

Molecular Docking-Based Evaluation of the Impact of Pharmaceutical Impurities on Visual Function

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This study investigated the potential impact of pharmaceutical impurities on key zebrafish eye proteins involved in the visual transduction of Opsin (PDB ID: 3CAP), transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H) via molecular docking approaches. A virtual ligand library of selected pharmaceutical impurities was constructed from PubChem, and docking was performed with PyRx to evaluate the binding affinities and interaction modes at the predicted active sites. The results revealed that pharmaceutical impurities, such as triphenyl phosphate (TPP), exhibit high binding affinity for the selected visual protein opsin, which transduces phosphodiesterase-6 with binding energies of -7.7 kcal/mol, -7.5 kcal/mol and -6.8 kcal/mol, suggesting possible disruption of protein function. This mechanistic insight highlights the risk impurities pose to visual protein integrity and function, which may contribute to ocular toxicity. This study demonstrated that docking is a rapid screening tool for assessing the safety profile of the effects of pharmaceutical impurities on ocular proteins. These findings provide a basis for further experimental validation and enhanced drug safety evaluations to minimize visual toxicity risks.

Keywords: Computational biology, Molecular Docking, Ocular toxicity, Pharmaceutical impurities, Zebrafish.

The maintenance of normal ocular function and vision critically depends on the structural and functional integrity of visual proteins. Pharmaceutical impurities unintended chemical entities present in drug formulations may interact adversely with these essential eye proteins, their function and posing significant risks to ocular health. A molecular-level understanding of these interactions is crucial for accurate safety evaluation of pharmaceuticals and for minimizing the risk of ocular toxicity.^{1,4,4}

Opsins are G protein-coupled receptors that absorb light through their covalently bound chromophore, retinal, triggering conformational changes that activate downstream signalling.^{2,0,2,3} Opsins possess seven transmembrane helices and play a central role in converting photon absorption into a biochemical signal by activating the G protein transducin.^{2,1} Transducin is a heterotrimeric G protein expressed in rod and cone photoreceptors.^{1,1} Upon activation by photoexcited opsin, the α -subunit exchanges GDP for GTP

and dissociates to activate phosphodiesterase-6 (PDE-6), leading to signal amplification (Bland, Clarke and Harden, 1976). Phosphodiesterase-6 hydrolyzes cyclic GMP (cGMP), leading to the closure of cGMP-gated ion channels and ultimately causing photoreceptor membrane hyperpolarization⁹ This cascade is essential for phototransduction and visual signal transmission.⁷ The unique barriers of the eye and the presence of membrane transporters can influence the accumulation of systemic drugs and their impurities within ocular tissues, increasing the risk of toxicity.^{1,6} Research using molecular docking has revealed significant interactions between drugs and ocular proteins, suggesting a role for impurities in disrupting protein function and potentially leading to visual impairment.^{1,5,2,5}

Molecular docking is a powerful *in silico* tool that predicts the binding interactions and affinities between small molecules and target proteins, offering insights into the potential toxicological impacts of pharmaceutical impurities on protein function. This technique has been widely used in drug discovery to identify molecular mechanisms and binding modes, guiding the assessment of drug safety profiles.^{1,9} Specifically, studies have applied molecular docking to evaluate the binding of drug molecules and impurities to ocular proteins, contributing to the understanding of their possible roles in ocular toxicity.⁵

This study aims to evaluate the binding interactions of selected pharmaceutical impurities with the key visual proteins opsin, transducin, and phosphodiesterase-6 via molecular docking approaches. By investigating the molecular interactions and binding affinities, this research seeks to provide a mechanistic understanding of how impurities might affect visual proteins, thereby contributing to improved drug safety assessments and ocular toxicity prediction.

MATERIALS AND METHODS

The identification of potential inhibitory compounds against the target proteins Opsin (PDB ID: 3CAP), transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H) was performed through a multistep *in silico* workflow involving ligand library construction, protein

preparation, active site prediction, and molecular docking.

Ligand selection and Library construction

A preliminary literature search was used to create a virtual ligand library. Pharmaceutical impurities, although often present in trace amounts, can significantly influence the efficacy, safety, and stability of drugs. Understanding their structures is crucial in drug design and toxicological assessments. In this study, we systematically created a structural library of selected pharmaceutical impurities via the PubChem database. The purity structures were retrieved from PubChem via their unique PubChem Compound Identifiers (CIDs), processed via Discovery Studio, and saved in the Protein Data Bank (PDB) format (Fig. 1). The final impurity library is stored in a dedicated data base.^{1,6}

Preparation of protein

Molecular docking is a fundamental technique in computer-aided drug design that requires the precise preparation of protein structures. The raw protein data obtained from the Protein Data Bank (PDB) often contain water molecules, cocrystallized ligands, and heteroatoms that may interfere with docking simulations. This study describes a standardized protocol for preparing protein structures by removing nonessential molecules and adding polar hydrogens via Discovery Studio Visualizer. The resulting created protein structures are stored in a dedicated folder for downstream virtual screening and docking experiments.^{1,5} This pipeline ensures reproducibility and structural integrity, facilitating accurate *in silico* modeling. The proteins that were prepared were opsin (PDB ID: 3CAP), transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H).

Identification of binding pockets

The ProteinPlus server provides a user-friendly platform for comprehensive analysis of protein structures, focusing on binding site prediction and druggability estimation. In this workflow, the protein structures of Opsin (PDB ID: 3CAP), Transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H) are uploaded in PDB form and utilized as the sole inputs. The DoGSiteScorer tool within ProteinPlus was then applied to the structure, automatically identifying potential binding pockets through topology

analysis (Fig. 3). For each detected pocket, the server presents an array of physicochemical and geometric features, including the crucial DogScore (druggability score) indicator.^{1,6} Visual inspection and downloadable result tables facilitate an in-depth assessment of the binding site characteristics. This integrated approach enables rapid evaluation of a protein's druggability and supports structure-based drug design endeavors (Fig. 3).

Molecular docking

Molecular docking simulations were performed to predict the binding affinity and orientation of the filtered ligand compounds within the predicted active site of the Opsin (PDB ID: 3CAP), transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H) proteins. PyRx software (version 0.8), a virtual screening tool that includes AutoDock Vina, was used to carry out the docking calculations.^{1,5,2,5} PyRx software was used to prepare and minimize the number of ligand molecules. The docking grid was created around the

selected active pocket coordinates: (opsin protein Dimension: X:33.1848 Y: 20.5538 Z: 40.4790, Transducin Dimensions: X:33.2673 Y:25.0000 Z: 35.4564) per phosphodiesterase-6 was subjected to entire grid formation (bind docking X:49.5148 Y:51.3781 Z:52.4954). Each ligand's docking simulation was then run, producing a variety of binding poses and matching binding affinity scores. These scores were used to rank the compounds; a higher projected binding affinity was indicated by lower (more negative) values. The top-scoring compounds were subsequently analyzed for their specific interactions, such as hydrogen bonding and hydrophobic interactions.^{2,1}

RESULTS

Binding Affinity Analysis

Molecular docking of the proteins Opsin (PDB ID: 3CAP), transducin (PDB ID: 6OY9) and phosphodiesterase-6 (PDB ID: 4F1H) with

