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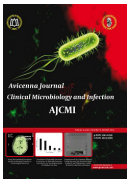

Original Article

Studying the Inhibitory Effects of Some Chalcone Derivatives on *Streptococcus mutans* Sortase A to Prevent Dental Caries: An In Silico Approach

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Abstract

Background: *Streptococcus mutans* is one of the most important microorganisms in tooth decay. Sortase A (SrtA) of *S. mutans* is responsible for the attachment of bacteria to the host cell and biofilm formation. Therefore, it seems necessary to investigate the inhibitors of this enzyme to prevent dental caries. Chalcones are always of interest in the medical community due to their wide range of biological activities. Many studies have reported that chalcone can help prevent caries. The present study was conducted to identify potential SrtA inhibitors with the chalcone skeleton.

Methods: The chalcone derivatives were obtained from the ZINC15, LEA3D, and PubChem databases, and then the selected compounds were optimized by HyperChem software. The affinity of these compounds to SrtA and total binding free energy (ΔG_{bind}) were estimated by the AutoDock 4.0 program. Finally, drug-likeness screening and absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the best ligands were obtained using online servers.

Results: Compared to chalcone, four of the studied ligands, including compounds 2, 7, 8, and 9 demonstrated high affinity for binding to *S. mutans* SrtA, with suitable drug-likeness and ADMET properties. Ligand 9 interacted with the key residues in the active site by the most negative ΔG_{bind} (-4.64 kcal/mol). The best conformation of this ligand had the most overlap with the chalcone.

Conclusion: By complementary both in vitro and in vivo studies on the inhibitory effects of compounds 2, 7, 8, and 9, the present study can be useful in controlling tooth decay and dental diseases.

Keywords: Chalcone derivatives, In silico, *Streptococcus mutans*, Sortase A, Molecular docking, Dental caries

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Introduction

Biofilm, the multidimensional complex structure, plays a highly important role in causing dental caries (1). The main component of the biofilm forming on the tooth surface is the Gram-positive bacterium called *Streptococcus mutans*. The sortase A (SrtA) of *S. mutans* is responsible for the attachment of microorganisms to the host cell wall and plaque formation. At the site of biofilm formation, *S. mutans* uses sugar compounds and during metabolism, converts them into acidic compounds, causing tooth decay (2,3).

Biofilm formation occurs in two ways, the first way requires the presence of sucrose. In this way, glycosyltransferases convert sucrose into polysaccharides and provide the conditions for plaque formation. In the second way, which is completely independent of this disaccharide, an interaction between surface protein Pac and agglutinins is created as a result of the catalytic activity of SrtA (4-6).

SrtA is an important group of transpeptidases located in the membrane of *S. mutans* (7). Microorganism surface proteins with conserved motif LPXTG are targeted as SrtA substrates. SrtA cleaves threonine and glycine peptide bond in surface proteins, and then in the catalytic site of SrtA, cysteine forms a peptide bond with a carbonyl end of threonine. Finally, this intermediate is transferred to the lipid II-surface protein to promote biofilm formation (7,8).

Considering the importance of SrtA in the pathogenesis of *S. mutans* and dental plaque formation, the investigation of its inhibitors has frequently been of interest to researchers (9-11). Although various antimicrobial compounds are commercially available to prevent and treat tooth decay,

dental caries has always been one of the problems of the medical community. However, due to drug resistance and occasionally the side effects of inhibitory compounds, it seems necessary to design new ligands with higher efficiency.

Chalcone, which is also called benzyl acetophenone, is a natural product belonging to flavonoid compounds (12-14). Chalcones can be isolated from plants belonging to *Leguminosae*, *Asteraceae,* and *Moraceae* species. Since ancient times, chalcones have been used in medical sciences due to their antibacterial, antifungal, and antiparasitic properties. Many studies have confirmed that chalcone can help prevent caries (14-17).

Molecular docking allows us to investigate the interactions between the ligand-protein complex and ΔG_{bind} (18). Previous studies represented that molecular docking suggests suitable compounds for enzyme inhibition, drug, and vaccine design (19-23).

