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Insilico Insights into Resveratrol as a Potential Inhibitor of Mycobacterium Tuberculosis Enoyl-ACP Reductase (InhA) Protein

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ABSTRACT

Mycobacterium tuberculosis, the causative agent of tuberculosis, is a global cause of death. Thus, the development of innovative treatment strategies is required. **Objective:** To develop in-silico drugs by phytochemicals to inhibit the Enoyl-ACP reductase (InhA) protein, which is essential for synthesizing mycobacterial cell walls. **Methods:** The 3D structure of InhA was taken from the Protein Data Bank. The Ramachandran plot validated the model with a score of 98.7% from the favoured Ramachandran plot. Computed Atlas of Surface Topography of Proteins was used to detect the active sites for ligand interaction. Resveratrol were selected based on existing studies and further listed for drug-likeness. Absorption, Distribution, Metabolism, Excretion, and Toxicity analysis showed the possibility of resveratrol as a drug candidate, with no violation of Lipinski rules and excellent absorption in the Gastrointestinal Tract. **Results:** The boiled egg model confirmed the ability of ligands to go through the blood-brain barrier. Toxicity predictions of resveratrol indicated low risks with several other systems of organs. Molecular docking with CB-Dock2 showed the strong binding of Resveratrol to InhA, with a Vina score equal to -8.8 kcal/mol. Further exploration of the docking complex by molecular docking simulation using the Integrated Management of the Public Distribution System was carried out, and the trajectory confirmed stable interaction and protein flexibility. **Conclusions:** It was concluded that resveratrol acts as a potent, non-toxic candidate for tuberculosis treatment and highlights its inhibition capacity of InhA. Results need future vitro and in vivo validation to develop this highly reliable therapeutic alternative for combating tuberculosis.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis that most often affects the lungs. It spreads through the air when an active TB infectious patient coughs, sneezes, or spits, and someone else inhales that air containing TB bacteria [1]. About 10 million people are infected due to Mycobacterium tuberculosis, and 5-10% of people will eventually get symptoms and develop TB disease [2]. While some tuberculosis patients struggle with treatment adherence, the implementation of Directly Observed Therapy (DOT) programs has been a significant success. These programs ensure compliance through supervised doses, either in clinics or communities, and have proven cost-effective in reducing tuberculosis cases [3]. The emergence of drug-resistant tuberculosis strains underscores the need for advancements in treatment research [4]. Promising avenues include fluoroquinolones and alternative pharmaceutical categories such as oxazolidinones (like linezolid). Additionally, investigations into immune system-targeted therapies, such as enhancing Bacillus Calmette–Guérin (BCG) or Mycobacterium vaccae vaccines, and sterilization via the citrate lyase pathway, show promise [5]. InhA, also known as Enoyl-ACP reductase, is involved in the fatty acid synthesis pathway of

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Mycobacterium tuberculosis, the causative agent of tuberculosis. This enzyme reduces enoyl-ACP to acyl-ACP, an essential step in elongating fatty acids required for mycolic acid biosynthesis [6]. Mycolic acids are long-chain fatty acids incorporated into the mycobacterial cell wall structure and provide the cell wall with its hydrophobic nature and resistance to most antibiotics [7]. The repression of InhA prevents the synthesis of mycolic acids and weakens the bacteria's cell wall, resulting in cell death. As a result, InhA is an attractive target for antitubercular agents, including isoniazid, which acts by blocking this enzyme to fight Mycobacterium tuberculosis infection [8]. Resveratrol is a phenolic compound in the stilbene family of bioactive agents identified primarily in grapes, berries, peanuts, and red wine. This, in turn, has generated much interest because of the interconnected multiple health uses and a highly effective antibacterial nature. Based on the information mentioned above, it can be concluded that Resveratrol acts against various bacteria, viruses, and fungi by affecting their cell walls and restricting their ability to synthesize their genetic material and essential metabolites [9]. This substance can also suppress the immune response and decrease inflammation, contributing to its excellent antimicrobial activity, thus pointing towards the need to use it in developing drugs for treating various infections. This distribution, combined with a broad-spectrum antimicrobial characteristic, stresses the possible use of Resveratrol in prevention and treatment [10]. The main objective of this study is to successfully design a phytochemical-based drug, employing computational approaches for treating Tuberculosis caused by Mycobacterium tuberculosis. The method used, namely silicon drug designing, is a relatively well-known method used for analyzing biological components with the help of computers. As a result, we gain suitable drug candidates and similar structural compounds that are promising and relatively economical compared to traditional drug designing methods. This study includes the analysis of virulent proteins produced by the mycobacterium and employs phytochemicals to help degrade the proteins. With the help of computational methods, current work was to analyze the drug interactions within the body through simulation, cutting down the time and resources required to produce successful drug candidates.