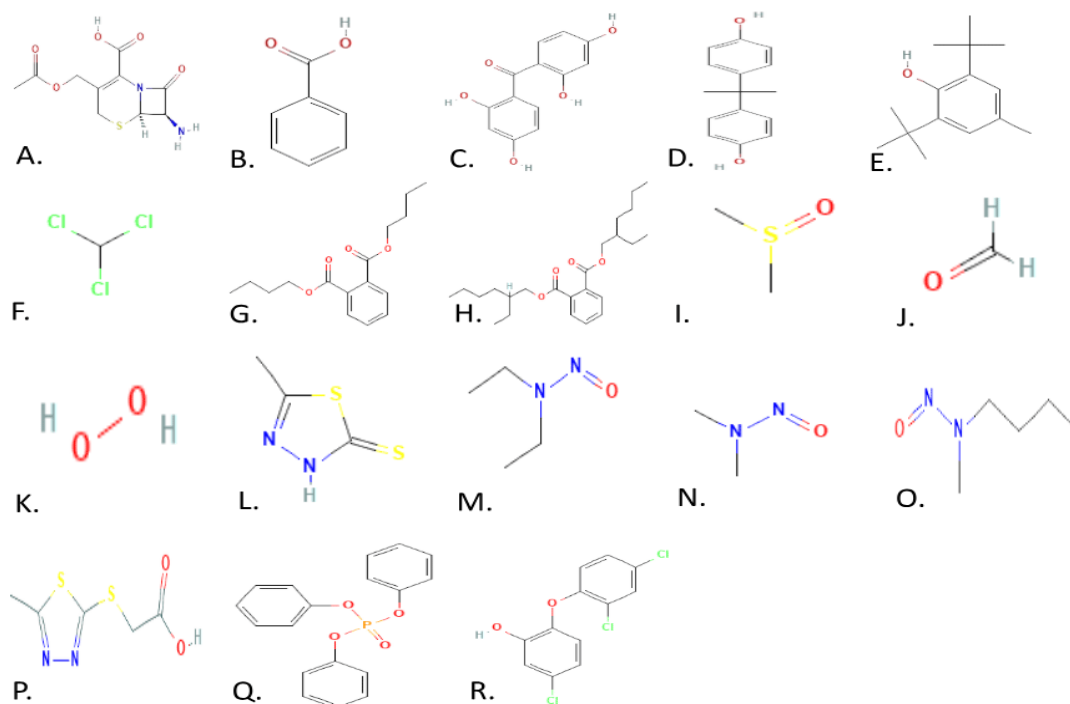


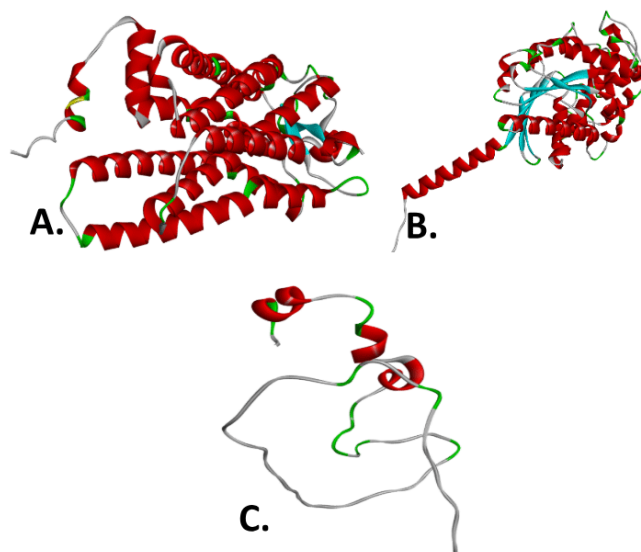
Fig. 1. (A): 7-amino cephalosporinic acid, (B): benzoic acid, (C): benzophenone2, (D): bisphenol, (E): butylhydroxytoluene, (F): chloroform, (G): dibutylphthalate, (H): diethylhexylphthalate, (I): dimethylsulfoxide, (J): formaldehyde, (K): hydrogen peroxide, (L): 2-mercapto-5-methyl-1,3,4- thiadiazole, (M): nitrosodiethylamine, (N): nitrosodimethylamine, (O): nitrosomethylaminobutyric acid, (P): thiadiazoleacetic acid, (Q): triphenyl phosphate, (R): tricolosan

pharmaceutical impurities revealed that triphenyl phosphate has the highest binding affinity (more negative score), with values of -7.7 kcal/mol, -7.5 kcal/mol and -6.8 kcal/mol, respectively (Table 2 and Fig 4). Supported by multiple stabilizing hydrogen bond and alkyl bond interactions.^{1,5,2,5}

Binding interaction between pharmaceutical impurities and opsin eye protein

Benzoic acid binds with opsin eye protein via hydrogen bonds (TRP A:173, PRO A:4), alkyl bond (ALA A:3) and pi-bond (TRY A:100, ILE A:172), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.1 Kcal/mol. Benzophenone-2 binds with opsin eye protein via hydrogen bonds (TRP A:171), alkyl bond (ALA A:3, ARG A:170) and pi-bond (TYR A:100, ILE A:172), demonstrating diverse non-covalent interactions and exhibiting binding affinity -7.0 Kcal/mol. Diethylhexyl phthalate binds with opsin eye protein via hydrogen bonds (no interaction), alkyl bond (VAL A:130, PHE A:139, PHE A:136, VAL A:123) and pi-bond (PHE A:126), demonstrating diverse non-covalent interactions and exhibiting binding

affinity -6.5 Kcal/mol. Bisphenol binds with opsin eye protein via hydrogen bonds (no interactions), alkyl bond (VAL A:130, PHE A:139, PHE A:136, VAL A:123) and pi-bond (PHE A:126), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.9 Kcal/mol. Butylhydroxytoluene binds with opsin eye protein via hydrogen bonds (no interactions), alkyl bond (PRO A:164, PRO A:163, CYS A:204) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.1 Kcal/mol. Chloroform binds with opsin eye protein via hydrogen bonds (no interaction), alkyl bond (ALA A:161, LEU A:115, LEU A:200) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.2 Kcal/mol. Dibutyl phthalate binds with opsin eye protein via hydrogen bonds (no interaction), alkyl bond (ALAA:25, ALAA:29, LEU A:32, LEU A:176, ARG A:279, ILE A:283, PHE A:287) and pi-bond (PHE A:36, PHE A:286), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.8 Kcal/mol. Dimethyl sulfoxide binds with opsin eye protein

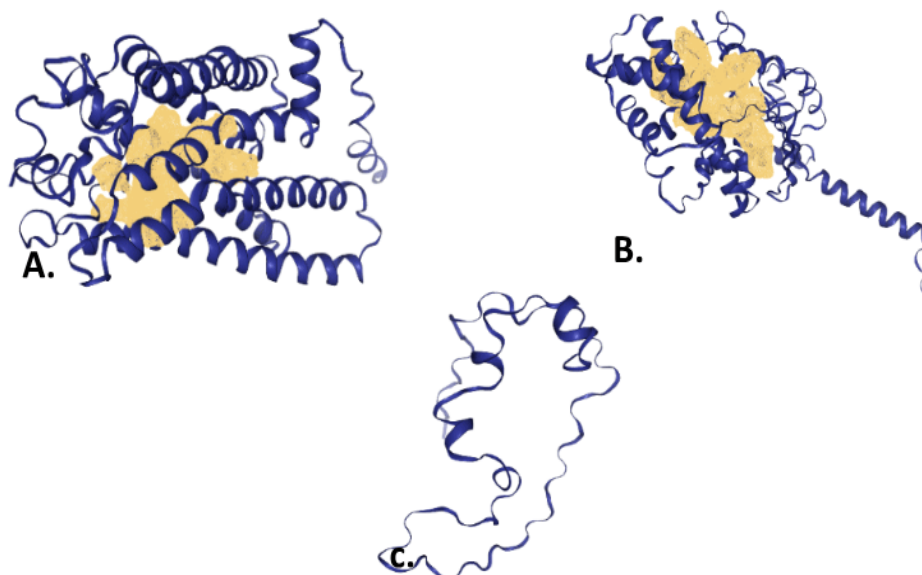


Protein	PDB ID	Resolution (Å)	Method	Chains Used	Waters Removed
Opsin	3CAP	2.9	X-ray	A	247
Transducin	6OY9	3.2	Cryo-EM	A,B,G	156
Phosphodiesterase-6	4F1H	2.6	X-ray	A	189

Fig. 2. (A) Opsin, (B) Transducin, (C) 6-Phosphodiesterase

via hydrogen bonds (ALAA:107,ALAA:108,TYR A:171), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.0 Kcal/mol. Formaldehyde binds with opsin eye protein via hydrogen bonds (LEU A:115), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -1.9 Kcal/mol. 2-mercapto-5-methyl-1,3,4-thiadiazole binds with opsin eye protein via hydrogen bonds (TYR A:171, PRO A:173), alkyl bond (ALA A:3,PHE A:17) and pi-bond (TYR A:100,ILE A:172), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.7 Kcal/mol. Nitrosodiethylamine binds with opsin eye protein via hydrogen bonds (ALA A:108), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity 4.4 Kcal/mol. Nitrosodimethylamine binds with opsin eye

protein via hydrogen bonds (SER A:111), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.5 Kcal/mol. Nitrosomethylaminobutyric acid binds with opsin eye protein via hydrogen bonds (ASN A:303,ASN A:66), alkyl bond (LYS A:304,VAL A:243) and pi-bond (no interactions), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.9 Kcal/mol. Thiadiazoleacetic acid binds with opsin eye protein via hydrogen bonds (GLU A:106,SER A:111,ALA A:107), alkyl bond (LEU A:200,PRO A:182,GLY A:110,CTS A:204) and pi-bond (TYR A:258,TYR A:261), demonstrating diverse non-covalent interactions and exhibiting binding affinity 5.7 Kcal/mol. Triclosan binds with opsin eye protein via hydrogen bonds (no interaction), alkyl bond (LEU A:176,ALA A:29,ILE A:283,PHE A:287) and pi-bond (PHE A:286,PHE A:36), demonstrating diverse non-covalent interactions and exhibiting binding



