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Research Article

SCREENING AND MOLECULAR DOCKING STUDIES OF CURCUMIN AND ITS DERIVATIVES AS INHIBITORS OF AMYLOID-β PROTEIN: A KEY PROTEIN IN ALZHEIMER'S DISEASE

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ABSTRACT

Objective: Alzheimer's disease (AD) is a progressive neurodegenerative disease. This disease is characterized by progressive cognitive deterioration along with declining activities of the day to day and behavioral changes. One of the important pathogenesis in AD is the chronic inflammation of neuron. Studies have demonstrated the associated inflammatory changes such as astrocytosis, microgliosis, and the presence of pro-inflammatory components that accompany the deposition of amyloid-β (Aβ) peptide. In Alzheimer patients, a chronic inflammation occurs because of activated macrophages and an increased amount of cell signaling proteins. *Curcuma longa* of Zingiberaceae family is cultivated spice in India and other Asian countries. Turmeric has a high content of curcuminoids and is recognized for its broad spectrum of biological activities. Molecular docking studies were performed by selecting various curcuminoids as Aβ inhibitors.

Methods: Docking studies help to understand the binding interactions of the protein with the ligands. Alzheimer's Aβ precursor(PDB ID: 1AAP) was selected as the crucial protein involved in AD and curcuminoids were selected as ligands. Molecular properties and quantitative structure-activity relationship (QSAR) model were calculated using Med Chem Designer and Build QSAR. Docking studies were performed using Autodock 4.0.

Results: Docking results indicate that curcuminoids can be considered as inhibitors of AAP as they interact with the active site region.

Conclusion: Docking analysis of auto dock binding energies and binding interactions of curcumin and its derivatives indicates that curcumin, curcumin bis acetate, bisdemethoxy curcumin, [18F] fluoropropyl substituted curcumin and tetrahydrocurcumin can be considered as probable inhibitors of AAP.

Keywords: Alzheimer's disease, *Curcuma longa*, Curcuminoids, Docking, Quantitative structure-activity relationship, Amyloid protein.

INTRODUCTION

Alzheimer's disease (AD) is one form of dementia that gradually increases with time. Dementia is a loss of brain function that occurs with certain diseases and aging. It was first discovered by German psychiatrist and neuropathology's Alois Alzheimer in 1906 and was named after him [1]. AD is characterized by the accumulation of the β-amyloid peptide (Aβ) and microtubule-associated protein tau within the brain. It affects the thinking, behavioral and physiological ability. Most often, AD is diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer occurs much earlier [2]. There are two types-early onset and late onset. Genetic, biochemical, and behavioral research suggest that physiologic generation of the neurotoxic Aβ peptide from sequential amyloid precursor protein (APP), proteolysis is the crucial step in the development of AD.

APP is a single-pass trans-membrane protein expressed at high levels in the brain and metabolized in a rapid and highly complex fashion by a series of sequential proteases, including the intramembranous γ-secretase complex, which also process other key regulatory molecules. The APP is one member of a family of related proteins that includes the amyloid precursor-like proteins (APLP1 and APLP2) in mammals [3]. Genetic studies of APP processing will be crucial to the development of therapeutic targets to treat AD [4].

Curcuma longa has shown numerous biological activities such as antioxidant [5], anti-inflammatory [6], anti-atherogenic [7], anti-psoriatic [8], anti-diabetic [9], immuno stimulatory [10], antibacterial [11], and anticancer effects [12]. This also contributes to the incorporation of the healing process of dermal wound and the prevention of AD [13].

Curcumin that accounts for 60-80% is a main coloring substance in *C. longa.* Other related compounds include the curcumin 4-4 diacetate, bisdemethoxy curcumin (BDMC), curcumin pyrazole and [18F] fluoropropyl-substituted. These are altogether known as curcuminoids belonging to a group of phenolic substances. Curcumin is an oil soluble pigment, practically insoluble in water at acidic and neutral pH, soluble in alkali. Preparations of water-soluble curcumin by incorporation into various surfactant micellar systems (acetone, methanol, and ethanol) have been reported. It is stable at high temperatures and in acids, but unstable in alkaline conditions and in the presence of light [14].