In this study, one of the most important enzymes involved in the biofilm formation, *S. mutans* SrtA, was chosen as a receptor to identify inhibitors with the chalcone skeleton by molecular docking, and then drug-likeness screening and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analyses were performed for selected ligands by online servers.

Materials and Methods

Selection of Compounds and Energy Optimization

The compounds that were selected for screening and analysis were based on the chalcone scaffold. Chalcone is an organic compound with an unsaturated ketone and two aromatic rings (14). Compounds in the simulation description format were downloaded from ZINC15, LEA3D, and PubChem databases, and then a suitable format was created using GaussView software, version 5.0. Finally, select compounds were optimized by HyperChem Professional software. For this purpose, the Polak-Ribière algorithm was used with a value of 0.1 for RMS.

Receptor Selection and Preparation

There are many structures of the crystallized *S. mutans* SrtA protein in the Protein Data Bank (PDB) site, but based on the study performed on the inhibition of SrtA by chalcone (17), PDB ID 4TQX was chosen for molecular docking. The three-dimensional crystal structures of the selected protein were downloaded from the Protein Data Bank in pdb format. Tetra ethylene glycol (PG4301), acetic acid (ACY302), sulfate ion (SO4303), and all water molecules were removed from the receptor structure using the Discovery Studio Visualizer software, and then it was employed as an input file in AutoDock tools, version 4.2.

Grid Box and Grid Center Determination

Based on the analysis of the crystal structure of 4TQX, there was no co-crystallized inhibitor in this structure. Discovery Studio Visualizer software was utilized to determine the position of the grid center. First, important amino acids of the active site were obtained using previous research (7,24), and then the center of those amino acids was used as the center of the grid. For calculating an optimal box size, the length of the ligands was calculated four times and employed as grid box size.

Molecular Docking Studies

Molecular docking studies can be applied to model the interaction between a ligand and a receptor, which allows us to determine the binding energy level and how the ligand is placed in the macromolecule (18). Docking was performed with AutoDock tools (ADT), version 4.2. After preparing protein and ligand files in pdb format, to create charge parameters for each atom in protein and ligand, pdbq files were created by adding Gasteiger and Kollman charges. Finally, auto-grid and auto-dock programs were performed after creating the grid and docking parameter files, respectively. Next, the types of clusters, ΔG_{bind}, and the best conformation were studied through the dlg output file.

Drug-Likeness Screening and Absorption, Distribution, Metabolism, Excretion, and Toxicity Properties

Drug-likeness screening and ADMET properties were performed for compounds that demonstrated good docking results. In addition to drug effectiveness, suitable physicochemical properties are extremely important in drug design. In the present study, Lipinski's rule of five (RO5) was used to study drug-likeness screening. According to the RO5, low absorption occurs in molecules with a molecular weight of more than 500 g/mol, the number of hydrogen bond acceptors of more than 10, the number of hydrogen bond donors of more than 5, and a lipophilicity factor (logP) more than 5 (25).

ADMET, which includes the main parameters of drug design (i.e., absorption, distribution, metabolism, excretion, and toxicity), was investigated using SwissADME and ProTox-II Server.

Results

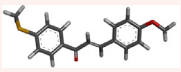
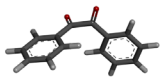
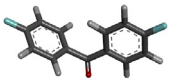
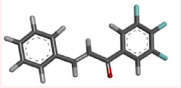
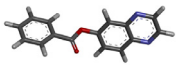
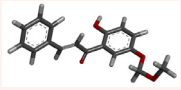
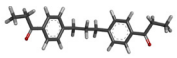
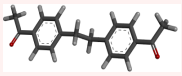
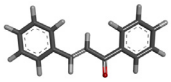
Selection and Energy Optimization of Compounds

In this study, ZINC15, LEA3D, and PubChem databases were employed to select compounds with the chalcone skeleton, and then the selected compounds were subjected to HyperChem to obtain the compound with the lowest energy. The ligand's name, molecular formula, database, ligand ID, and structural details of the selected compounds are presented in Table 1.