Protein	Pocket Center (X,Y,Z)	DogScore
A.Opsin (3CAP)	(33.18, 20.55, 40.48)	0.72
B.Transducin	(33.27, 25.00, 35.46)	0.68
C.PDE-6 (4F1H)	(49.51, 51.38, 52.50)	0.75

Fig. 3. Binding Pocket Prediction

affinity -7.6 Kcal/mol. Triphenyl phosphate binds with opsin eye protein via hydrogen bonds (no interaction), alkyl bond (VAL A:130, ILE A:211) and pi-bond (PHE A:126, VAL A:123), demonstrating diverse non-covalent interactions and exhibiting binding affinity -7.7 Kcal/mol. 7-aminocephalosporanic acid binds with opsin eye protein via hydrogen bonds (ASN A:66, ASN A:303, GLN A:305), alkyl bond (ALA A:239, VAL A:243) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.2 Kcal/mol (Table 3 and Fig 5.6)^{1,5,2,5}

Binding interaction between pharmaceutical impurities and transducin eye protein

Benzoic acid binds with transducin eye protein via hydrogen bonds (LEU A:114, LYS A:273), alkyl bond (ALA A:139) and pi-bond (ASP A:146), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.1 Kcal/mol. Benzophenone-2 binds with transducin eye protein via hydrogen bonds (SER A:140, ASP A:146), alkyl bond (ALA A:139) and pi-bond (VAL A:269), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.9 Kcal/mol. Diethylhexyl phthalate binds with transducin eye protein via hydrogen bonds

(no interaction), alkyl bond (PHE A:136, ALA A:139) and pi-bond (VAL A:269), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.0 Kcal/mol. Bisphenol binds with transducin eye protein via hydrogen bonds (LEU A:144, ASP A:227), alkyl bond (LYS A:273, LYS A:276) and pi-bond (ALA A:139), demonstrating diverse non-covalent interactions and exhibiting binding affinity -7.3 Kcal/mol. Butylhydroxytoluene binds with transducin eye protein via hydrogen bonds (no interaction), alkyl bond (ALA A:139, MET A:238, VAL A:269, LYS A:273) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.0 Kcal/mol. Chloroform binds with transducin eye protein via hydrogen bonds (LYS A:266), alkyl bond (ALA A:322, VAL A:269, CYS A:321, ALA A:322) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -2.6 Kcal/mol. Dibutyl phthalate binds with transducin eye protein via hydrogen bonds (ALA A:139, SER A:140, GLE A:137), alkyl bond (PHE A:136, ALA A:277) and pi-bond (LYS A:273, LYS A:276, MET A:228), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.9 Kcal/mol. Dimethyl sulfoxide binds

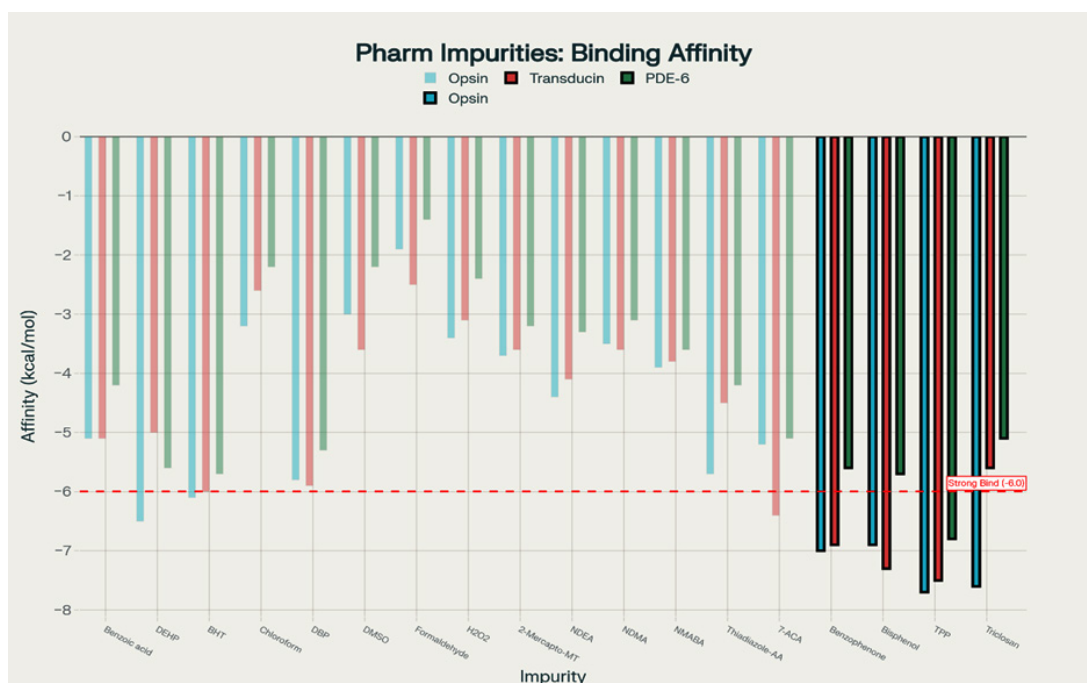


Fig. 4. Binding affinity comparison between triphenyl phosphate and visual proteins