Curcumin (diferulomethane), derived from the rhizome of *C. longa*, can inhibit Aβ aggregation [15]. It was proposed that the reason for specific binding to the Aβ-peptide and facilitating the inhibition of fibril formation was the symmetric and compact structure of curcumin [16]. Its structural similarity to a beta-sheet breaker, N,N'- bis (3- hydroxyphenyl) pyridazine-3,6-diamine, named RS-0406 (novel inhibitor) could be one of the reasons for its inhibition characteristics. The important structural feature of the molecule is the two aromatic end groups and any alterations in these groups were found to affect its activity. Curcumin, like chrysamine G (CG), being lipophilic, is able to cross the blood brain barrier (BBB) which facilitates its binding to plaques. Its specific binding to the beta-sheet structures of the plaques formed by aggregation of various different proteins indicate that it's binding is not dependent on amino acid sequence of the proteins, but rather it's conformation-dependent. Experiments have shown that curcumin, which is nontoxic is able to cross the blood-brain barrier due to its high hydrophobicity, can interfere with Aβ oligomerization better than ibuprofen and naproxen. Clinical trials are going in phases II and III for curcumin [17] and ginkgo biloba [18], respectively. Congo red,

CG and thioflavin S have shown to bind with amyloid plaques with high affinity, and they can also prevent the formation of b-A fibrils. They are not able to cross the BBB, so they were not found suitable for treatment of AD [19]. Hence, it is worthwhile to address this problem by computer simulations to shed more light [20].

METHODS

Preparation of protein and ligand

The three-dimensional (3D) structure of Alzheimer's Aβ precursor protein (PDB ID: 1AAP) was obtained from protein database PDB [21]. 39 compounds (curcumin and its derivatives) were obtained from NCBI Pub Chem [22]. The ligands were obtained in SDF format and converted into PDB using Spdbv [23]. The CID numbers of the selected ligands are given in Table 1.

Active site analysis

The active site analysis of 1AAP was performed using LIGSITE [24]. It is an online server for automatic identification of residues given its 3D coordinate.

Calculation of physicochemical properties and quantitative structure-activity relationship (QSAR) studies

The properties of curcuminoids ligands such as molecular weight, hydrogen bond acceptor, hydrogen bond donors, log p value and number of atoms were obtained using Med Chem designer™ 2.5.0.8 (Simulations Plus, Inc. - www.simulations-plus.com) that also analyzes the number of violations or deviations from Lipinski's rule. QSAR Model was generated using Build QSAR [25].

Docking studies

The binding mode and interactions of 1AAP with curcuminoids were studied using Auto Dock 4.0 [26]. Docking was performed for 30 compounds which were considered for further studies. The docking results were analyzed using software Accelrys Discovery Studio 3.5 [27] to understand the protein ligand interactions.

RESULTS

Calculation of physicochemical properties

The physicochemical properties of curcuminoids are indicated in Table 1. Based on the rule of five 9 compounds namely 7, 8, 9, 12, 13, 14, 15, 26 and 39 were eliminated for docking studies.

Docking studies of ligands

Docking was performed with 30 compounds, and only 20 compounds resulted in least binding energy. The binding energies of curcuminoids are indicated in Table 2.

QSAR model generation

Log P was used as representative of lipophilic property.The binding energy obtained from docking was used as one of the variable.

Multiple linear regression (MLR)

MLR was performed using the Build QSAR. The statistical values, multiple correlation coefficient (r), cross-validation q2, standard errors (s) and standard error of prediction were used to evaluate the QSAR model. A total of 20 curcumin compounds were used as data set (Table 2). The final QSAR equation is shown in Fig. 1.

DISCUSSION

Binding interactions

The binding mode obtained by the docking studies of AAP and curcumin and its derivatives were analyzed using Discovery Studio 2.0. The binding pocket include Glu7, Gln8, Asp24, Val25, Thr26, Glu3, Val4, Ser6, Ala9, Glu10, Thr11, Gly12, Tyr22, Asp24. Five most potent compounds, i.e. 969516 (curcumin), 6441419 (curcumin bis acetate), 5315472 (BDMC), 11947775 ([18F] fluoropropyl substituted curcumin) and 124,072 (tetrahydrocurcumin) exhibited good binding energy and interacted with the binding pocket.