This study aims to construct befitting therapeutic molecules for treating Tuberculosis, simultaneously testing any toxic or otherwise adverse effects the drug may have.

METHODS

The tertiary structure for InhA (Enoyl-ACP reductase) was obtained from Research Collaboratory for Structural Bioinformatics (RSCB) Protein Data Bank (PDB ID: 2NSD), where 3D structures of various proteins, found experimentally through X-ray crystallography, are submitted [11]. The tertiary structure of Enoyl-ACP reductase (also known as Enoyl-acyl carrier protein reductase) retrieved through the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RSCB-PDB) was validated through PROCHECK Ramachandran Plot [12]. PROCHECK predicts the stereochemical quality of a protein by analyzing the torsion angle distribution of various protein residues on a 2D plane. The binding sites of the Enoyl-ACP reductase protein were identified from the Computed Atlas of Surface Topography of Proteins (CASTp). These binding sites are the protein's active site where ligands will interact with the protein [13]. Resveratrol from grapes (Vitis vinifera) was chosen based on its antimicrobial properties based on existing literature. The Signed Distance Fields (SDF) formatted 3D models were downloaded from PubChem [14]. Pharmacokinetic properties consist of ADMET, which is an acronym for Absorption, Distribution, Metabolism, Excretion, and Toxicity, meaning how the body absorbs the drug, how it is distributed across the body systems, what metabolic reactions become part of, after how long it is excreted from the body and if it imposes any toxic effects on the body. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) analysis monitors the properties of any ligand selected as a drug candidate. The ADME were analyzed using the Swiss ADME tool [15], whereas the toxicity analysis was conducted using the Pro Tox II Server [16]. Docking is the interaction study between the ligand and the target protein; CB-Dock2 was used to analyze the docking patterns of Resveratrol with the target protein [17]. The results from CB-Dock2 provide five cavities by default and docking possibilities in each. To simulate the molecular docking interactions between ligand and protein, Integrated Management of Public Distribution System (iMODS) software was operated with default parameters for the most appropriate dock complex selected [18].

RESULTS

This method predicts a protein's structure by comparing it to a previously known structure. These known structures are frequently determined using techniques like X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and cryo-electron microscopy. Protein 3D structure is downloaded in the Protein Data Bank (PDB) file and after that Discovery Studio tool was employed for protein purification to remove water molecules and ligands attached to the protein to avoid any hindrance for further analysis. The Ramachandran Plot showed a quality score, with most favoured Rama score of 98.7 including both the favourable and most favourable regions. In the

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Ramachandran Plot, the red colour represents the most favoured region, while the yellow colour indicates the favored region. The majority of protein residues fall within the red region, suggesting that the protein structure has high quality. The 3D structure of InhA protein taken from PDB is seen in Figure 1a. The Expasy ProtParam Tool helps analyze numerous characteristics depicting the physical and chemical properties of InhA. Physiochemical properties showed that protein is stable as its instability index is below 40, if this value was above 40 then protein would be unstable. The aliphatic index showed how many aliphatic amino acids are present in the protein primary sequence and its predicted value is 99.85. The Grand Average of Hydropathicity (GRAVY) indicates that the protein is hydrophobic, as its value is 0.152, if the GRAVY value were negative, it would suggest that the protein is hydrophilic. The Ramachandran Plot is seen in figure 1b.