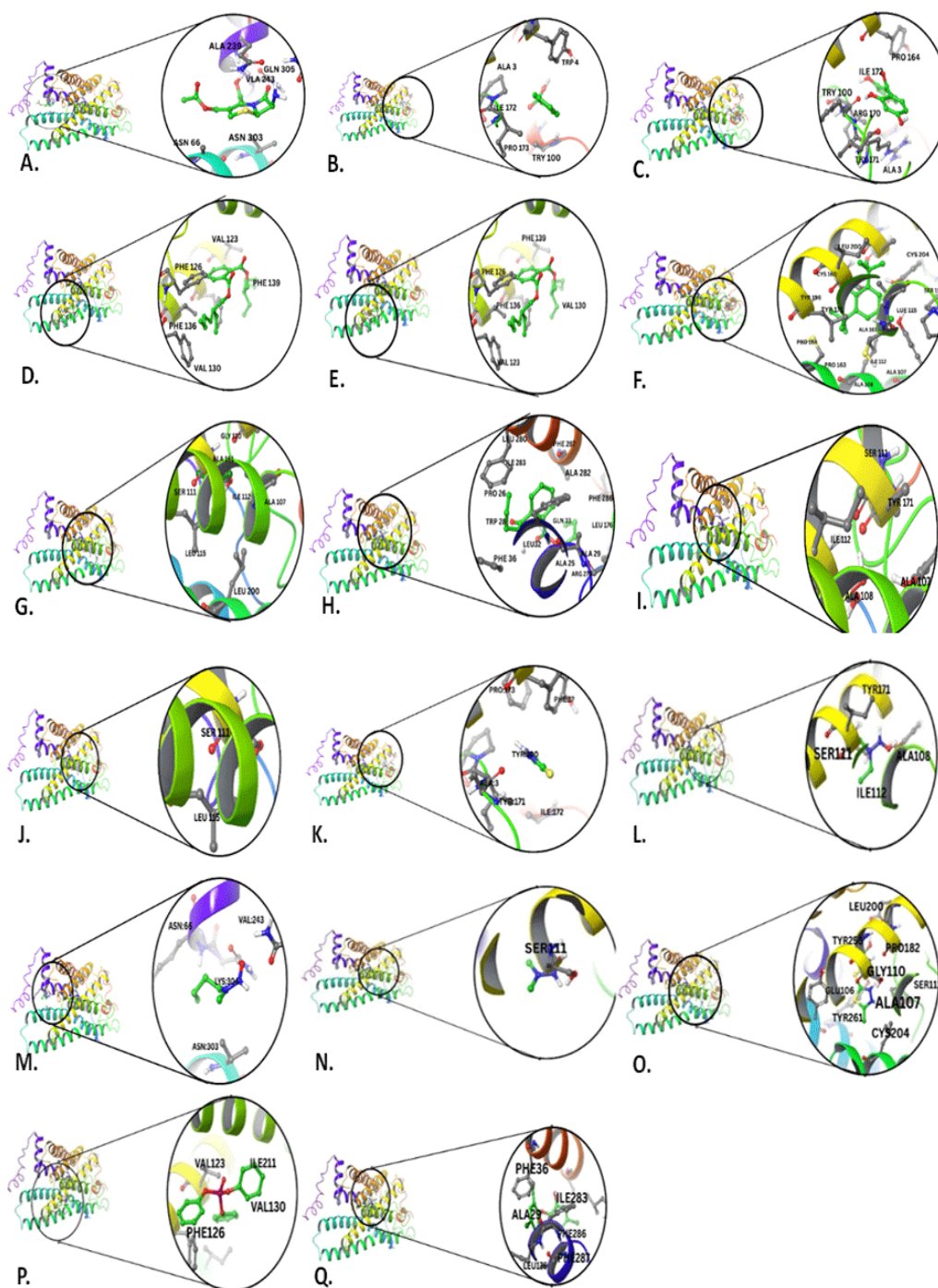


Fig. 5. 3D Interaction Models Of Opsin Protein And Impurities : (A):7-aminocephalosporonicacid, (B):benzoicacid, (C):benzophenone2, (D):diethylhexylphthalate, (E):bisphenol, (F):butylhydroxytoluene, (G):chloroform, (H):dibutylphthalate, (I):dimethylsulfoxide, (J):formaldehyde, (K):2-mercapto-5-methyl-1,3,4-thiadiazole, (L):nitrosodiethylamine, (M):nitrosomethylbutyricacid, (N):nitrosodimethylamine, (O):thiadiazoleaceticacid, (P):triphenylphosphite,(Q):triclosan

with transducin eye protein via hydrogen bonds (LYS A:42, ALA A:37), alkyl bond (no interaction) and pi-bond (ASP A:196), demonstrating diverse non-covalent interactions and exhibiting binding affinity – 3.6 Kcal/mol. Formaldehyde binds with transducin eye protein via hydrogen bonds (SER A:40, GLY A:41, LYS A:42), alkyl bond (no interaction) and pi-bond (null), demonstrating diverse non-covalent interactions and exhibiting binding affinity -2.5 Kcal/mol. 2-mercapto-5-methyl-1,3,4-thiadiazole binds with transducin eye protein via hydrogen bonds (PHE A:185), alkyl bond (ARG A:193) and pi-bond (GLN A:184, ASN A:191), demonstrating diverse non-

covalent interactions and exhibiting binding affinity – 3.6 Kcal/mol. Nitroso diethylamine binds with transducin eye protein via hydrogen bonds (ARG A:172), alkyl bond (ALA A:322, LYS A:266) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity – 4.1 Kcal/mol. Nitrosodimethylamine binds with transducin eye protein via hydrogen bonds (ARG A:174), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.6 Kcal/mol. Nitrosomethylaminobutyric acid binds with transducin eye protein via hydrogen bonds (PHE

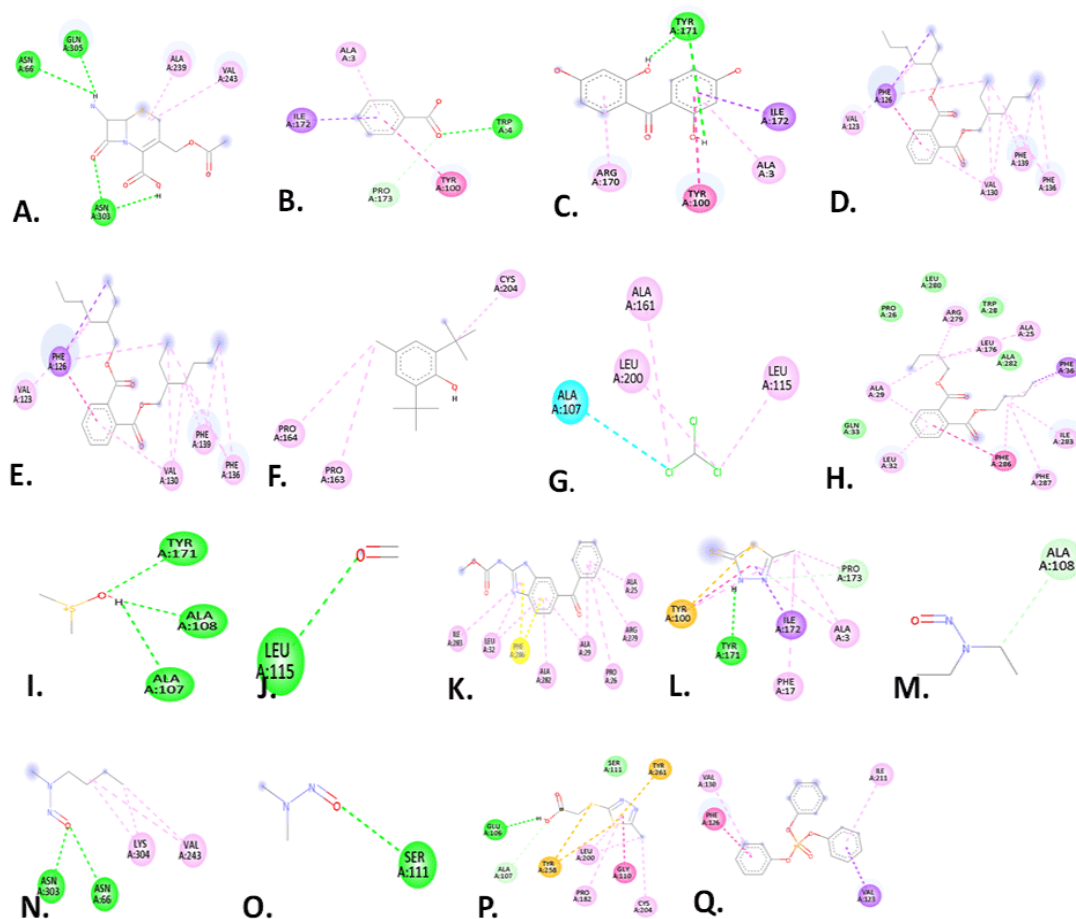


Fig. 6. (A) 2D Interaction Models Of Opsin Protein And Impurities 7-aminocephalosporonic acid, (B) benzoic acid, (C) Benzophenone2, (D) diethylhexylphthalate, (E) bisphenol, (F) butylhydroxytoluene, (G) chloroform, (H) dibutylphthalate, (I) dimethylsulfoxide, (J) formaldehyde, (K) mebendazole, (L) 2-mercapto-5-methyl-1,3,4-thiadiazole (M) nitrosodiethylamine, (N) nitrosomethyl aminobutyric acid, (O) nitrosodimethylamine, (P) thiadiazoleacetic acid (Q) triclosan

