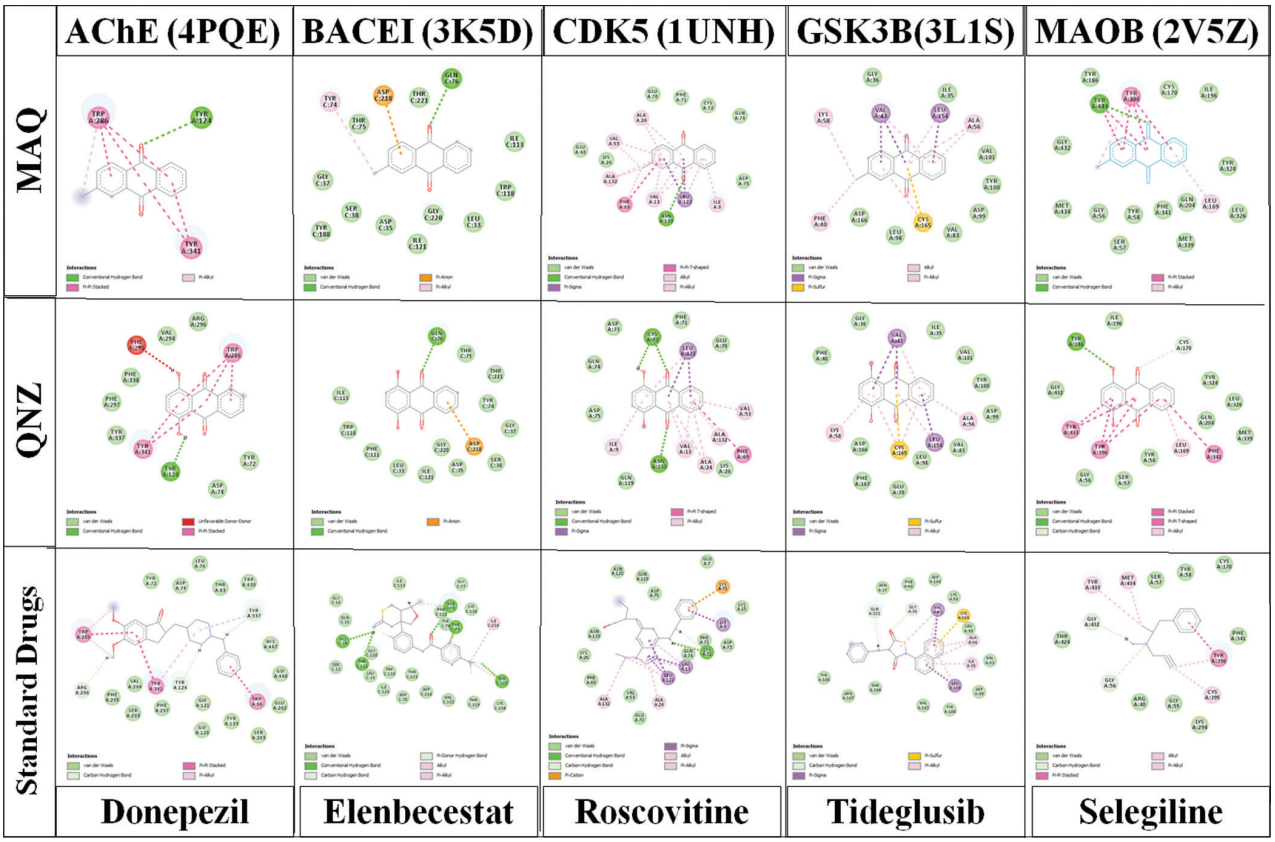


Figure 3: 2D structures of anthraquinones and standard compounds with binding targets