Fig. 2 depicts the interactions of curcumin with Tyr22, Asp24, Thr26, Thr11, Ala9, Gln8, Phe33, Phe34. Curcumin mostly formed

QSAR Equation

BINDING ENERGY = - 0.6371 (± 0.3817) LogP - 12.9449 (± 1.5277)

(n = 20 ; R = 0.637 ; s = 0.792 ; F = 12.296 ; p = 0.0025 ; Q2 = 0.220 ; SPress = 0.907 ; SDEP = 0.883)

Fig. 1: Quantitative structure-activity relationship model

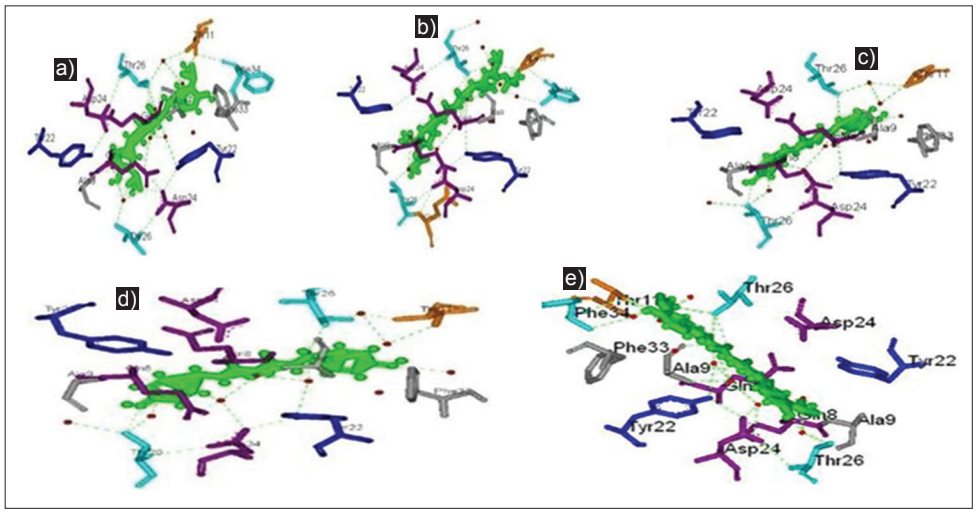

Fig. 2: a) Curcumin, (b) curcumin bis acetate, (c) bisdemethoxy curcumin, (d) fluropropyl substituted curcumin, (e) tetrahydrocurcumin (ligands are represented in green)

Table 1: Physicochemical properties of curcumin and its derivatives

S. No.	CID number	S+logP	S+logD	MlogP	MWt	HBDH	M_NO	T_PSA	Rule of 5
1	5469424	3.261	3.085	2.537	338.362	2	5	83.83	0
2	5469426	3.142	2.813	2.033	354.362	3	6	104.06	0
3	147439	3.118	3.004	2.318	308.336	2	4	74.6	0
4	5315472	3.118	3.004	2.318	308.336	2	4	74.6	0
5	5469425	2.973	2.582	1.805	340.335	4	6	115.06	0
6	11947775	3.948	3.672	3.005	428.46	1	6	82.06	0
7	46173989	0.069	−0.772	−2.658	692.675	8	16	251.36	3
8	11526601	1.247	0.601	−0.173	530.532	5	11	172.21	2
9	46926318	0.069	−0.772	−2.658	692.675	8	16	251.36	3
10	124072	3.038	2.504	2.405	372.421	2	6	93.06	0
11	71752447	3.038	2.504	2.405	372.421	2	6	93.06	0
12	71315012	1.257	−1.121	−0.557	544.516	5	12	189.28	2
13	71315013	1.257	−1.121	−0.557	544.516	5	12	189.28	2
14	44195235	4.194	4.056	2.117	502.524	3	8	122.52	1
15	46926100	0.257	−0.623	−2.147	692.675	8	16	251.36	3
16	6441419	3.471	3.184	1.758	452.464	0	8	105.2	0
17	44452370	4.102	4.005	2.67	472.498	3	7	113.29	0
18	54597187	4.854	4.743	3.625	492.479	2	6	93.06	0
19	44451939	4.548	4.468	3.429	456.498	2	6	93.06	0
20	2889	3.368	3.097	2.256	368.389	2	6	93.06	0
21	25111343	3.162	2.862	2.138	467.522	3	8	125.15	0
22	11474949	4.118	3.822	2.689	396.443	2	6	93.06	0
23	969516	3.368	3.097	2.256	368.389	2	6	93.06	0
24	6477182	3.822	3.715	2.178	396.443	0	6	71.06	0
25	16727530	4.728	4.582	2.859	448.519	0	6	71.06	0
26	45028269	5.842	5.215	2.208	588.659	0	8	105.2	1
27	11351170	5.541	5.517	3.72	440.502	2	6	76.74	0
28	44451964	5.063	5.013	3.571	485.5	2	9	122.56	0
29	44451990	5.698	5.667	3.819	458.492	2	6	76.74	0
30	57396347	3.368	3.097	2.256	368.389	2	6	93.06	0
31	53464495	3.368	3.097	2.256	368.389	2	6	93.06	0
32	45276266	2.691	2.44	1.93	412.442	2	7	102.29	0
33	71314377	5.791	5.468	3.514	482.653	1	6	82.06	0
34	14578495	4.285	4.244	2.676	364.403	3	6	87.6	0
35	23640558	4.285	4.244	2.676	364.403	3	6	87.6	0
35	23640558	4.285	4.244	2.676	364.403	3	6	87.6	0
36	24766776	2.072	1.801	1.515	425.441	3	8	125.15	0
37	44451940	5.58	5.554	3.159	470.528	2	7	85.97	0
38	45276267	2.134	1.96	1.09	456.496	2	8	111.52	0
39	71315948	8.275	7.959	4.153	596.917	0	6	71.06	2