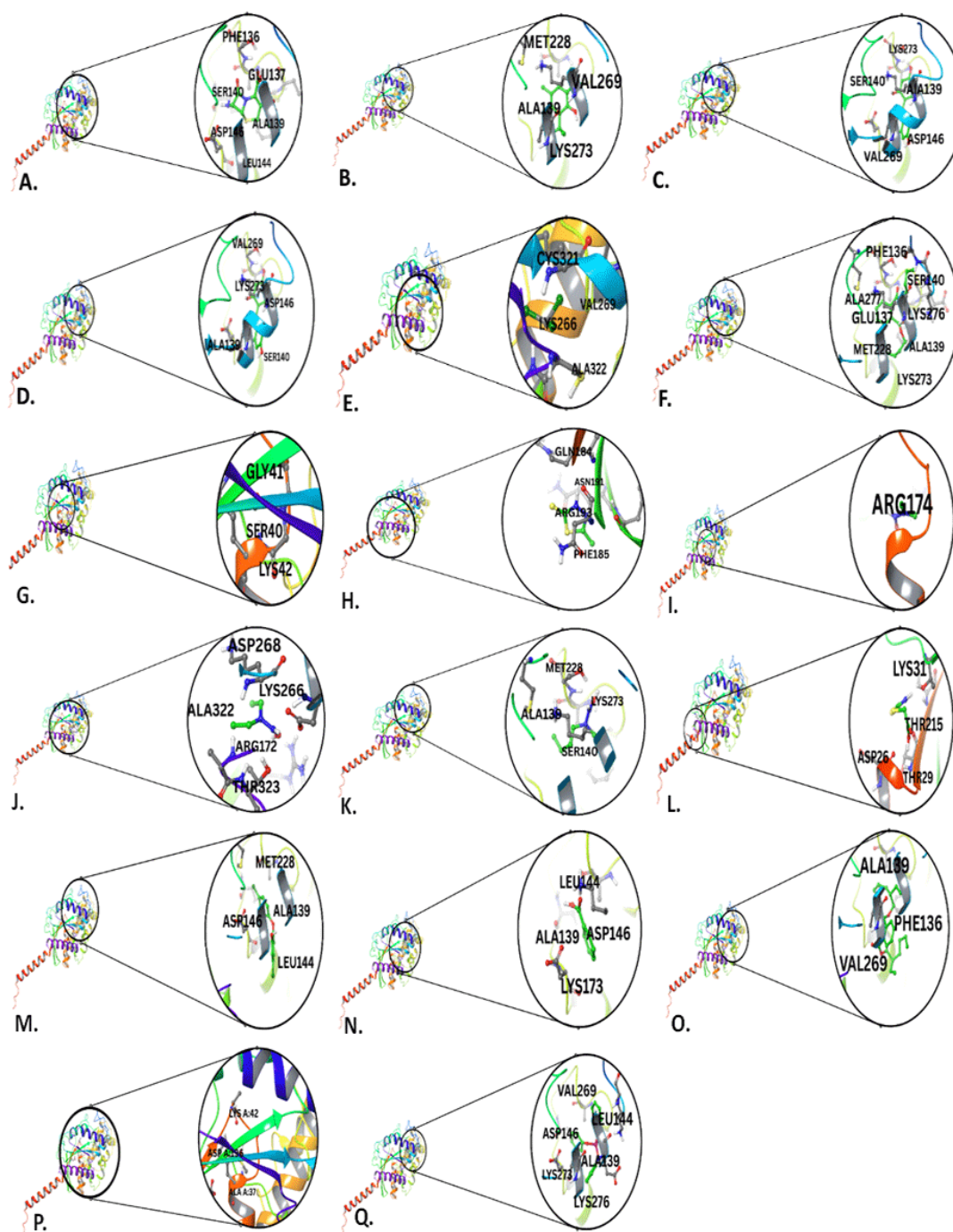


Fig. 7. 3D Interaction Models Of Transducin Protein And Impurities. (A): 7-aminocephalosporin, (B): butylhydroxytoluene, (C): benzophenone2, (D): bisphenol, (E): chloroform, (F): dibutylphthalate, (G): formaldehyde, (H): 2-mercapto-5-methyl-1,3,4-thiadiazole, (I): nitrosodimethylamine, (J): nitrosodiethylamine, (K): nitrosomethylbutyric acid, (L): thiadiazoleacetic acid, (M): triclosan (N): Benzoic Acid (O): Diethyl hexylphthalate (P): Dimethyl sulfoxide (Q): Triphenylphosphate

A:136,ALA A:139,SER A:140), alkyl bond (MET A:228,LYS A:270) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.8 Kcal/mol. Thiadiazoleaceticacid binds with transducin eye protein via hydrogen bonds (ASP A:26,LYS A:31,THR A:215), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.6 Kcal/mol. Triclosan binds with transducin eye protein via hydrogen bonds (no interaction), alkyl bond (ALA A:139 LEU A:144,MET A:228) and pi-bond (ASP A:146), demonstrating diverse non-covalent interactions and exhibiting binding affinity – 5.6 Kcal/mol. Triphenyl phosphate binds with transducin eye protein via hydrogen bonds (no interaction), alkyl

bond (LEU A:144,VAL A:269,LYS A:276) and pi-bond (LEU A:144ASP A:146,LYS A:273), demonstrating diverse non-covalent interactions and exhibiting binding affinity – 7.5 Kcal/mol.7-Aminocephalosporanicacid binds with transducin eye protein via hydrogen bonds (GLU A:137), alkyl bond (PHE A:136) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.4 Kcal/mol. (Table 5 and Fig 7,8)^{1,5,2,5}

Binding interaction between pharmaceutical impurities and phosphodiesterase-6 eye protein

Benzoic acid binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36), alkyl bond (ARG A:33,PRO A:55) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting

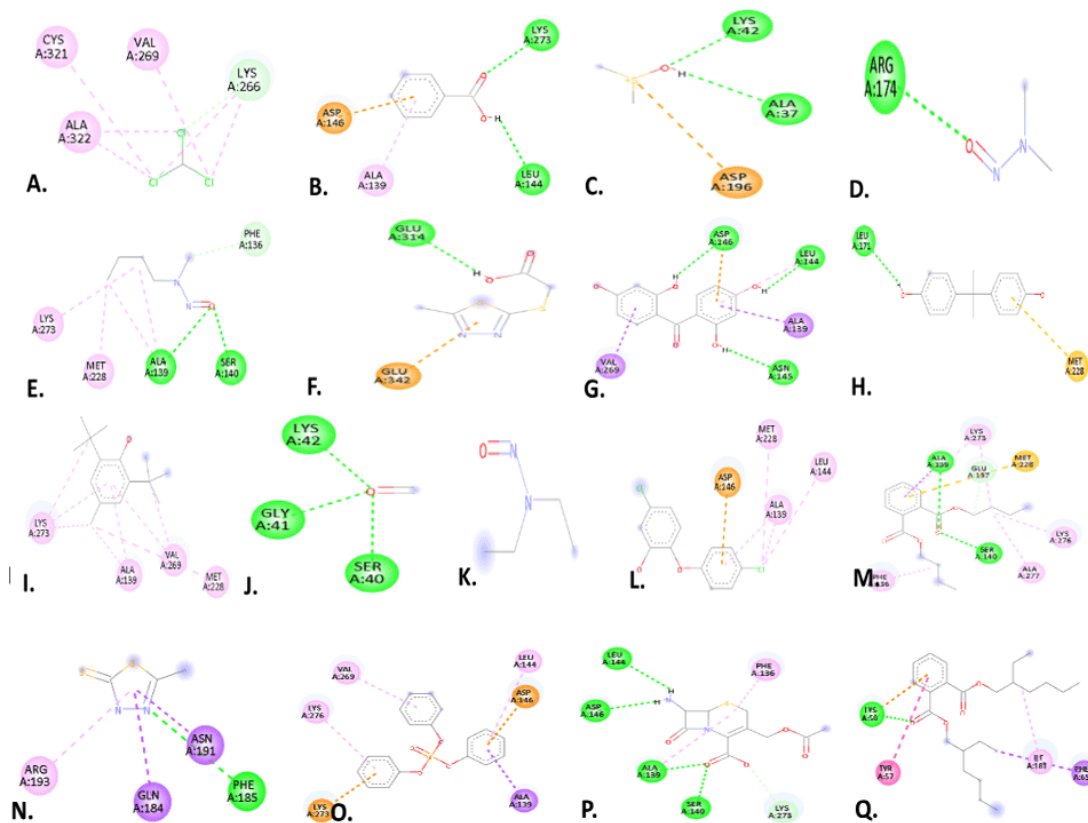


Fig. 8. 2D Interaction Models Of Transducin Protein And Impurities (A):chloroform, (B):benzoicacid, (C):dimethylsulfoxide, (D):nitrosodimethylamine, (E):nitrosomethylaminobutyricacid, (F):thiadiazoleaceticacid, (G):benzophenone, (H):bisphenol, (I):butylhydroxytoluene, (J):formaldehyde, (K):nitrosodiethylamine, (L):triclosan, (M):dibutylphthalate, (N):2-mercapto-5-methyl-1,3,4-thiadiazole, (O):triphenylphosphate, (P):7-aminocephalosporanicacid, (Q):diethylhexylphthalate