Receptor Selection and Preparation

The crystal structure of *S. mutans* SrtA with access code 4TQX as a receptor was downloaded from https://www.rcsb.org. The crystal structure of 4TQX with resolution 1.37 Å was reported by Wallock-Richards et al. This enzyme consists of a catalytic part with 8 beta sheets and a tail at the N-terminal end with a helix structure (17). The final structure of the protein after preparation with

Table 1. List of Studied Compounds

Ligand Number	Ligand Name	Molecular Formula	Database	Ligand ID	Structure
1	(E)-3-(4-methoxyphenyl)-1-(4-methylsulfanylphenyl)prop-2-en-1-one	$C_{17}H_{16}O_2S$	Zinc15	ZINC4252637	
2	1,2-diphenylethane-1,2-dione	$C_{14}H_{10}O_2$	PubChem	8651	
3	2-(2-Phenylethyl)-3-phenylaziridine	$C_{16}H_{17}N$	Zinc15	ZINC205482393	
4	bis(4-fluorophenyl)methanone	$C_{13}H_8F_2O$	PubChem	9582	
5	(E)-3-phenyl-1-(3,4,5-trifluorophenyl)prop-2-en-1-one	$C_{15}H_9F_3O$	PubChem	101571323	
6	6-quinoxalinyl benzoate	$C_{15}H_{10}N_2O_2$	LEA3D	g9_mol30	
7	(E)-1-[2-hydroxy-5-(methoxymethoxy) phenyl]-3-phenylprop-2-en-1-one	$C_{17}H_{16}O_4$	PubChem	11335085	
8	1-[4-[3-(4-propanoylphenyl)propyl]phenyl] propan-1-one	$C_{21}H_{24}O_2$	PubChem	134887749	
9	1-[4-[2-(4-acetylphenyl)ethyl]phenyl] ethanone	$C_{18}H_{18}O_2$	PubChem	13100	
10	Chalcone	$C_{15}H_{12}O$	PubChem	637760	

Discovery software is shown in Figure 1.

Grid Box and Grid Center Determination

Amino acids such as cysteine 205 (Cα: X = 5.81, Y = 29.70, and Z = -13.33) and arginine 213 (Cα: X = 4.36, Y = 31.66, and Z = -17.16) are important residues in the catalytic site of the enzyme. SrtA recognizes and cleaves the amide bond of the substrate by cysteine 205 and stabilizes the transition state by arginine 213 (7,24). Therefore, the center of these two residues (X = 5.48, Y = 29.14, and Z = -16.244(was used as the grid center. Using Discovery Studio Visualizer software, the size of the grid box was determined to be 40 × 40 × 40.

Molecular Docking Studies

A molecular docking study was performed to analyze the affinity and orientation of chalcone derivatives in the active site of SrtA (18). The results related to the binding energy, intermol energy, electrostatic energy, and torsional energy of all compounds are provided in Table 2. A more negative binding energy indicates a stronger interaction between the ligand and receptor. Compared to chalcone, compounds 2, 7, 8, and 9 showed a high affinity for binding to *S. mutans* SrtA. The results of the clustering histogram of compounds represented that ligand 9 has the most negative binding energy, and 88/100 conformations are placed in cluster 1. The best conformation of ligand 9 has the most overlap with the chalcone (Figure 2A). Discovery Studio Visualizer and LigPlus software were employed to study the hydrogen bonds and hydrophobic interactions between ligands and amino acids in the active site of the enzyme, respectively. These interactions are summarized in Table 3, and the binding mode of ligand 9 is illustrated in Figure 2.

Drug-Likeness Screening and Absorption, Distribution, Metabolism, Excretion, and Toxicity Prediction

Based on the results (Table 4), all the selected compounds

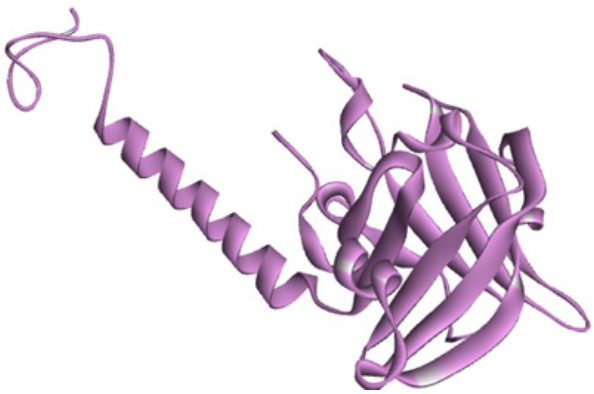


Figure 1. The Final Structure of the Receptor After Preparation.