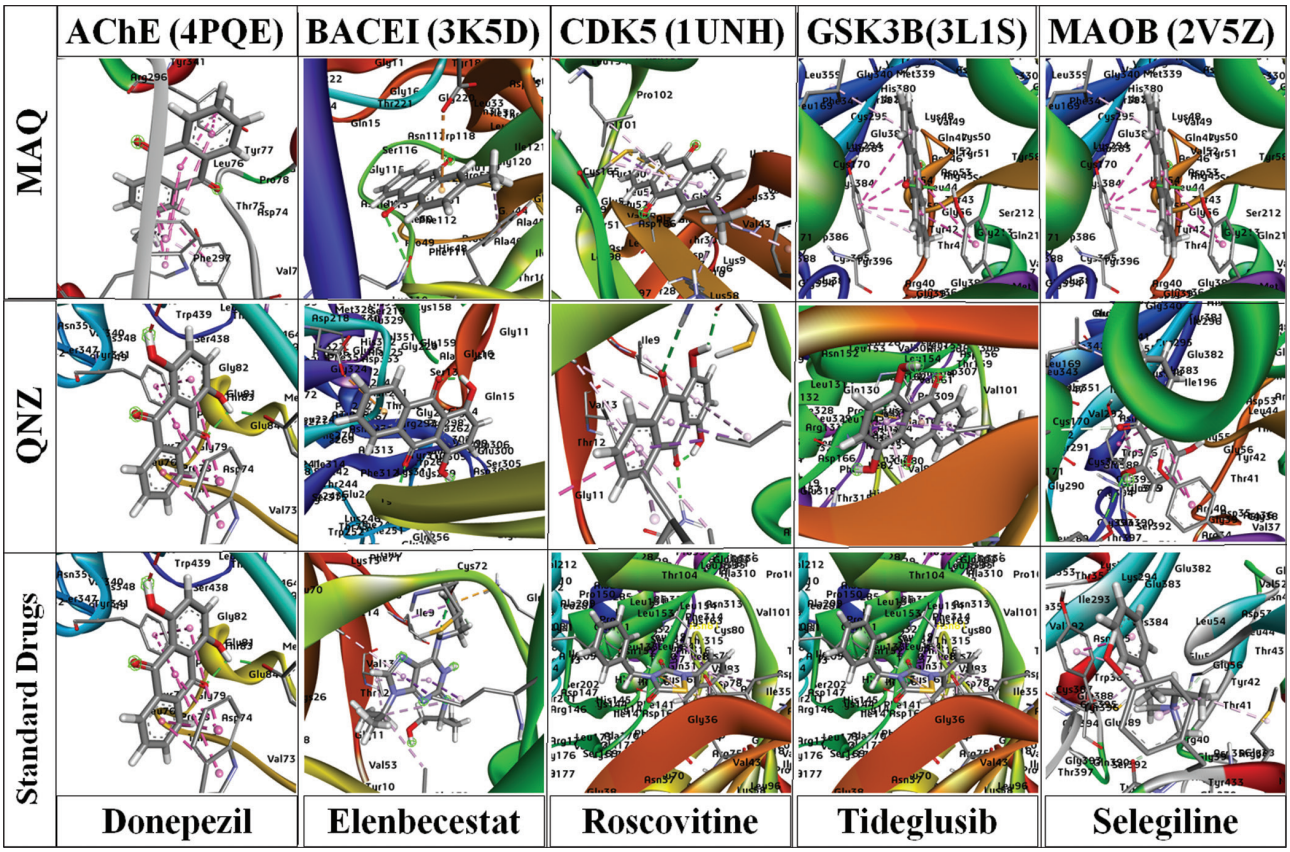


Figure 4: 3D structures of anthraquinones and standard compounds with binding targets

Table 5: MAQ, QNZ, and standard drugs binding energies and interaction of amino acid with following protein AChE, CDK5, MAOB, GSK3β, and BACE1 targets