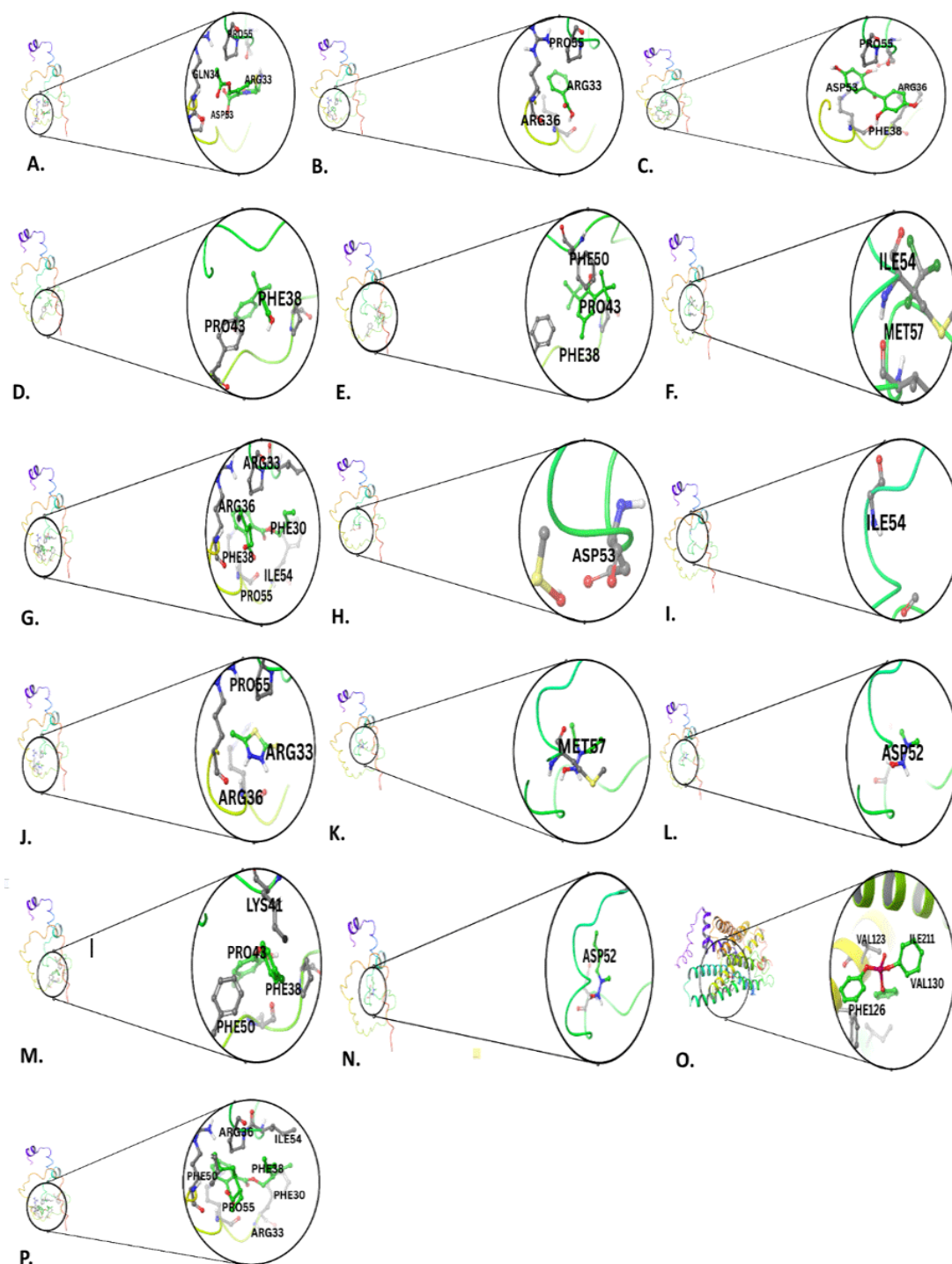


Fig. 9. 3D Interaction Models Of 6- Phosphodiesterase Protein And Impurities (A):7-aminocephalosporonicacid, (B):benzoicacid, (C)benzophenone2, (D):bisphenol, (E)butylhydroxytoluene, (F):chloroform, (G):dibutylphthalate, (H):dimethylsulfoxide, (I):formaldehyde, (J)2-mercapto-5-methyl-1,3,4-thiadiazole(K):nitrosodiehtylamine (L):nitrosodimethylamine, (M):triclosan, (N):nitrosomethylbutyricacid, (O):triphenylphosphate (P):diethyl hexyl pthalate

binding affinity -4.2 Kcal/mol. Benzophenone 2 binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36, ASP A:53), alkyl bond (PRO A:55) and pi-bond (PHE A:38), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.6 Kcal/mol. Diethylhexyl phthalate binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36), alkyl bond (PRO A:55, ILE A:54, ARG A:33) and pi-bond (PHE A:30, PHE A:38, PHE A:50), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.6 Kcal/mol. Bisphenol binds with phosphodiesterase-6 eye protein via hydrogen bonds (no interaction), alkyl bond (PRO A:43)

and pi-bond (PHE A:38), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.7 Kcal/mol. Butylhydroxytoluene binds with phosphodiesterase-6 eye protein via hydrogen bonds (no interaction), alkyl bond (PHE A:38, PRO A:43) and pi-bond (PHE A:50), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.7 Kcal/mol. Chloroform binds with phosphodiesterase-6 eye protein via hydrogen bonds (no interaction), alkyl bond (MET A:57, ILE A:54) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity 2.2 Kcal/mol. Dibutylphthalate binds with phosphodiesterase-6 eye protein via

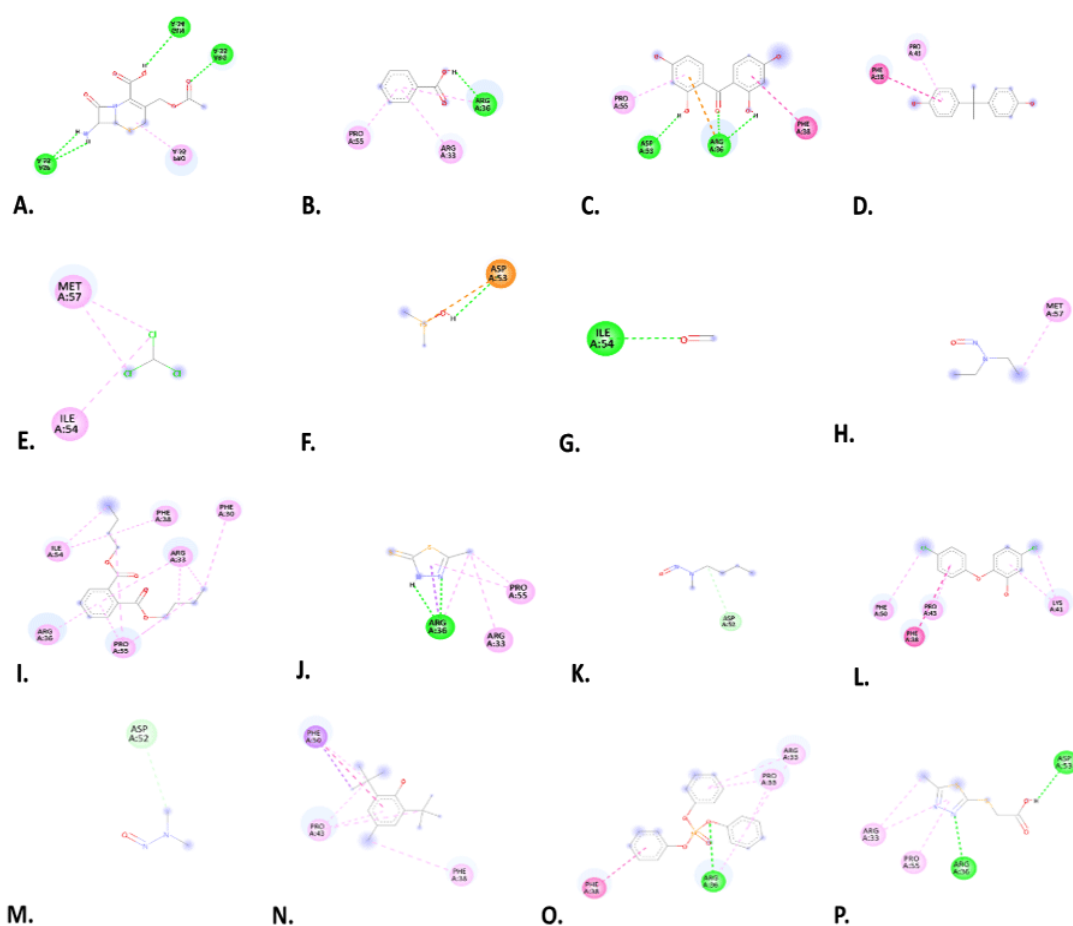


Fig. 10. 2D Interaction Models Of 6-Phosphodiesterase Protein And Impurities (A):aminocephalosporonicacid, (B):benzoicacid, (C):benzophenone2, (D):bisphenol, (E):chloroform, (F):dimethylsulfoxide, (G):formaldehyde, (H):nitrosodiethylamine, (I):dibutylphthalate, (J):2-mercapto-5-methyl-1,3,4-thiadiazole, (K):nitrosomethylbutyricacid, (L):triclosan, (M):nitrosodimethylamine, (N):butylhydroxytoluene, (O):triphenylphosphate, (P):thiadiazoleaceticacid

hydrogen bonds (no interaction), alkyl bond (PHE A:30, ARG A:33, PHE A:38 ILE A:54) and pi-bond (ARG A:36, PRO A:55), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.3 Kcal/mol. Dimethyl sulfoxide binds with phosphodiesterase-6 eye protein via hydrogen bonds (ASP A:53), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -2.2 Kcal/mol. Formaldehyde binds with phosphodiesterase-6 eye protein via hydrogen bonds (ILE A:54), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions

and exhibiting binding affinity -1.4 Kcal/mol. 2-mercapto-5-methyl-1,3,4-thiadiazole binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36), alkyl bond (ARG A:33) and pi-bond (PRO A:55), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.2 Kcal/mol. Nitrosodiethylamine binds with phosphodiesterase-6 eye protein via hydrogen bonds (no interaction), alkyl bond (MET A:57) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.3 Kcal/mol. Nitrosodimethylamine binds with phosphodiesterase-6 eye protein via hydrogen bonds (ASP A:52), alkyl bond

