Clinical Management and Therapeutic Strategies for the Thyroid-Associated Ophthalmopathy: Current and Future Perspectives

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Supplementary file S1:

A. Molecular docking

1.1. Ligand retrieval

The 3D structures of all the selected ligands: Curcumin (CID: 969516), Withaferin A (CID: 265237), Resveratrol (CID: 445154), Scopolamine (CID: 3000322), Quercetin (CID: 5280343), and Berberine (CID: 2353) were retrieved from the NCBI PubChem compound database in SDF format followed by optimization using Discovery Studio.

1.2. Protein preparation

The 3 dimensional (3D) crystal structures of hyaluronan protein (PDB: 1UUH) were obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank [71]. Prior to docking analysis, active binding site of the protein were determined using MetaPocket 2.0 server [72]. Molegro Virtual Docker (MVD-3.0.0) has been used for the molecular docking selected phytoconstituents with hyaluronan protein [73].

1.3. Results

Considering the significance of natural compounds in drug discovery, molecular docking of selected phytoconstituents hyaluronan protein was studied and docking results are given in terms of Mol Dock Score, Rerank Score, H Bond energy and interacting residues (Table S1). Docking results demonstrated that all the selected phytoconstituents have significant interaction with hyaluronan protein. Form all the

selected molecules; Curcumin found to have a highest binding affinity (Moldock score -388.512 kJ/mol, rerank score -175.97 kJ/mol and hydrogen bond energy-16.9319 kJ/mol) compared to the other phytoconstituents. Therefore, it is predicted that Curcumin will be a potential molecule if synthesized and tested for the treatment of TAO.

Table S1. Molecular docking results are indicated with maximum binding affinity of phytoconstituents with hyaluronan protein along with Moldock Score and other parameters.

S.No.	Ligand	MolDock Score (kJ/mol)	Rerank Score (kJ/mol)	H-Bond (kJ/mol)	Interacting residues
1.	**Curcumin**	-388.512	-175.97	-16.9319	TYR42, LEU70, TYR79, ILE91, ILE96, THR102, GLY103
2.	**Withaferin A**	-155.622	-72.0896	-9.57009	TYR42, GLU67, LEU70, TYR79, ILE91, HIS92, ILE96, TYR114
3.	**Resveratrol**	-151.749	-98.1192	-14.4029	GLU67, LEU70, TYR79, ILE91, ILE96, LEU107, TYR114
4.	**Scopolamine**	-149.866	-10.5868	-7.85712	TYR42, GLU67, LEU70, SER71, CYS77, TYR79, ILE88, ARG90, ILE91, ILE96,CYS97, THR102, GLY103
5.	**Quercetin**	-143.609	-113.004	-13.9648	TYR42, LEU70, ILE91, THR102, LEU107, TYR114
6.	**Berberine**	-140.038	-87.6962	-4.05775	TYR42, GLU67, LEU70, SER71, ILE91, ILE96, GLY103, VAL104

Moldock score was calculated by summing the external ligand interaction (protein–ligand interaction) and internal ligand interaction score. Rerank Score provides an estimate of the strength of the protein–ligand interaction. H Bond energy is Hydrogen bonding energy (kJ/mol).

Interacting residues are amino acid present on binding site and involved in various interactions with ligand.

B. *In silico* pharmacokinetics

Drug likeness and ADME parameters of Curcumin, Withaferin A, Resveratrol, Scopolamine, Quercetin and Berberine were investigated by SwissADME server [74]. In Lipinski filter analysis, ligand having MW >500 g/mol, Hydrogen-bond-accepting atoms >10, hydrogen-bond-donating atoms >5, and CLogP >5 were not considered as a good orally active agent. Molecules violating more than one of these rules may have problems with oral bioavailability. Results demonstrated that all molecules follow the Lipinski rule to be orally active compounds. For pharmacokinetics analysis, these molecules were considered for various parameters ranging from GI absorption to skin permeation (Table S2). Pharmacokinetics results demonstrated that all the six phytoconstituents have high GI absorption, skin permeability whereas resveratrol and berberine showed good blood brain barrier permeability compared to the curcumin, withaferin A, scopolamine, and quercetin.

Table S2: Drug likeness and pharmacokinetics properties of Curcumin, Withaferin A, Resveratrol, Scopolamine, Quercetin and Berberine.

Parameters studied	Curcumin	Withaferin A	Resveratrol	Scopolamine	Quercetin	Berberine
1. Lipinski analysis for drug likeness						
Mass	368.38 g/mol	470.60 g/mol	228.24 g/mol	303.35 g/mol	302.24 g/mol	336.36 g/mol
Hydrogen bond donor	2	2	3	1	5	0

Hydrogen bond acceptors	6	6	3	5	7	4
LogP	3.27	3.39	1.71	2.74	1.63	3.62
Molar Refractivity	102.80	127.49	67.88	83.48	78.03	94.87
2. Pharmacokinetics						
GI absorption	High	High	High	High	High	High
BBB permeation	No	No	Yes	No	No	Yes
P-gp substrate	No	Yes	No	No	No	Yes
CYP1A2 inhibitor	No	No	Yes	No	Yes	Yes
CYP2C19 inhibitor	No	No	No	No	No	No
CYP2C9 inhibitor	Yes	No	Yes	No	No	No
CYP2D6 inhibitor	No	No	No	Yes	Yes	Yes
CYP3A4 inhibitor	Yes	No	Yes	No	Yes	Yes
Log K_p (skin permeation)	-6.28 cm/s	-6.45 cm/s	-5.47 cm/s	-7.45 cm/s	-7.05 cm/s	-5.78 cm/s

C. Gene network analysis for thyroid associated ophthalmopathy

The gene expression data of various genes that involved in pathogenesis of TAO has been collected from articles available on PubMed. These studies were further scrutinized according to the information given under the Methods section. A total 43 studies were

included for final evaluation and literature search revealed that 94 genes were up-regulated while 5 genes were found to be down-regulated during TAO (Table S3). A biological network of genes and associated pathways was analysed using GeneMANIA webserver and Reactome pathway database.