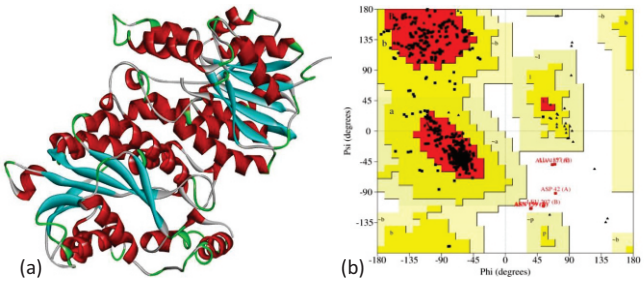

Figure 1: (a) 3D structure of InhA Protein, (b) Ramachandran of Plot Protein Validation

Details extracted from the ProtParam results page are shown in table 1.

Table 1: Physiochemical Properties of InhA

Physiochemical PropertiesInhA Protein	InhA Protein
No. of Amino Acids	269
Molecular Weight	28527.84
Theoretical pI	5.73
Total No. of Negatively Charged Residues	26
Total No. of Positively Charged Residues	22
Formula	$C_{1269}H_{2029}N_{349}O_{375}S_{11}$
Total No. of Atoms	4033
Extinction Coefficient	30940 M^{-1} cm^{-1}
Estimated Half-life	30 hours (mammalian reticulocytes, in vitro) >20 hours (yeast, in vivo) >10 hours (Escherichia coli)
Instability Index	39.29 (Stable)

STRING results provide protein-protein interactions based on which gene ontology functions are determined Each of the nodes (circles) is coloured which means the particular protein of interest (InhA) has a first shell or level of interaction with all the other proteins. InhA interacts with 10 different proteins by being involved in various biological processes such as fatty acid elongation, mycolic acid biosynthesis process, fatty acid biosynthesis process and response to antibiotics and these results are shown in figure 2.

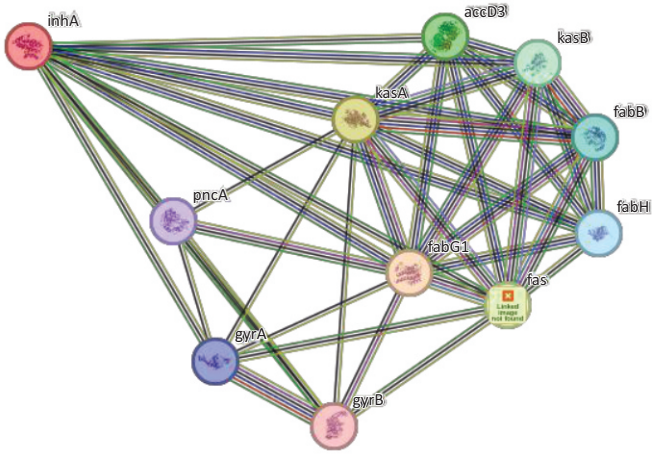

Figure 2: STRING Analysis of the Protein InhA (Enoyl-ACP Reductase)

In the case of InhA, Richards' and Connolly's surfaces are visibly marked red where the ligands can interact with the protein pockets. The residues in pocket are as follows:SER13, GLY14, ILE15, ILE16, THR17, SER19, SER20, ILE21, ALA 22, THR39, GLY40, PHE41, ASP42, ARG43, ILE47, LEU63, ASP64, VAL65, GLN66, HIS93, SER94, ILE95, GLY96, PHE97, MET98, PRO99, GLN100, MET103, GLY104, LYS118, ILE122, SER123, MET147, ASP148, PHE149, MET155, PRO156, ALA157, TYR158, MET161, LYS165, LEU168, ALA191, GLY192, PRO193, ILE194, THR196, LEU197, ALA198, MET199, ALA201, ILE202, VAL203,GLY205, ALA206, LEU207, ILE215, LEU218, GLU219, TRP222, MET232. Binding site identification results are indicated in figure 3.