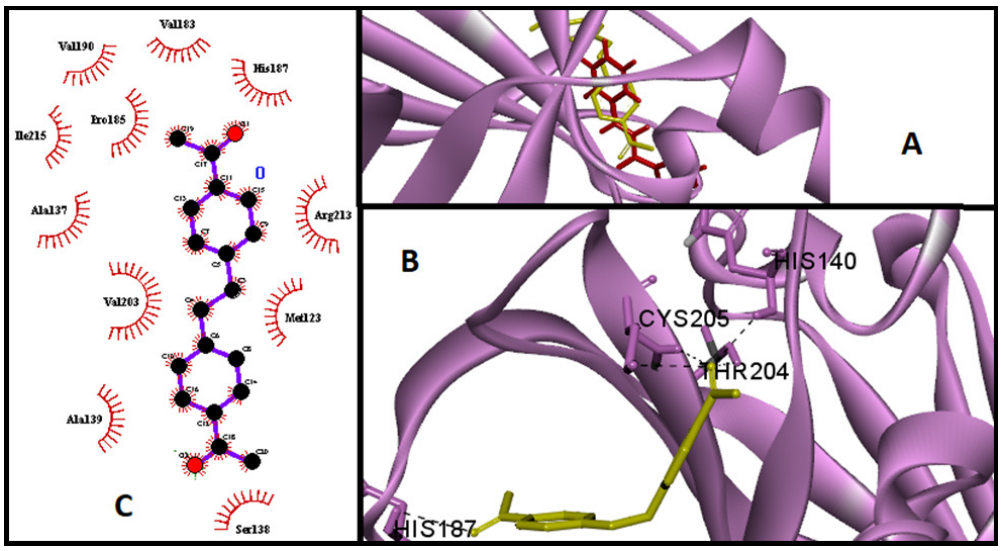


Figure 2. (A) Docked Conformations of Chalcone (Red) and Compound 9 (Yellow) in the Active Site of SrtA (violet), (B) Hydrogen Bonding, and (C) Hydrophobic Interactions of Compound 9 Analyzed by Discovery Studio and LigPlus, respectively.

had acceptable drug-likeness properties and follow Lipinski's RO5.

The values of lipophilicity, molecular weight, and the topological polar surface area (TPSA) indicated good membrane permeability with high to moderate water solubility. The molecular weight of all compounds is less than 500 g/mol.

Membrane permeability was limited when the TPSA was greater than 140 Å2. The results (Table 4) also revealed that the TPSA value of all selected compounds is less than 140 Å². According to the RO5, an oral drug should have a LogP value < 5. The lipophilicity value of all ligands was less than 5.

The ADMET properties of the selected compound are presented in Table 5. In this study, all selected compounds exhibited high gastrointestinal absorbance and blood-brain barrier penetration, and no compounds were the substrates of permeability glycoprotein. The results of the Cytochrome P450 inhibitor demonstrated that all the compounds are inhibitors for CYP2C19. Compounds 2, 7, and chalcone did not interact with CYP2D6 and CYP3A4, while compounds 8 and 9 were potential inhibitors. Among all compounds, only compound 7 could inhibit CYP2C9. Compounds 7, 8, and 9 were the inhibitors of CYP1A2.

Based on ProTox-II server results, all compounds were non-active to mutagenicity, immunotoxicity, cytotoxicity,

Table 2. Docking Results Based on Energy Values in kcal/mol

Ligand Number	Binding Energy	Intermol Energy	Electrostatic Energy	Torsional Energy
1	-2.73	-4.22	0.38	-0.24
2	-4.09	-4.99	-0.06	0.89
3	-3.65	-5.43	0.33	1.79
4	-3.49	-4.09	-0.07	0.6
5	-3.45	-4.34	0.0	0.89
6	-3.82	-4.72	-0.15	0.89
7	-4.32	-6.41	-0.19	2.09
8	-4.59	-6.97	-0.07	2.39
9	-4.64	-6.13	0.01	1.49
Chalcone	-3.88	-4.77	0.06	0.89