Table 2: Variables used for QSAR model

S. No.	CID	Compound	S+logP	Autodock binding energy
1	969516	Curcumin	3.368	−13.68
2	5315472	BDMC	3.118	−13.11
3	14578495	Curcumin pyrazole	4.285	−14.85
4	6477182	Dimethylcurcumin	3.822	−13.22
5	5469426	Demethyl curcumin	3.142	−14.12
6	5469425	Didemethyl curcumin	2.973	−12.73
7	124072	Tetrahydrocurcumin	3.038	−12.03
8	45276267	Di-O-(2-hydroxyethyl) curcumin	2.134	−14.14
9	45276266	CHEMBL1088638	2.691	−13.91
10	6441419	Curcumin bis-acetate	3.471	−13.41
11	44451964	CHEMBL258632	5.063	−13.06
12	16727530	allyl-curcumin	4.728	−14.72
13	11351170	N-phenylpyrazole curcumin	5.541	−15.41
14	44451990	CHEMBL258739	5.698	−14.98
15	44451940	CHEMBL410334	5.58	−15.58
16	11474949	Ethyl curcumin	4.118	−14.18
17	44452370	Chembl260079	4.102	−14.02
18	44451939	4-benzylidene curcumin	4.548	−14.48
19	11947775	[18FP]-curcumin	3.948	−13.98
20	2889	Curcumin	3.368	−13.38

H-bonds with the alanine residues present in the amyloid protein and were capable of binding to the aliphatic amino acids residues at various positions within the protein, mainly $A\beta_{12\text{-}28}$ [28]. Our study is in correlation with these findings since curcumin is binding to the residues at positions 22, 24, 26 respectively. CURCUMIN bis diacetate with Tyr22, Asp24, Thr26, Thr11,Ala9, Gln8, Glu27, Phe33, Phe34, BDMC with Tyr22, Asp24, Thr26, Thr11, Ala9, Gln8, Phe33, (18F) fluoropropyl-substituted curcumin with Tyr22, Asp24, Thr26, Thr11, Ala9, Gln8, Phe33, and tetrahydro curcumin with Thr11, Thr26, Asp24, Tyr22, Ala9, Gln8, Phe32, Phe33 curcumin and its derivatives formed H bonds with the fragment $A\beta_{9\text{-}33}$.

CONCLUSION

Present study aimed at identifying curcuminoids as AAP inhibitors. A total of 39 curcuminoids were selected for studies. These compounds were retrieved from NCBI PubChem. The physico chemical properties and binding energies were calculated using Med Chem Designer and Auto Dock 4.0. A QSAR model was generated using Build QSAR for 20 compounds after applying the principle of rule of five. Based on these analyses and Auto dock studies the best binding mode was obtained with least energy value. The interaction with active site residues obtained from Discovery Studio Visualizer 2.0 indicate that curcumin, curcumin bis acetate, BDMC, fluoropropyl substituted curcumin) and tetrahydrocurcumin can be considered as the most probable inhibitors of the Alzheimer's Precursor Protein. Curcumin reveals the

therapeutic role in management of epileptic seizures and other neurological disorders and hence we conclude that curcumin and its derivatives can be considered as probable drug candidates [29].

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