Targets	Drugs	Docking Score (kcal/mol)	Hydrophilic	Hydrophobic	RMSD
AChE (4PQE)	MAQ	−9.9	--	TRP286,TYR341,TYR72	0.23
	QNZ	−9.6	TYR124 (3.17, 2.60), TYR337 (2.85)	TRP286, TYR341, PHE297	0.22
	Donepezil (AChEI)	−8.6	TYR337:OH (3.90924)	TRP286 ,TYR341,PHE297	0.47
CDK5 (1UNH)	MAQ	−10	ASN133:HD22(2.13377)	LEU122:CD2,LEU122:CD1,PHE69, ALA132	0.75
	QNZ	−9.1	ASN133 (2.17), GLU70 (2.51).	ALA24, VAL53, ALA132, ILE9, VAL13, LEU122, PHE69.	0.77
	Roscovitine (CDK5I)	−8.1	ASN133:HD22 (2.16436), LYS26:HZ3 (2.68857)	VAL13:CG2, GLN74:CA, ILE9:CG2, LEU122:CD2, PHE69	0.89
MAOB (2V5Z)	MAQ	−10.5	TYR433:HH (2.46433)	TYR396, TYR433.	0.31
	QNZ	−9.9	CYS170 (2.05, 3.56)	LEU169, TYR396, TYR433, PHE341	0.34
	Selegiline (MAO-BI)	−7.1	TYR396:HH (3.32022)	TYR433,TYR396,TYR433,TYR58, PHE341.	0.6
GSK3B (3L1S)	MAQ	−9.4	--	VAL43:CG1,ILE35:CD1,LEU154:CD2,VAL43:CG2, PHE40, CYS165:SG.	0.79
	QNZ	−8.9	ASP166 (2.46),	LYS58, ALA56, VAL43, LEU154, CYS165	0.78
	Tideglusib (GSK-3βI)	−9.2	GLY36:CA (3.17875)	LEU154:CD2,VAL43:CG1,CYS165:SG	0.75
BACE1 (3K5D)	MAQ	−8.2	GLN76 (3.05)	ASP218,TYR74	0.82
	QNZ	−7.8	THR221 (2.19), GLN 76 (2.36)	TYR74	0.99
	Elenbecestat (3K5D)	−9.9	THR75 (2.17), THR221(2.19), THR319 (2.70), THR222 (2.35)	LYS214, THR75, TYR188, GLN76, ASP218, ILE216	0.35

Table 6: Percentage response of Acetylcholine, MAQ, QNZ alone and combination with ACh on chick ileum

DOSE (mL)	% Response							
	ACh (100 μg)	DPZ (20 μg) +Ach	QNZ (100 μg)	MAQ (100 μg)	QNZ + Ach		MAQ + ACh	
					QNZ (20 μg) +ACh	QNZ (40 μg) +Ach	MAQ (20 μg) + ACh	MAQ (40 μg) +ACh
0.1	44.23	80.76	1.92	1.92	17.30	38.46	13.46	15.38
0.2	73.07	96.15	1.92	1.92	21.15	50	19.23	28.84
0.4	80.76	103.84	1.92	1.92	42.30	63.46	26.92	34.61
0.8	100	113.46	1.92	1.92	51.92	63.46	32.69	42.30

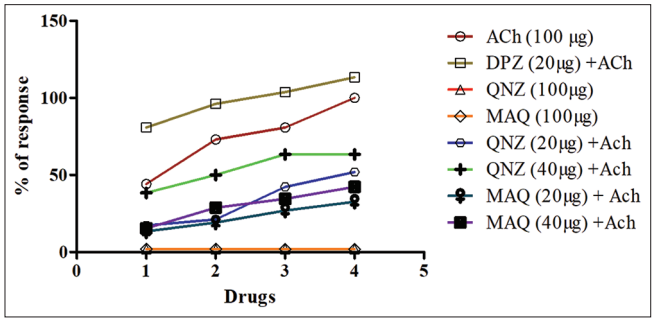


Figure 5: Effect of anthraquinones on chick ileum

of −9.6, −9.1, −9.9, −8.9, and 7.8 kcal/mol on AChE, CDK5, MAOB, GSK3β, and BACE, respectively, compared with standards compounds. Elenbecestat showed greater binding energy of −9.9 on BACE1 when compared with tested compounds.

Ex vivo Studies

The results for the chick ileum contractility studies are mentioned in Table 6, Figures 5 and 6. It is observed that there is a synergistic effect of acetylcholine along with the AQ MNQ and QNZ, indicating potency. The contractions of excised chick ileum were increased with MAQ and QNZ in the presence of ACh when compared to ACh alone. The increased contractions are due to inhibition of acetylcholinesterase; therefore, the metabolism of Ach was decreased, availability of ACh levels is high and more amount of ACh act on chick ileum and cause muscle contraction. It would be considered as evidence for treating dementia associated with AD.