Table 3. Docking Results Based on Interaction Analysis

Ligand Number	Amino Acids Involved in Hydrogen Bonds	Amino Acids Involved in Hydrophobic Interaction
1	-	Ala139, His140, Thr204, Ser138, Cys205, Leu116, Ala137, Pro185, Thr184, Val183, His187, Arg213, Val203, Leu111, and Met123
2	His140, Thr204, and Cys205	Ser138, Val203, Arg213, Ala137, Ala139, Ala208, and Asp207
3	-	Val190, Ile215, Thr184, Cys205, Met123, Ala137, Leu116, Val203, Ser138, Thr204, Arg213, His187, Pro135, and Val183
4	Thr204 and Cys205	Ala139, Leu116, Ser138, Arg213, Ala137, and Val203
5	Arg213	Ile191, Val190, Met123, Val203, Ala137, Ser138, and Leu116
6	Arg213	Ile191, Val190, Pro185, His187, Val203, Cys205, Thr204, Ser138, Leu116, Ala137, and Met123
7	His140, Thr204, and Cys205	Leu116, Ala139, Ser138, Thr117, Arg213, Val203, Ala137, Met123, Asp112, and Asn113
8	His140, Thr204, and Cys205	Ser138, Ala139, Leu116, Ala137, Leu111, Arg213, Met123, Val188, Val190, Ile191, His187, Thr184, Val203, Pro185, and Val183
9	His140, His187, Thr204, and Cys205	Ser138, Ala139, Val203, Ala137, Ile215, Pro185, Val190, Val183, His187, Arg213, and Met123
Chalcone	His140, Thr204, and Cys205	Met123, Ala137, Leu116, Arg213, Ser138, ALa139, and Val203

Table 4. Drug-Likeness Screening

Ligand Number	Molecular Weight (g/mol)	H-bond Acceptors	H-bond Donors	Lipophilicity	TPSA (Å²)	Water Solubility
2	210.23	2	0	3.38	34.14	High
7	284.31	4	1	3.81	55.76	Moderately soluble
8	308.41	2	0	4.99	34.14	Moderately soluble
9	266.33	2	0	3.51	34.14	High
Chalcone	208.26	1	0	3.08	17.07	High

Note. TPSA: Topological polar surface area; LogP: Partition coefficient in octanol/water.

Table 5. ADMET Properties

Ligand Number	ADME Properties				Toxicity				
	GI Absorption	BBB Permeant	P-gp Substrate	CYP Inhibitor	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
2	High	Yes	No	CYP2C19 inhibitor	Inactive	Inactive	Inactive	Inactive	Inactive
7	High	Yes	No	CYP1A2 inhibitor CYP2C19 inhibitor CYP2C9 inhibitor	Inactive	Inactive	Inactive	Inactive	Inactive
8	High	Yes	No	CYP3A4 inhibitor CYP1A2 inhibitor CYP2C19 inhibitor CYP2D6 inhibitor	Inactive	Inactive	Inactive	Inactive	Inactive
9	High	Yes	No	CYP3A4 inhibitor CYP1A2 inhibitor CYP2C19 inhibitor CYP2D6 inhibitor	Inactive	Inactive	Inactive	Inactive	Inactive
Chalcone	High	Yes	No	CYP2C19 inhibitor	Inactive	Inactive	Inactive	Inactive	Inactive

Note. ADMET: Absorption, distribution, metabolism, excretion, and toxicity; BBB: *Blood-brain barrier*; CYP: Cytochrome *P450*; GI: Gastrointestinal; P-gp: Permeability glycoprotein.

carcinogenicity, and hepatotoxicity.

Discussion

Tooth decay is one of the most common and important global oral health problems (26); it is directly related to the formation of a sticky layer on the teeth, which is called plaque. Considering the importance of *S. mutans* SrtA in the formation of dental plaques (1-3), in this study, new compounds with the chalcone skeleton were studied to obtain potential inhibitors of this enzyme.