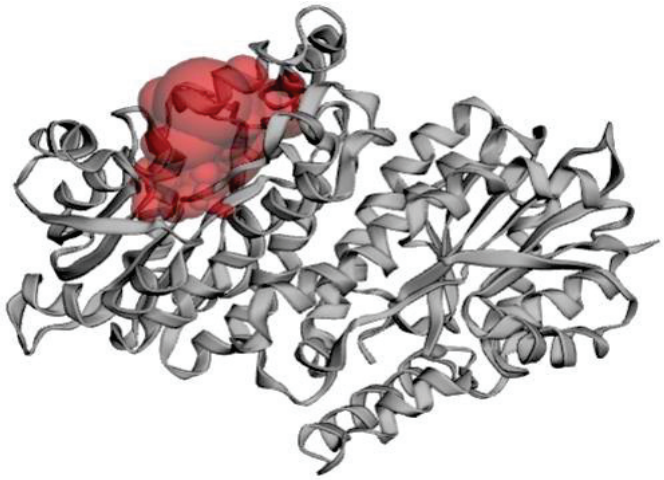

Figure 3: Active Site Identification by Castp. Highlighted Regions in Red Are the Active Sites

Resveratrol had the best Vina Score, -8.8 kcal/mol. The docking interaction of top ligand Resveratrol with targeted protein is represented in Figure 4a. A 2D visualization of docking is shown in figure 4b.

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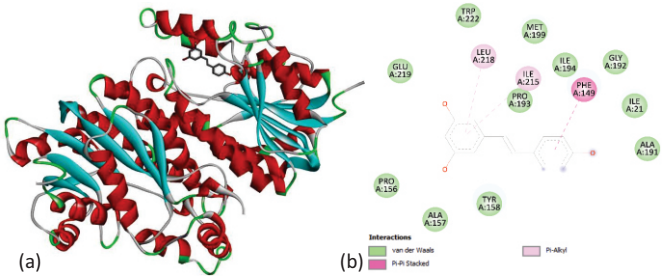

Figure 4: (a) 3D Visualization of Docking Interaction of Top Ligand Resveratrol with InhA, and (b) 3D Visualization of Docking Interaction

Various physiochemical parameters including GI absorption, bioavailability score and water solubility was analyzed using ADMET. No violation of Lipinski's rule was demonstrated by Resveratrol. Four ligands, e.g., epigallocatechin gallate (ECG), curcumin, berberine, and resveratrol, were selected and further listed for their drug-likeness. ADMET analysis showed the possibility of resveratrol as a drug candidate, with no violation of Lipinski rules and excellent absorption in the Gastrointestinal Tract. The list of physiochemical parameters for Resveratrol is seen in table 2.

Table 2: ADMET Properties for Resveratrol

Category	Property	Value
Physicochemical Properties	Formula	C14H12O3
	Molecular weight	228.24 g/mol
	Num. heavy atoms	17
	Num. arom. heavy atoms	12
	Fraction Csp3	0.00
	Num. rotatable bonds	2
	Num. H-bond acceptors	3
	Num. H-bond donors	3
	Molar Refractivity	67.88
	TPSA	60.69 Å^2
Lipophilicity	Log Po/w (iLOGP)	1.71
	Log Po/w (XLOGP3)	3.13
	Log Po/w (WLOGP)	2.76
	Log Po/w (MLOGP)	2.26
	Log Po/w (SILICOS-IT)	2.57
	Consensus Log Po/w	2.48
Water Solubility	Log S (ESOL)	-3.62
	Solubility (ESOL)	5.51e-02 mg/ml ; 2.41e-04 mol/l
	Class (ESOL)	Soluble
	Log S (Ali)	-4.07
	Solubility (Ali)	1.93e-02 mg/ml ; 8.44e-05 mol/l
	Class (Ali)	Moderately soluble
	Log S (SILICOS-IT)	-3.29
	Solubility (SILICOS-IT)	1.18e-01 mg/ml ; 5.16e-04 mol/l
	Class (SILICOS-IT)	Soluble
	GI absorption	High
	BBB permeant	Yes
Pharmacokinetics	P-gp substrate	No
	CYP1A2 inhibitor	Yes
	CYP2C19 inhibitor	No
	CYP2C9 inhibitor	Yes
	CYP2D6 inhibitor	No
	CYP3A4 inhibitor	Yes
	Log Kp (skin permeation)	-5.47 cm/s
Drug likeness	Lipinski	Yes; 0 violation
	Ghose	Yes
	Veber	Yes
	Egan	Yes
	Muegge	Yes
	Bioavailability Score	0.55
Medicinal Chemistry	PAINS	0 alert
	Brenk	1 alert: stilbene
	Leadlikeness	No; 1 violation: MW<250
	Synthetic accessibility	2.02
Toxicity	Hepatotoxicity	Inactive
	Neurotoxicity	Inactive
	Nephrotoxicity	Active
	Respiratory toxicity	Inactive
	Cardiotoxicity	Active
	Carcinogenicity	Inactive
	Immunotoxicity	Inactive
	Mutagenicity	Inactive
	Cytotoxicity	Inactive
	BBB-barrier	Inactive
	Ecotoxicity	Inactive
	Clinical toxicity	Inactive
	Nutritional toxicity	Inactive

