Research Paper E-ISSN NO : 2455-295X | VOLUME : 7 | ISSUE : 8 | AUGUST 2021

GAMMA-D-CRYSTALLIN EYE LENS PROTEIN IS TARGETED FOR SENILE CATARACT- AN IN SILICO ANALYSIS

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ABSTRACT:

Age-related or senile cataract is one of the important reasons for blindness in half of the world. Opacification of the inner core region of the lens that focuses the light on the retina is inevitable with aging. Gamma-D-crystallin protein is present in the middle layer of the lens, which undergoes biochemical and physiological changes leading to opacification of the lens. The primary treatment of senile cataract includes surgery, but surgical treatment is not accessible by most people, so identification of anti-cataract drugs to treat cataract is focused in the present study. The main objective of the present study is to predict the tentative binding parameters like ligand chemistry, receptor flexibility and the scoring function of ligand-receptor complex and predominant binding mode (s) of a ligand with the target protein - Gamma-D-crystallin. Drug compounds are retrieved from the PubChem database. The 3D structure of Gamma-D-crystallin protein was retrieved from the PDB ID 2G98. Drug compounds are screened primarily for ADMETox properties, and docking studies were performed using Glide module of Schrodinger software. Docking studies of 3D model of Gamma-D-crystallin protein with ligands revealed that most of them have good binding affinity and maximum G score. In particular, the drug compound salsalate showed -9.21 Kcal/mol of G.score and formed 3 numbers of hydrogen bonds with the residues Arg79,Gln54. It is found to have prominent interactions.

KEYWORDS:

SENILE CATARACT, GAMMA CRYSTALLIN PROTEIN, EYE LENS PROTEIN, IN SILICO ANALYSIS, DOCKING STUDIES.

INTRODUCTION

Age related or senile cataract is the reason behind the 51% of world blindness in people above 50 years, according to WHO and Global Burden of Disease, Injuries and Risk Factors Study (GBD).[1,2,3] The National Blindness and Visual Impairment Survey India 2015-19 reported that 66.2% of blindness in India is caused by cataracts. A cataract is an opacification of the inner ophthalmic crystallin lens that focuses light on the retina together with the cornea. Lens has the potential to change the focussing range and confers its refractive index by one third.[4] Degradation of transparency of the crystallin lens will lead to the development of cataract. Classification of cataract types is based on its location on the lens. Nuclear cataract is hardening of the inner core middle region of the lens caused due to ageing. Cortical cataract is found in the lens fibres around the nucleus that extend from outer to inner core region. It mainly occurs in diabetics. Sub capsular cataracts develop under the lens capsule in the posterior cortical layer and are caused by the intake of steroids, susception to microwave radiation, and also diabetics.[5] Senile cataract is the key reason for visual impairment in age advanced person. Modern surgical treatment of cataract comprises the removal of the opaqued lens and replacement with a new transparent ocular lens. Although cataract surgery is a common procedure practiced worldwide, surgical complications, health care cost, secondary opacification, requires the alternate method of treating senile cataracts.[6]

The human eye lens comprises outer epithelial cells and inner network of crystallin proteins that maintain unique refractive index and transparency of the lens.[7] On the advancement of age, the middle layer crystallin proteins denature and degrade, resulting in the movement of epithelial cells to the inner region, gradually develops the opacification on the lens.[8] Age-related cataract genesis is caused by the failure of α-crystallin chaperone activity and post-translational modifications (PTMs) of the crystallin proteins.[9] Successive depletion of thiol residues leads to fragmentation of in crystallin proteins, which is associated with lens opacification.[10]

Senile cataracts can be treated surgically, but it includes surgical complications like posterior capsular rupture, vitreous loss, posterior loss of lens fragments, injury to cornea, iris and lens.[11,12] Also post-surgical complications like cystoid macular edema, increased intraocular pressure, posterior capsular opacification, and protein leakage from the breakdown of the blood–aqueous barrier.[13] Pharmacological treatment in delaying the process of opacification of lens leads to the development of anti-cataract drugs. Based on in vivo studies of anti-cataract drug compounds incubated with eye crystalline lens, revealed eye solution comprising of 1% N-acetylcarnosine (NAC) can be used as an ophthalmic prodrug, other such protein stabilisers like bendazac and hydroxybendazac inhibits the progress of cataract development.[14,15]