Several studies reported that chalcone can inhibit SrtA activity. Using in vitro methods, Li et al investigated the inhibitory activity of chalcone against SrtA. Their results represented that chalcone with a half maximal inhibitory concentration of 28.41 ± 5.34 µM occupies the enzyme's active site (27). The findings of Wallock-Richards et al and Zhang et al. revealed that the plant's natural product chalcone is an inhibitor of SrtA and can effectively inhibit *S. mutans* biofilm formation (17,28).

Due to the important role of the chalcone ketone group in enzyme inhibition, derivatives with O, N, F, and S functional groups were selected from the ZINC15, LEA3D, and PubChem databases. GaussView software was used to convert the 3D structure of the selected compounds into the appropriate input file for HyperChem software.

After energy optimization, the molecular docking study was performed for the screening of compounds based on the mode of interactions and the binding energy.

In the active site of *S. mutans* SrtA, peptide bond cleavage and transition state stabilization require the presence of cysteine 205 and arginine 213, respectively (7,24). Considering the main role of these residues in the enzyme's catalytic activity, the biological activity of the enzyme will be inhibited if the compound interacts with them.

Wang et al elucidated that astilbin forms a hydrogen bond with arginine 213 and interferes with the catalytic activity of SrtA. In this research, similar to our study, 4TQX was chosen as the receptor (29). According to Luo et al, cysteine 205 and arginine 213 participated in the interaction between all studied inhibitors and *S. mutans* SrtA (7).

Based on our docking results, all compounds interact with these two key amino acids. H-bond interaction with cysteine 205 and hydrophobic interaction with arginine 213 were observed in the docking result of ligands 2, 4, 7, 8, 9, and chalcone. Arginine 213 interacts with ligands 5 and 6 by hydrogen bonding. Ligands 1 and 3 are also connected to these amino acids through a hydrophobic bond.

Among the studied compounds, some of the derivatives (2, 7, 8, & 9) have more negative binding energy and higher affinity than chalcone ($\Delta G_{binding} < -3.88$ kcal/mol). The structure analysis of these compounds indicates that the presence of an additional carbonyl (C = O) group in the ligand can be effective in receptor affinity. Compound 9 (with $\Delta G_{bind} = -4.64$ kcal/mol) was predicted as the most potent inhibitor. The Ser138, Ala139, Val203, Ala137, Ile215, Pro185, Val190, Val183, His187, Arg213, Met123

His140, His187, Thr204, and Cys205 in the active site of *S. mutans* SrtA were the sites for hydrogen bonding and hydrophobic interactions with compound 9.

Santana de Oliveira et al studied the antibacterial properties of *Siparuna guianensis* by the molecular docking approach and reported that the main compound of the essential oil (Atractylone) binds to SrtA via Ile215, Val190, Ile191, Val188, Arg213, and Val203 (30). It was found that compound 9 and Atractylone shared common interactions with Ile215, Val190, Arg213, and Val203 residues.

Finally, 1,2-diphenylethane-1,2-dione (compound 2), (E)-1-[2-hydroxy-5-(methoxymethoxy) phenyl]-3-phenylprop-2-en-1-one (compound 7), 1-[4-[3-(4-propanoylphenyl)propyl]phenyl]propan-1-one (compound 8), and 1-[4-[2-(4-acetylphenyl)ethyl]phenyl] ethanone (compound 9) were selected for further analyses.

A computer-based drug-likeness screening and the ADME properties of the selected ligands revealed that all compounds have high absorption capability and acceptable drug-likeness profiles based on Lipinski's RO5. Additionally, a toxicity study showed that none of the selected ligands were toxic.

Conclusion

In the current study, the structural binding features of chalcone derivatives with *S. mutans* SrtA were analyzed, and then several parameters such as absorption, distribution, metabolism, excretion, toxicity, and drug-likeness were studied for the selected compounds.

Based on the results, compounds 2, 7, 8, and 9 (with the appropriate results of docking, drug-likeness, and ADMET properties) can act as SrtA inhibitors and prevent biofilm formation and tooth decay. However, future validation by both in vitro and in vivo studies is inevitable in this regard.

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Methodology: Azizeh Asadzadeh.
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Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The authors declare that they have no conflict of interest.

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