Figure 5a shows that the lower modes are associated with lower eigenvalues suggesting that; they are easier to deform than higher modes that are associated with higher eigenvalues and therefore need more energy to deform. This is a general behaviour and implies that the results obtained from the simulation are in line with the expected physical response. Higher modes (Mode 1 and Mode 2) have higher variances as indicated in Figure 5b. Also, the total variance rises gradually with the number of modes, indicating the gradual addition of motion. These trends indicate the simulation is valid and by the existing theories. Figure 5c indicates the deformability of atoms in a simulation. The peaks indicate varying levels of deformability among the atoms, with some atoms showing high deformability close to 1, and others much lower, closer to 0. This variability suggests differences in the flexibility or structural properties of the atoms within the simulated molecular system. Figure 5d indicates the B-factor value, the red line representing the Normal Mode Analysis (NMA) closely follows the trend of the grey bars from the Protein Data Bank (PDB) data, indicating that the simulation accurately captures the atomic mobility compared to the experimental data. This alignment suggests that the

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simulation results are reliable. Figure 5e represents an elastic network model, showing which pairs of atoms are connected by springs. The dots indicate the stiffness of these springs, with darker greys representing stiffer springs. Based on the heat map, the results appear to be good. Figure 5f shows the strong red diagonal line indicates consistent and strong interactions between residues, which is generally a positive sign in protein simulations. This suggests that the simulation has captured the expected residue-residue interactions accurately.

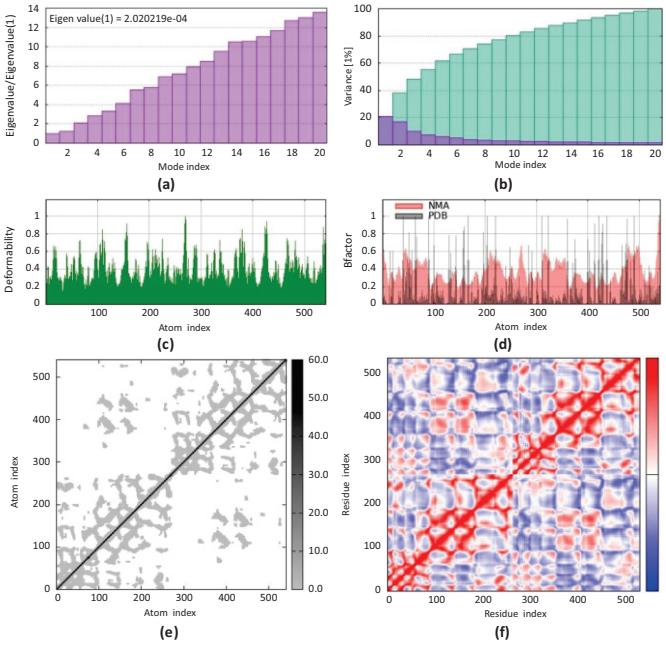

Figure 5: Results of MD simulation by iMODS. (a) Eigenvalues representing stiffness of the protein, (b) Variance of the protein structure, (c) Graphs represent deformability, (d) Graphs representing B-factor, (e) Elastic network also provides information on protein strength and stiffness, and (f) Covariance depicts the motion of pairs and groups of residues.