The present study involves the identification of drug

compounds and their effects on gamma-D-crystallin protein in the treatment of senile cataract**.** Reports suggested pirenoxine, aspirin, aspirin like drugs, sorbinil, naltrexone can be used as anticataract drugs.[16,17,18,19] With reference to the studies, the drug compounds that are similar in structure and properties to the reported drugs were chosen for this study for the identification of new drugs in the treatment of senile cataract.

MATERIALS AND METHODS

TARGET PROTEIN SELECTION AND IDENTIFICATION OF ACTIVE SITE

Gamma-D-crystallin protein (complementary ID 2G98) was retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/). The protein structure and other reports were obtained from (http://www.rcsb.org/pdb/). The active site pocket for the Crystallin protein was predicted using bioinformatics tool, Ligsite, an online tool available at http:/projects.biotec.tu-dresden.de/pocket/.

SELECTION OF DRUG COMPOUNDS

Drug compounds that have pharmacological and biological activities that obey pharmacokinetic properties were selected. Based on the articles published, prescribed medications are naltrexone, bendazac and hydroxybendazac. Hence, compounds that have similar structure and properties to that of drug compounds were selected. Around fifty compounds were retrieved from Pubchem data base in 2D.sdf file format for further analysis.

ADMETox analysis

Qikprop, (RRID:SCR_014906) a Schrodinger module is used for drug likeness analysis, for studying ADMETox properties. Primary results for Thirty-one compounds were obtained after screening in qikprop module and found that the drug compounds of six in number that satisfy ADMETox properties. These six compounds were selected and subjected to docking studies.

DOCKING STUDIES

The glide module **(**RRID:SCR_000187**)** of Schrodinger software was used to perform Protein-ligand interactions. First, unwanted water molecules were removed from the protein. Next, the energy was minimised, and, finally, the structure was optimized before docking using a glide module. The small molecules or drug compounds were organized by neutralizing charged groups, tautomerized and improved chirality. The PyMol viewer software was used to study the interactions between D crystallin protein and drug compound. The glide module was used to observe the ability for a drug compound to interact with Gamma crystallin protein, conclusively PyMol (RRID:SCR_000305) software was used to identify hydrogen bonds.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

RESULTS AND DISCUSSION

Drug compounds were analysed for ADMETox properties and observed in a specific range were tabulated in Table 1. Number of non-trivial (not CX3), non-hindered (not alkene, amide, small ring) rotatable Bond (Rotor), Molecular Weight, Computed dipole moment of the molecule (dipole), surface area solvent accessibility (SASA), number of hydrogen bonds donated and accepted, octanol/water coefficient, blood/brain barrier, metabolic involvement, percent human oral absorption was included for investigation. Percentage for human oral absorption was intended, and most of the compounds showed higher absorption range. Those compounds that follow ADMETox parameters are selected for further docking studies. An automated docking program, SYSDOC was a widely used tool for docking studies for decades.[20] Due to the compatibility and advancement of Schrodinger software, a glide module from Schrodinger software was used in this study. The results revealed Glide score (G.score), interacting residues, bond length and interaction type with that of targeted Gamma crystallin protein.(Table 2) Consequently, in silico pharmacokinetic evaluation was performed in 1,2,4-triazole and 1,3,4-oxadiazole and interpreted as these compounds were a good contender as a drug.[21] In addition, inhibitory action of quercetin glycosides by computational docking studies was performed by Syed Aun Muhammad and Nighat Fatima (2015).[22]