Table S3: List of up-regulated and down-regulated genes and their cellular functions in thyroid associated ophthalmopathy analysed by GeneMANIA webserver.

Associated genes	Function	Reference
Up regulated genes		
TLR9, USF1, IRS2, IL12B, PPARG, IGF1, ADIPOQ	Cellular response to insulin stimulus	1,2,3,4
IGF1, IGF1R	Insulin receptor binding	2
PPARG, NFKB1, NFKBIA, ADIPOQ	Regulation of cholesterol transport	4,6
TLR9, IL12B, IRF5, IL23R, IFNG	Positive regulation of interleukin-12 production	1,3,6,7,8,9
TLR9, IL12B, CD34, IL23R	Regulation of interleukin-10 production	1,3
CCL3,WNT5A	Positive regulation of interleukin-1 production	2
TLR7, TLR9, IL12B, CCL3, CCL5, IL2, IL6, IL21, PTGS2, PIK3AP1, TNFSF11, WNT5A, ADIPOQ	Regulation of inflammatory response	10,1,3,2, 11,12,13,4
IL1A, SFRP1, PTGS2, CD34, WNT5A	Positive regulation of angiogenesis	14,2,13
PDGFB, CCL3, TNF, TNFSF11, CYR61, ADIPOQ	Regulation of ERK1 and ERK2 cascade	15,5,4
PPARG, USF1, STAT3, ADIPOQ	Glucose homeostasis	4,16,17
IRS2, TNF, IL12B	Regulation of lipid catabolic process	2,5,3
SFRP1, CXCL10 , CXCL11 , SLIT2	Glycosaminoglycans binding	2,11,18
ABCG1, PPARG	Cholesterol storage	19,4
TLR7, TLR9, IL12B, IL10, IL12, NFKBIA	Regulation of NF-κβ import into nucleus	20,1,3,5
TLR9, IL12B, RARA, CCL3, IFNG, CD34,	Tumor necrosis factor	1,3,21,11,7,

ZEP36, ADIPOQ	super family cytokine production	8,9,4
SFRP1, IRS2, IL2, IL6, IL10, CTLA4, TGFB1	Regulation of B cell activation	2,7,22,4
TLR9, IL6R, IL6, WNT5A	Positive regulation of interleukin-6 production	1,7,2
PDGFB, NFKB1, TGFB1, HAS2	Hyaluronan biosynthetic process	15,5,4
CCL2, CCL3, CCL4, CCL5, CCL7	CCR chemokine receptor binding	11
TLR9, IRF1, IRF5, IRF7, NFKB1, NFKBIA	Positive regulation of type I interferon production	1,23,6,5
TLR9, IL2, IL6, IL6R, IL10, 1L12A, IL12B, IL21, IL22, CCL3, CCL4, CCL5, CCL7, CXCL3, CXCL8, CXCL9, CXCL10, CXCL11, TGFB1, TGFB2,TNFSF11	Cytokine receptor binding	1,7,3,24,25,11,4,5
IL1A, IL2, IL6, IL10, 1L12A, IL12B, CCL3, CCL4, CCL5, CCL7, CXCL3, CXCL9, CXCL10, CXCL11, TGFB2, TNFSF11, ADIPOQ	Cytokine activity	7,12,3,11,4
TLR7, TLR9, IL1A, IL2, IL12B, IL6, IL6R, IL10, IL22, CCL2, CCL3, CCL5, PTX3, CXCL8, NFKB1, FOS, TGFB1, PTGS2, PIK3AP1, TNFSF11, ADIPOQ, WNT5A	Inflammatory response	10,1,12,3,25,11,26,5,4,13,2
PDGFB, 1L12A, IL12B, IL6R, CCL5, PPARG, IFNG, STAT1, IGF1, ADIPOQ	Regulation of smooth muscle cell proliferation	15,3,7,12,11,4,8,9,17,2
THY1, IL2, IL6, 1L12A, IL12B, IL10, IL23R, IL21, RARA, CCL5, ICAM1, S1PR1, CTLA4, IGF1, IRF1, CD8A, HLA-DRA, TNFSF11	T cell activation	27,12,3,28,24,21,11,29,22,2,6,30,4
THY1, 1L12A, IL12B, RARA, CCL2, CCL5, IL2, IL6, CD4, CTLA4, IL21, IL23R, IGF1, HLA-DRA, TNFSF11	Positive regulation of T cell activation	27,3,21,11,22,24,28,2,30,4
1L12A, IL12B, IL17A, IL23R, IL2, IL21, IFNG, TGFB1	Interleukin-17 production	3,9,28,7,8,4
DEFA1, DEFA1B, DEFA3, TIMD4, SCD, FADS1	-	31
CRBPI, CLN2, HEXB, GNAS1	-	32,33,34
CASQ1, CASQ2, HSD11B1	-	35,36
ABCG1, ACTA2, SFRP2, TNC	-	19
SOCS3, ERA	-	37,38

TPX2, CDCA5, PRC1, KIF23, GTF2F1, SMC3, USF1, ZNF263	-	16
CYR61, PTGS2, EGR1, ZFP36, SCD	-	39
Down-regulated genes		
NR5A1	-	39
GM-CSF, IL-13	-	40
TIM-3	-	41
HBD-2	-	42
CD103	-	43

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