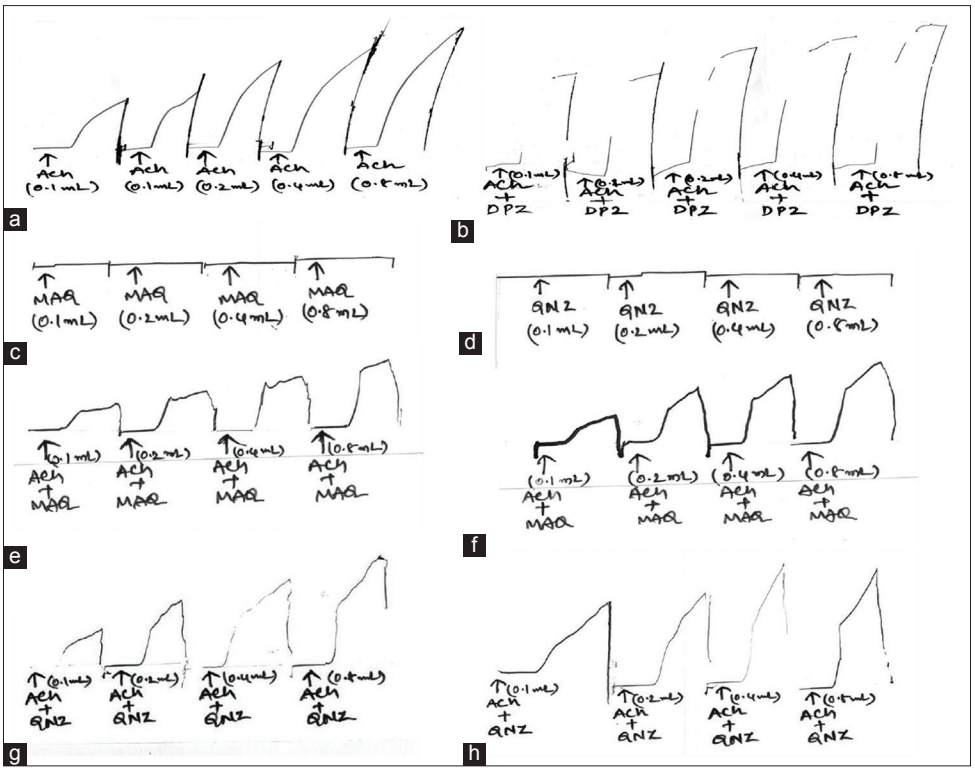


Figure 6: Effect of isolated compounds, acetylcholine and donepezil on chick ileum. (a) ACh response; (b) DPZ response, (c) 2-Methyl Anthraquinone (MAQ) response; (d) Quinizarine (QNZ) response; (e) MAQ (20 μg) and ACh combination response; (f) MAQ (40 μg) and ACh combination response; (g) QNZ (20μg) and ACh combination response; (h) Effect of QNZ (40 μg); and ACh combination response

CONCLUSION

The drugs for AD are AChE inhibitors, which are synthetic molecules with a high risk of adverse effects. Neuroprotective action of AQ was proven through the mechanism inhibition of tau aggregation.[22,23] Hence, this study was focused on natural molecules as alternatives to synthetic medicine. Conclusively, this study stands unique in its assent that MAQ and QNZ, possessed acceptable drug likeliness, ADMET properties. MAQ and QNZ have the high ability to interact with AChE, CDK5, MAOB, and GSK3β except on BACE1. The results were compared with the promising effect on chick ileum muscle contractility. Thus, MAQ and QNZ should be promising compounds and possesses a potentiality to develop as drugs for the treatment of AD.

ACKNOWLEDGMENT

The authors are thankful to the management of Chebrolu Hanumaiah Institute of Pharmaceutical Sciences for providing facilities.