The overall Glide score were experiential in the range of -6.49 to -9.21Kcal/mol. The compound salsalate showed -9.21 Kcal/mol of G.score and formed 3 number of hydrogen bonds with the residues Arg79,Gln54. Incorporating Glide module for docking studies is most common among researchers. 19 The compound had two hydrogen bond interaction with Arg79 with bond length of 2.0 Å and 1.7 Å, whereas Gln54 had hydrogen bond interaction with bond length 2.4 and Leu144 had oxygen bond interaction with bond length 1.8 Å. The compound 2-Amino-3-oxophenoxazine-1-carboxylic acid showed -7.96 Kcal/mol of G.score and formed 4 number of hydrogen bonds with the residues Trp156, Tyr156, Leu144, Gln54, Tyr150, Arg79 with bond length 3.0,1.9,3.4,2.1,1.9,1.7 Å, respectively. The compound 1-Amino-5-hydroxybenzo[a]phenoxazin-2-one showed -7.67 Kcal/mol of G.score and the formed 2 number of hydrogen bonds with the residues Tyr150, Tyr45 with bond length 2.2 Å. The compound also formed 2 number of oxygen bonds with Asp155, Leu144 with bond length 2.2 and 2.4 Å respectively. The compound Pirenoxine showed 6.67 Kcal/mol of G.score and formed 2 number of oxygen bonds with Tyr50 and Gly154 with bond length 2.8 and 3.5 Å respectively. Also, it formed 1 number of Hydrogen bond with Gly52 with bond length 2.0 Å. The compound 1-Hydroxy-5-oxo-5H-pyrido[3,2-a]
phenoxazine-3-carboxylate showed -6.63 Kcal/mol of G.score and formed one hydrogen bond and one oxygen bond to the Gly52 and Tyr50 residues with bond length 2.0

and 2.9 Å respectively. The compound 2,6-Dimethoxybenzoic acid -6.49 Kcal/mol of G.score and formed 3 number of hydrogen bonds with the residues Arg167 with bond length 2.2, 2.8 Å and formed one number of hydrogen bonds with the residue Tyr62 with bond length 2.0. The interaction of Gamma crystallin protein with salsalate alone is shown in figure 3.1. Docking studies for several compounds of different origin were also done. For exitance, docking studies for phenolic compounds identified from wild mushrooms were analysed.[23]

TABLE 1 ADMETOX PROPERTIES OF DRUG COMPOUNDS

Molecule	Rotor	Molecular weight	Dipole	SASA	donorHB	acceptHB	QPlogPoct	QPlogPw	QPlogP o/w	QPlogBB	#metabolicreactions	Human Oral Absorption	Percent Human Oral Absorption	Rule of Five	Rule of Three
normal values	0.0 - 15.0	130.0 - 725.0	1.0 - 12.5	300.0 - 1000.0	0.0 - 6.0	2.0 - 20.0	8.0 -35.0	4.0 - 45.0	-2.0 - 6.5	-3.0 - 1.2	1.0 - 8.0	1,2 or 3 for low,medium or high	(<25% is poor) (>80% is high)	Max.4	Max.3
Pirenoxine (4846)	1	308.25	2.135	387.04	2	8	13.999	13.872	-0.339	-0.958	1	2	54.802	0	0
Salsalate (5161)	4	258.23	5.698	393.682	1	4.25	10.078	8.113	1.451	-1.012	1	3	70.002	0	0
2,6-Dimethoxybenzoic acid(15109)	3	182.176	4.096	475.271	1	3.5	9.085	6.545	1.942	-1.074	3	3	77.163	0	3
1-Hydroxy-5-oxo-5H-pyrido[3,2-a] phenoxazine-3-carboxylate(24868267)	1	308.25	4.628	387.039	2	8	14.369	13.872	-0.339	-0.958	1	2	54.802	0	1
2-Amino-3-oxophenoxazine-1-carboxylic acid(117846692)	2	256.217	4.539	340.434	2	5.5	11.337	10.759	0.154	-0.754	2	2	62.129	0	2
1-Amino-5-hydroxybenzo[a]phenoxazin-2-one(150932900)	2	278.267	3.591	366.507	2.5	5.25	12.591	11.486	0.308	-0.469	3	3	80.364	0	2
N-Acetyl-DL-cysteine (94364)	0	163.191	5.387	330.863	2	3.2	8.768	7.297	0.207	-0.2	1	3	82.695	0	0
Aspirin (2244)	3	180.16	2.785	351.238	1	4.75	7.962	7.791	0.192	-0.91	0	2	63.9	0	0
Bendazac (2313)	1	282.298	4.088	574.761	3	6.4	16.743	13.931	1.111	-0.65	2	3	89.102	0	0
Methyl salicylate (4133)	2	152.149	3.221	372.539	0	1.75	4.622	3.241	1.492	-0.232	2	3	95.983	0	0
Tiopronin (5483)	1	163.191	5.046	430.102	4.6	3.4	13.626	11.484	0.069	-0.748	1	3	77.831	0	0
Histidine (6274)	1	155.156	5.816	366.801	4.333	2.333	12.16	10.211	0.006	-1.189	1	2	58.273	0	0
Phenyl salicylate (8361)	3	214.22	1.808	333.413	0	2.25	5.679	4.344	1.515	-0.027	1	3	100	0	0
Ethyl salicylate (8365)	3	166.176	2.314	303.744	1	3.75	6.434	6.367	0.223	-0.233	1	3	86.262	0	0
Triflusal (9458)	3	248.158	2.822	382.961	1	4.75	8.523	7.468	0.606	-0.816	0	3	67.379	0	0