DISCUSSION

The computational method applied in this research was quite helpful in identifying resveratrol as a possible drug for the treatment of TB. The high score from the Ramachandran plot that was used to analyze the quality of the structural model of the target protein InhA proves that the method used to model the protein's three-dimensional structure is accurate. It is crucial to validate the structure because it means that the interactions that will be studied will be grounded in a proper and correct model [19]. The binding sites of the InhA protein were predicted and estimated using the CASTp tool and were entirely accurate. These are the regions of the protein that the ligand, the compound used in this case, resveratrol, can interact with to affect the protein [20]. More elaborative analysis of this interaction was done with the help of CB-Dock2, and the findings indicated that resveratrol has a high affinity for these active sites on InhA. The Vina score of the given structure is -8.8 kcal/mol Thus these docking studies imply a good firm and stable interaction between resveratrol and InhA protein, which is a favourable implication for its therapeutic value [21]. When ADMET was carried out in more detail, this was even more positive for using resveratrol as a drug. Based on the ADMET analysis, it was observed that the compound has good permeability, especially across the gastrointestinal tract; besides, it does not violate any of Lipinski's rule of five. Toxicity analysis revealed that this drug candidate does not cause significant harm to any body organs, except for minor toxicity in the heart and kidneys, which is considered negligible. This, therefore, means that resveratrol has properties that are suitable as far as the bioavailability and solubility of the compound, which are factors that determine the effectiveness of any drug. Moreover, the acute and sub-chronic toxicity predictions demonstrated that the risks associated with the potential adverse effects are pretty low, which is beneficial since the use of supplements in therapeutic regimens is of great concern [22]. The molecular dynamics simulations performed in this study using the iMODS provided more details on the dynamic behaviour of the resveratrol-InhA complex in a physiological environment. Furthermore, these simulations have helped substantiate the assumption that the interaction between the resveratrol and InhA is both essential and temporal. It was concluded from the analysis of the simulations that resveratrol can interact with the target protein and will not lead to the disruption of the docking complex. This stability is essential for the compound to work in a biological system; thus, resveratrol can continue to inhibit in the long run [23]. The study conducted by Singh and Pandey reveals Gravacridone diol from the Rutaceae family with the best binding affinity of -10. 80 kcal/mol is better than the known inhibitor triclosan(-7. 33 kcal/mol) [24]. The current study focuses on Resveratrol as a possible inhibitor, and the docking score is -8.8 kcal/mol, describing the different physicochemical properties and the interaction residues. Both studies use ADMET profiling and molecular dynamics simulations to assess the compounds' stability and drug-likeness. However, study shows that Gravacridonediol has a higher binding energy and better pharmacokinetic profile than the first compound. Both research studies are based on identifying inhibitors for the InhA protein in Mycobacterium tuberculosis [24]. Thus, based on results, resveratrol can be a potential anti-TB drug. However, to prove the efficiency and reliability of the method, it is necessary to pass to the computational level. Further diagnostic and research studies, including clinical experiments (in vitro and in vivo), are needed to support these findings. These experimental validations will help confirm that resveratrol can inhibit InhA in a living organism and check the side effects of resveratrol. Moving from in-silico to in-vitro and then to in-vivo is a giant leap in the drug development

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process to come up with a new treatment for tuberculosis.

CONCLUSIONS

It was concluded that TB remains a global health challenge because the FDA has endorsed no medications for concomitant therapy, and the InhA protein plays a significant role in the disease. In our study, Resveratrol was considered an antioxidant polyphenol with antimicrobial characteristics. These digital simulations showed that it has a tight binding affinity for the active site of InhA and forms a complex that inhibits the proper function of InhA and, hence, the growth of Mycobacterium tuberculosis. Similarly, Resveratrol was endowed with good pharmacodynamics, such as not being toxic, crossing the blood-brain barrier, and good GI absorption; thus, it remains one of the best for optimization. Consequently, in light of this study, using Resveratrol as a new treatment for TB can help improve the patient's health status and lessen the infection rate.

Authors Contribution

Conceptualization: OU, NH
Methodology: OU, NH, A
Formal analysis: OU, NH, AQM,
Writing review and editing: FA, MM, EJ, SF

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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