REFERENCES

1. Magalingam KB, Radhakrishnan A, Ping NS, Haleagrahara N. Current concepts of neurodegenerative mechanisms in Alzheimer's disease. Biomed Res Int 2018.
2. Kumar A, Singh A. A review on Alzheimer's disease pathophysiology and its management: An update. Pharmacol Rep 2015;67:195-203.
3. Alzheimer's Association. 2018 Alzheimer's disease facts and figures. Alzheimers Dement 2018;14:367-429.
4. Cummings J, Lee G, Ritter A, Zhong K. Alzheimer's disease drug development pipeline: 2018. Alzheimers Dement (N Y) 2018;4:195-214.
5. Jung HA, Ali MY, Jung HJ, Jeong HO, Chung HY, Choi JS. Inhibitory activities of major anthraquinones and other constituents from *Cassia obtusifolia* against β-secretase and cholinesterases. J Ethnopharmacol 2016;191:152-60.
6. Convertino M, Pellarin R, Catto M, Carotti A, Caflisch A. 9,10-Anthraquinone hinders beta-aggregation: How does a small molecule interfere with Abeta-peptide amyloid fibrillation? Protein Sci 2009;18:792-800.
7. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Hum Mol Genet 2010;19:R12-20.
8. Lahiri DK, Farlow MR, Greig NH, Sambamurti K. Current drug targets for Alzheimer's disease treatment. Drug Dev Res 2002;56:267-81.
9. Pires DE, Blundell TL, Ascher DB. pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. J Med Chem 2015;58:4066-72.
10. Jain G, Bodakse SH, Namdev K, Rajput MS, Mishra S. Development of an *ex vivo* model for pharmacological experimentation on isolated tissue preparation. J Adv Pharm Technol Res 2012;3:176-81.
11. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, *et al*. The protein data bank. *Nucleic Acids Res* 2000;28:235-42.
12. Dym O, Unger T, Toker L, Silman I, Sussman JL. Crystal Structure of Human Acetylcholinesterase. Israel: Israel Structural Proteomics Center; 2014.
13. Mapelli M, Massimiliano L, Crovace C, Seeliger MA, Tsai LH, Meijer L, *et al*. Mechanism of CDK5/p25 binding by CDK inhibitors. J Med Chem 2005;48:671-9.
14. Binda C, Wang J, Pisani L, Caccia C, Carotti A, Salvati P, *et al*.

Structures of human monoamine oxidase B complexes with selective noncovalent inhibitors: Safinamide and coumarin analogs. J Med Chem 2007;50:5848-52.
15. Arnost M, Pierce A, ter Haar E, Lauffer D, Madden J, Tanner K, *et al*. 3-Aryl-4-(arylhydrazono)-1H-pyrazol-5-ones: Highly ligand efficient and potent inhibitors of GSK3beta. Bioorg Med Chem Lett 2010;20:1661-4.
16. Hanessian S, Shao Z, Betschart C, Rondeau JM, Neumann U, Tintelnot-Blomley M. Structure-based design and synthesis of novel P2/P3 modified, non-peptidic beta-secretase (BACE-1) inhibitors. Bioorg Med Chem Lett 2010;20:1924-7.
17. Kiss R, Sandor M, Szalai FA. http://Mcule.com: A public web service for drug discovery. J Cheminform 2012;4:P17.
18. Studio D. Discovery Studio. Accelrys [2.1]. 2008.
19. Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, *et al*. MolProbity: More and better reference data for improved all-atom structure validation. Protein Sci 2018;27:293-315.
20. Burn JH. Observations on isolated muscles. In: Practical Pharmacology. Oxford: Blackwell Scientific Publications; 1952.
21. Arunakumar C, Vidyadhara S, Nagaraju B, Sandeep D, Vineela S. *Ex-vivo* anti-Alzheimer's and wound healing activity of methanolic extract of *Mimosa tenuiflora*. Invent Impact Ethnopharmacol 2017;2017:1-7.
22. Liu T, Jin H, Sun QR, Xu JH, Hu HT. Neuroprotective effects of emodin in rat cortical neurons against beta-amyloid-induced neurotoxicity. Brain Res 2010;1347:149-60.
23. Pickhardt M, Gazova Z, von Bergen M, Khlistunova I, Wang Y, Hascher A, *et al*. Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments *in vitro* and in cells. J Biol Chem 2005;280:3628-35.