2-Methoxybenzoic acid (11370)	2	152.149	4.194	415.376	1	2.75	7.543	6.136	1.689	-1.043	2	3	75.34	0	0
Acetylcysteine (12035)	0	163.191	5.387	330.863	2	3.2	8.768	7.297	0.207	-0.2	1	3	82.695	0	0
Benzydamine (12555)	0	309.41	2.912	647.505	3	3	16.301	10.365	3.755	0.544	2	1	100	0	1
Butyl salicylate (16330)	3	194.23	2.566	385.241	1	3.75	8.162	6.483	1.078	-0.208	2	3	93.272	0	0
Methyl 2-methoxybenzoate (61151)	2	166.176	5.517	515.227	0	2.75	7.572	4.55	2.023	-0.579	2	3	100	0	0
D-Histidine (71083)	1	155.156	5.816	366.801	4.333	2.333	12.16	10.211	0.006	-1.189	1	2	58.273	0	0
N-Acetyl-D-cysteine (94364)	0	163.191	5.387	330.863	2	3.2	8.768	7.297	0.207	-0.2	1	3	82.695	0	0
Anserine (112072)	0	240.261	9.506	464.309	6.5	6.2	20.588	17.399	-0.686	-0.757	1	3	60.435	1	0
Mercaptopropionylglycine (165505)	1	163.191	6.78	437.793	5.4	5.6	16.643	14.877	-0.598	-0.787	1	3	73.171	0	0
Carnosine (439224)	0	226.235	6.381	413.803	6.5	5.2	18.01	16.444	-1.052	-0.953	1	2	54.501	1	0
Bucillamine (656604)	0	223.305	7.144	395.213	5	3	14.705	11.611	0.359	-0.105	1	3	85.02	0	0
n-Acetyl-l-cysteinate (7043949)	0	163.191	2.26	330.863	3	4.2	10.032	9.747	-0.211	-0.2	1	3	80.245	0	0
N-Acetylcarnosine (9903482)	0	268.272	3.128	447.387	5	6.7	17.198	15.605	-0.505	-0.702	1	3	60.425	1	0
N-Monoacetylcystine (12049111)	1	282.329	8.209	493.756	5	4.2	17.588	13.732	0.16	-2.031	1	2	36.385	1	1
N-(3-Sulfanylpropanoyl)-L-alanine (13036940)	1	177.218	0.388	425.251	6.4	5.6	16.845	16.181	-0.655	-0.516	1	3	76.094	0	0
3,4-Dihydro-2H-[1,3]oxazino[3,2-b] indazole (13664293)	0	174.202	0.402	275.028	1	2	5.548	4.886	1.088	0.421	0	3	100	0	0