Table 1. Predicted Binding Sites Residues of Visual Protein

Visual eye proteins	Protein Binding sites
Opsin	GLA A:7, ASN A:48, ILE A:72, SER A:73, ALA A:75, ILE A:78, PHE A:79, SER A:83, GLU A:106, ALA A:107, MET A:109, GLY A:110, SER A:111, ILE A:112, ALA A:113, GLY A:114, ALU A:115, VAL A:116, THR A:117, LEU A:121, TRP A:154, SER A:169, TRY A:171, ILE A:172, PRO A:173, GLU A:174, CYS A:180, GLY A:181, PRO A:182, ASP A:183, TRP A:184, PYR A:185, SER A:193, PYR A:196, PHR A:197, LEU A:200, LEU A:201, CYS A:204, THE A:205, VAL A:249, MET A:250, SER A:253, THE A:254, CYS A:257, TYR A:258, ALA A:259, PRO A:260, TYR A:261, ALA A:262, THR A:264, ALA A:265, MET A:266, PHE A:268, VAL A:281, PRO A:284, ALA A:285, SER A:288, LYS A:289, SER A:291, CYS A:292, TYR A:294, ASN A:295, PRO A:296, ILE A:298
Transducin	GLY A:36, ALA A:37, GLY A:38, GLU A:39, SER A:40, GLY A:41, LYS A:42, SER A:43, THR A:44, VAL A:46, LYS A:47, LYS A:50, GLN A:54, TYR A:57, PHE A:65, ILE A:69, ASN A:72, THR A:73, GLN A:75, SER A:76, ALA A:79, ARG A:82, ALA A:83, THR A:86, PHE A:136, GLU A:137, ARG A:138, ALA A:139, SER A:140, GLU A:141, TYR A:142, GLN A:143, LEU A:144, ASN A:145, ASP A:146, SER A:147, ALA A:148, GLY A:149, TYR A:151, LEU A:152, LEU A:171, ARG A:172, SER A:173, ARG A:174, VAL A:175, LYS A:176, THR A:177, ILE A:181, THR A:183, ASP A:196, VAL A:197, GLY A:198, GLY A:199, GLN A:200, ARG A:201, ARG A:204, PHE A:219, ILE A:220, ALA A:221, ALA A:222, SER A:224, ALA A:225, ASP A:227, MET A:228, VAL A:229, LEU A:230, VAL A:231, GLU A:232, ARG A:238, MET A:239, GLU A:241, SER A:242, LEU A:245, ASN A:265, LYS A:266, ASP A:268, VAL A:269, GLU A:272, LYS A:273, LYS A:276, ALA A:277, PHE A:303, CYS A:321, ALA A:322, THR A:323,
Phosphodiesterase-6	PHE A:30, LYS A:31, GLN A:32, ARG A:33, GLN A:34, THR A:35, ARG A:36, GLN A:37, PHE A:38, LYS A:39, SER A:40, LYS A:41, PRO A:42, PRO A:43, LYS A:44, ILE A:47, GLY A:49, PHE A:50, GLY A:51, ASP A:52, ASP A:53, ILE A:54, PRO A:55, MET A:57, GLU A:58, GLY A:59, LEU A:60, GLY A:61, ILE A:64, THR A:65

(no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -3.1 Kcal/mol. Nitrosomethylaminobutyric acid with phosphodiesterase-6 eye protein via hydrogen bonds (ASP A:52), alkyl bond (no interaction) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -4.2 Kcal/mol. Thiadiazoleacetic acid binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36, ASP A:53), alkyl bond (ARG A:33) and pi-bond (PRO A:55), demonstrating diverse non-covalent interactions and exhibiting binding affinity -4.2 Kcal/mol. Triclosan binds with phosphodiesterase-6 eye protein via hydrogen bonds (no interaction), alkyl bond (PRO A:43, PHE A:50) and pi-bond (PHE A:38, LYS A:41), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.1 Kcal/mol. Triphenyl phosphate binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:36), alkyl bond (no interaction) and pi-bond (ARG A:33, PRO A:55, PHE A:38), demonstrating diverse non-covalent interactions and exhibiting binding affinity -6.8 Kcal/mol. 7-Amino cephalosporanic

acid binds with phosphodiesterase-6 eye protein via hydrogen bonds (ARG A:33, GLN A:34, ASP A:53), alkyl bond (PRO A:55) and pi-bond (no interaction), demonstrating diverse non-covalent interactions and exhibiting binding affinity -5.1 Kcal/mol (Table 6 and Fig 9,10)^{1,5,2,5}

DISCUSSION

This study explored how pharmaceutical impurities, chemical byproducts or contaminants in drug formulations can interact with critical visual proteins such as opsin, transducin, and phosphodiesterase-6. These proteins are essential for the phototransduction pathway, the process by which light signals are converted into electrical signals in the eye. These findings suggest that several impurities can strongly bind to these proteins, potentially disrupting their normal functions and posing risks to visual health.^{2,3} Among the tested impurities, triphenyl phosphate exhibited the strongest binding affinity for all three proteins, with binding energies of -7.7 kcal/mol (opsin), -7.5 kcal/mol (transducin) and -6.8 kcal/mol (phosphodiesterase). Other impurities, such as triclosan, benzophenone-2, and bisphenol, also

Table 2. Pharmaceutical Impurities and their Binding Affinity with Visual Eye Protein

Pharmaceutical Impurities	Binding affinity of Opsin (Pdb Id:3CAP) Kcal/mol	Binding affinity of Transducin (Pdb Id:60Y9) Kcal/mol	Binding affinity of 6-Phosphodiesterase (Pdb Id :4F1H) Kcal/mol
Benzoic acid	-5.1	-5.1	-4.2
Benzophenone-2	-7	-6.9	-5.6
Diethylhexylphthalate	-6.5	-5	-5.6
Bisphenol	-6.9	-7.3	-5.7
Butylhydroxytoluene	-6.1	-6	-5.7
Chloroform	-3.2	-2.6	-2.2
Dibutylphthalate	-5.8	-5.9	-5.3
Dimethylsulfoxide	-3	-3.6	-2.2
Formaldehyde	-1.9	-2.5	-1.4
Hydrogen peroxide	-3.4	-3.1	-2.4
Triphenylphosphate	-7.7	-7.5	-6.8
2-Mercapto-5-methyl-1,3,4-thiadiazole	-3.7	-3.6	-3.2
Nitrosodiethylamine	-4.4	-4.1	-3.3
Nitrosodimethylamine	-3.5	-3.6	-3.1
Nitrosomethylaminobutyric acid	-3.9	-3.8	-3.6
Thiadiazoleacetic acid	-5.7	-4.5	-4.2
Triclosan	-7.6	-5.6	-5.1
7-aminocephalosporinic acid	-5.2	-6.4	-5.1

Table 3. Opsin and Pharmaceutical Impurities interactions and their type of bond interaction