TABLE 2 INTERACTION OF DRUG COMPOUNDS WITH GAMMA-D-CRYSTALLIN PROTEIN

Name of the ligand	Residues interaction	Bond length (å)	No of bonds formed	G. score (kcal/mol)
Pirenoxine(4846)	TYR 50(O=O)	2.8	3	-6.67
	GLY52 (O-H)	2.0		
	GLN154(O=O)	3.5		
Salsalate(5161)	ARG79(O-H)	2.0	4	-9.21
	ARG79(O-H)	1.7		
	GLN54(O-H)	2.0		
	LEU144(H-O)	1.8		
2,6-Dimethoxybenzoic acid(15109)	ARG167(O-H)	2.2	4	-6.49
	ARG167(O-H)	2.4		
	ARG167(O-H)	2.8		
	TYR-62(O-H)	2.0		
1-Hydroxy-5-oxo-5H-pyrido[3,2-a]phenoxazine-3-carboxylate(24868267)	GLY52(O-H)	2.0	2	-6.63
	TYR50(O=O)	2.9		
2-Amino-3-oxophenoxazine-1-carboxylic acid(117846692)	TRP156(0=0)	3.0	6	-7.96
	TYR156(O-H)	1.9		

	LEU144(0=0)	3.4		
	GLN54(0-H)	2.1		
	TYR150(O-H)	1.9		
	ARG79(O-H)	1.7		
1-Amino-5-hydroxybenzo[a]phenoxazin-2-one(150932900)	ASP155(H-O)	2.2	4	-7.67
	TYR150(O-H)	2.2		
	LEU144(H-O)	2.4		
	TYR45(O-H)	2.2		

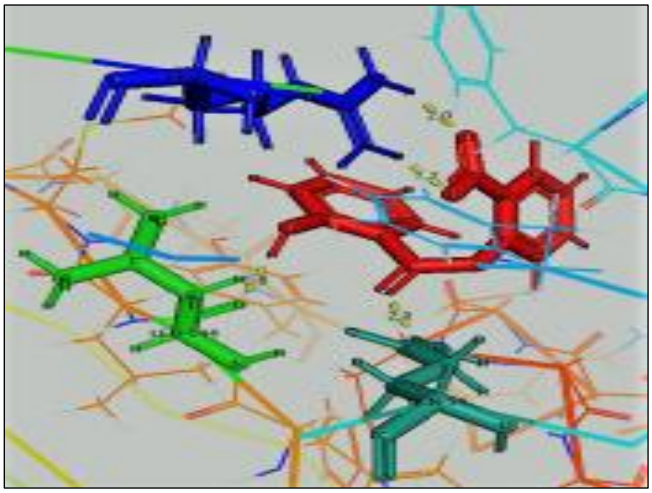

FIG. 1 THE INTERACTION OF GAMMA CRYSTALLIN PROTEIN WITH SALSALATE

REFERENCES

1. Moncef Khairallah, Rim Kahloun, Rupert Bourne, Hans Limburg, Seth R. Flaxman, Jost B. Jonas, Jill Keeffe, Janet Leasher, Kovin Naidoo, Konrad Pesudovs, Holly Price, Richard A. White, Tien Y. Wong, Serge Resnikoff, Hugh R. Taylor. (2015) Vision Loss Expert Group of the Global Burden of Disease Study Investigative, Ophthalmology & Visual Science October. Vol.56, 6762-6769.

2. Yasemin Budama-Kilinc, Rabia Cakir-Koc, Serda Kecel-Gunduz, Yagmur Kokcu, Bilge Bicak, Hande Mutlu, Aysen E. (2018) Novel NAC-loaded poly (lactide-co-glycolide acid) nanoparticles for cataract treatment: preparation, characterization, evaluation of structure, cytotoxicity, and molecular docking studies. PeerJ 6:e4270.