S. No	Pharmaceutical Impurities	H-bond	Interaction Alkyl bond	Pi - bond
1	Benzoicacid	TRP A:4,PRO A:173	ALA A:3	TRY A:100, ILE A:172
2	Benzophenone2	TRY A:171	ALA A:3,ARG A:170	TYR A:100, ILE A:172
3	Diethylhexylphthalate	No interaction	VAL A:130,PHE A:139,PHE A:136,VAL A:123	PHE A:126
4	Bisphenol	No interaction	VAL A:130,PHE A:139,PHE A:136,VAL A:123	PHE A:126
5	Butylhydroxytoluene	No interaction	PRO A:164,PRO A:163,CYS A:204	No interaction
6	Chloroform	No interaction	ALA A:161,LEU A:115,LEU A:200	No interaction
7	Dibutylphthalate	No interaction	ALA A:25,ALA A:29,LEU A:32,LEU A:176, ARG A:279,ILE A:283,PHE A:287	PHE A:36,PHE A:286
8	Dimethylsulfoxide	ALA A:107,ALA A:108, TYR A:171	No interaction	No interaction
9	Formaldehyde	LEU A:115	No interaction	No interaction
12	2-Mercapto-5-methyl-1,3,4-thiadiazole	TYR A:171,PEO A:173	ALA A:3,PHE A:17	TYR A:100, ILE A:172
13	Nitrosodiethylamine	ALA A:108	No interaction	No interaction
14	Nitrosodimethylamine	SER A:111	No interaction	No interaction
15	Nitrosomethylaminobutyricacid	ASN A:303,ASN A:66	LYS A:304,VAL A:243	No interaction
16	Thiadiazoleaceticacid	GLU A:106,SER A:111,ALA A:107	LEU A:200,PRO A:182,GLY A:110,CTS A:204	TYR A:258, TYR A:261
17	Triclosan	No interaction	LEU A:176,ALA A:29,ILE A:283,PHE A:287	PHE A:286,PHE A:36
18	Triphenylphosphate	No interaction	VAL A:130,ILE A:211	PHE A:126,VAL A:123
19	7-aminocephalosporinicacid	ASN A:66,ASN A:303 GLN A:305	ALA A:239,VAL A:243	No interaction

Table 4. Transducin eye protein and Pharmaceutical Impurities interactions and their type of bond interaction

S. No	Pharmaceutical Impurities	H-Bond	Interactions Alkyl-I-Bond	PI-Bond
1	Benzoic acid	LEU A:144,LYS A:273	ALA A:139	ASP A:146
2	Benzophenone2	SER A:140,ASP A:146	ALA A:139	VAL A:269
3	Diethylhexylphthalate	No interaction	PHE A:136,ALA A:139,	VAL A:269
4	Bisphenol	LEU A:144,ASP A:227	LYS A:273,LYS A:276	ALA A:139
5	Butylhydroxytoluene	No interaction	ALA A:139, MET A:228,	No interaction
			VAL A:269,LYS A:273	
6	Chloroform	LYS A:266	ALA A:322,VAL A:269,	No interaction
			CYS A:321,ALA A:322	
7	Dibutylphthalate	ALA A:139,SER A:140,GLE A:137	PHE A:136,ALA A:277	LYS A:273,LYS A:276,MET A:228
8	Dimethylsulfoxide	LYS A:42,ALA A:37	No interaction	ASP A:196
9	Formaldehyde	SER A:40,GLY A:41,LYS A:42	No interaction	NULL
12	2-Mercapto-5-methyl-1,3,4-thiadiazole	PHE A:185	ARG A:193	GLN A:184,ASN A:191
13	Nitrosodiethylamine	ARG A:172	ALA A:322,LYS A:266	No interaction
14	Nitrosodimethylamine	ARG A:174	No interaction	No interaction
15	Nitrosomethylaminobutyric acid	PHE A:136,ALA A:139,SER A:140	MET A:228,LYS A:273	No interaction
16	Thiadiazoleacetic acid	ASP A:26, LYS A:31,THR A:215	No interaction	No interaction
17	Triclosan	No interaction	ALA A:139,LEU A:144,MET A:228	ASP A:146
18	Triphenylphosphate	No interaction	LEU A:144,VAL A:269, LYS A:276	LEU A:144, ASP A:146,LYS A:273
19	7-aminocephalosporinic acid	GLU A:137	PHE A:136	No interaction

Table 5. Phosphodiesterase-6 eye protein and Pharmaceutical Impurities interactions and their type of bond interaction

SN	Pharmaceutical Impurities	-Bond	Interactions Alkyl-Bond	PI-Bond
1	Benzoicacid	ARG A:36	ARG A:33, PRO A:55	No interaction
2	Benzophenone2	ARG A:36,ASP A:53	PRO A:55	PHE A:38
3	Diethylhexylphthalate	ARG A:36	PRO A:55,ILE A:54,ARG A:33	PHE A:30,PHE A:38,PHE A:50
4	Bisphenol	No interaction	PRO A:43	PHE A:38
5	Butylhydroxytoluene	No interaction	PHE A:38,PRO A:43	PHE A:50
6	Chloroform	No interaction	MET A:57,ILE A:54	No interaction
7	Dibutylphthalate	No interaction	PHE A:30,ARG A:33,PHE A:38,ILE A:54	ARG A:36, PRO A:55
8	Dimethylsulfoxide	ASP A:53	No interaction	No interaction
9	Formaldehyde	ILE A:54	No interaction	No interaction
12	2-Mercapto-5-methyl -1,3,4-thiadiazole	ARG A:36	ARG A:33	PRO A:55
13	Nitrosodiethylamine	No interaction	MET A:57	No interaction
14	Nitrosodimethylamine	ASP A:52	No interaction	No interaction
15	Nitrosomethylaminobutyricacid	ASP A:52	No interaction	No interaction
16	Thiadiazoleaceticacid	ARG A:36,ASP A:53	ARG A:33	PRO A:55
17	Triclosan	No interaction	PRO A:43,PHE A:50	PHE A:38,LYS A:41
18	Triphenylphosphate	ARG A:36	No interaction	ARG A:33,PRO A:55,PHE A:38
19	7-aminocephalosporinicacid	ARG A:33,GLN A:34,ASP A:53	PRO A:55	No interaction

presented moderate to high affinities, indicating a possible competitive or inhibitory effect on normal ligand binding and physiological function.^{2,10,7,2,4}

These interactions are supported by stabilizing hydrogen bonds, alkyl bonds, and pi-bond networks, emphasizing the likelihood of structural and functional protein perturbation by these impurities. Strong binding of pharmaceutical impurities to visual proteins may interfere with phototransduction, the crucial process underpinning visual signalling. The predicted interaction sites correspond to known active or binding regions of the proteins, supporting possible disruptive biochemical consequences. The detailed molecular interactions observed can affect protein folding and function, thereby increasing the risk of drug-induced ocular toxicity. This study emphasizes the importance of impurity profiling and toxicological assessment in the drug development process to prevent unintended visual side effects in clinical or preclinical settings.^{1,3,8}

Docking-based computational methods provide a rapid *in silico* approach to predict possible harmful interactions, supporting regulatory and quality control efforts. Incorporating this workflow can improve early screening for ocular safety risks associated with pharmaceutical impurities, potentially reducing later-stage toxicological complications. The results are based solely on *in silico* docking studies and require experimental validation through biochemical or *in vivo* studies to confirm the real biological impact. The selection of protein structure, docking parameters, and software-induced biases may influence binding predictions; thus, cross-platform validation is suggested. Further integration with molecular dynamics and toxicity assays would provide deeper mechanistic and safety insights.^{1,5,2,5}

CONCLUSION

This study successfully applied molecular docking to evaluate the binding interactions of selected pharmaceutical impurities with key visual proteins, namely, opsin, transducin, and phosphodiesterase-6, in zebrafish. These results indicate that several impurities, such as triphenyl phosphate, have significant binding affinities for these proteins, which is supported by hydrogen bonds and hydrophobic interactions. These strong

interactions suggest the potential for impurities to disrupt protein function, which may impair visual signalling and phototransduction processes, which are critical for normal vision. However, this study is limited by its *in silico* experimental validation through biochemical assays, and *in vivo* models are needed to confirm the physiological relevance of these interactions.

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This research does not involve any clinical trials.

Author contributions

Pasala Praveen Kumar: Design; Rolla Kondaraju Gari Raghavendra Darshan: Performed the project, Docking, Writing; Shaik Muskan, Siddamurthi Samba Siva Reddy and Darla Preethi: Collected data related to the project; Rolla Kondaraju Gari Raghavendra Darshan and Kamini Sai Kumari: Docking; Shaik Muskan, Siddamurthi Samba Siva Reddy and Darla Preethi: Visualization; Rolla Kondaraju Gari Raghavendra Darshan: Wrote the paper; Ghanagiri

venkataramanappa gar parameswar: Gives tips during the project.

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