3. Pascolini D & Mariotti SP. (2012) Global estimates of visual impairment. Br J Ophthalmol. 96: 614–618.

4. Hamdy Abdelkader, Raid G. Alanyac and Barbara Pierscionek. (2015) Age-related cataract and drug therapy: opportunities and challenges for topical antioxidant delivery to the lens, Journal of Pharmacy and Pharmacology. Volume 67, Issue 4, Pages 537–550

5. Vinson JA. (2006) Oxidative stress in cataracts. Pathophysiology 13, 151–162.

6. Rosenthal R. (2002) Outcome indicators for cataract. In: Rosenthal R, ed. Health Outcome Indicators. London: University of Oxford. 1–86

7. Bloemendal H, de Jong W, Jaenicke R, Lubsen NH, Slingsby C, Tardieu A. (2004) Ageing and vision: structure, stability and function of lens crystallins. Prog Biophys Mol Biol.86: 407–485.

8. Liu XF, Hao JL, Xie T, Malik TH, Lu CB, Liu C, Shu C, Lu CW, Zhou DD. (2017) Nrf2 as a target for the prevention of age-related and diabetic cataracts by against oxidative stress. Aging Cell. 16, 934–942.

9. Harding J. (1991) The ageing lens. In: Harding J, ed. Cataract: Biochemistry, Epidemiology and Pharmacology. London: Chapman and Hall. 71–81.

10. Wilmarth P, et al. (2006) Age-related changes in human crystallins determined from comparative analysis of post-translational modifications in young and aged lens: does deamidation contribute to crystallin insolubility? J Proteome Res. 5: 2554–2566.

11. Higashide T, Sugiyama K. (2008) Use of viscoelastic substance in ophthalmic surgery - focus on sodium hyaluronate. Clin Ophthalmol 2(1):21-30.

12. Duan P, Liu Y, Li J. (2017) The comparative efficacy and safety of topical non-steroidal anti-inflammatory drugs for the treatment of anterior chamber inflammation after cataract surgery: a systematic review and network meta-analysis. Graefes Arch Clin Exp Ophthalmol 255(4):639–649.

13. Dick HB, Schwenn O, Krummenauer F, Krist R, Pfeiffer N. (2000) Inflammation after sclerocorneal versus clear corneal tunnel phacoemulsification. Ophthalmology. 107(2):241–247

14. Yasemin Budama-Kilinc, Rabia Cakir-Koc, Serda Kecel-Gunduz, Yagmur Kokcu, Bilge Bicak, Hande Mutlu, Aysen E. (2018) Novel NAC-loaded poly (lactide-co-glycolide acid) nanoparticles for cataract treatment: preparation, characterization, evaluation of structure, cytotoxicity, and molecular docking studies. PeerJ 6:e4270.

15. Shen H, et al. (2010) Synthesis and biological evaluations of novel bendazac lysine analogues as potent anticataractagents. Bioorg Med Chem Lett. 20: 2115–2118.

16. Testa M, et al. (1987) Higher efficacy of flunoxaprofen over bendazac and other non-steroidal anti-inflammatory drugs in treatment of cataract. CurrTherap Res 42: 182–189.

17. Maggs JL, Park BK. (1988) Drug-protein conjugates XVI studies of sorbinil metabolism: formation of 2- hydroxysorbinil and unstable protein conjugates. BiochemPharmacol. 37: 743–748

18. Tauseef M, et al. (2008) Antioxidative action of aspirin on endothelial function in hypercholesterolaemic rats. Basic Clin Pharmacol Toxicol. 103: 314–321

19. Abdelkader H, et al. (2011) New therapeutic approaches in treatment of diabetic keratopathy. Clin Exp Opthalmol. 39: 259–270.

20. Pang YP & Kozikowski AP. (1994) Prediction of the binding sites of huperzine A in acetylcholinesterase by docking studies. Journal of Computer-Aided Molecular Design 8(6), 669–681.

21. Sampark S Thakkar, Parth Thakor, Hiren Doshi, Arabinda Ray. (2017) 1,2,4-Triazole and 1,3,4-oxadiazole analogues: Synthesis, MO studies, in silico molecular docking studies, antimalarial as DHFR inhibitor and antimicrobial activities. Bioorganic & Medicinal Chemistry 25 4064–4075

22. Muhammad S & Fatima N. (2015) In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. Pharmacognosy Magazine 11(42), 123.

23. Alves, Ferreira, Froufe, Abreu, Martins, Pintado. (2013) Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. Journal of Applied Microbiology 115, 346-35.