European Review for Medical and Pharmacological Sciences 2023; 27: 5301-5309

Revolutionizing treatment for toxic shock syndrome with engineered super chromones to combat antibiotic-resistant *Staphylococcus aureus*

M. NAVEED[1], N. AIN[1], T. AZIZ[2], I. ALI[1], M. AQIB SHABBIR[1], K. JAVED[1], M. ALHARBI[3], A. ALSHAMMARI[3], A.F. ALASMARI[3]

[1]Department of Biotechnology, Faculty of Science and Technology, University of Central Punjab, Lahore, Pakistan
[2]Department of Agriculture, University of Ioannina, Arta, Greece
[3]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

Abstract. – OBJECTIVE: *Staphylococcus aureus*-induced toxic shock syndrome (TSS) is a rare, but potentially fatal disease with limited treatment options. The emergence of antibiotic-resistant strains has led to a pressing need for the development of effective therapies. This study aimed to identify and optimize potential drug candidates against toxic shock syndrome by targeting the pathogenic toxin protein using chromones as lead compounds.

MATERIALS AND METHODS: In this study, 20 chromones were screened for their ability to bind to the target protein. The top compounds were further optimized through the addition of cycloheptane and amide groups, and the resulting compounds were evaluated for their drug-like properties using chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling.

RESULTS: Among the compounds screened, 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone exhibited the highest binding affinity with a molecular weight of 341.40 g/mol and a binding energy of -10.0 kcal/mol. The optimized compound exhibited favorable drug-like properties, including high water solubility, synthetic accessibility, skin permeation, bioavailability, and gastrointestinal absorption.

CONCLUSIONS: This study suggests that chromones can be engineered to develop effective drugs against TSS caused by S. aureus. The optimized compound has the potential to be a promising therapeutic agent for the treatment of TSS, providing new hope for patients suffering from this life-threatening disease of toxic shock syndrome.

Key Words:
Chromones, Toxic shock syndrome, Drug discovery, *Staphylococcus aureus*, Antibiotic resistance.

Introduction

In 1978, the earliest description of toxic shock syndrome was given, while reports were released in the 1980s[1]. TSS incidence in the United States is estimated to be between 0.8 and 3.4 per 100,000 people. Staphylococcal toxic shock syndrome (STSS) is a rare side effect of *Staphylococcus aureus* infection where bacterial toxins TSS toxin-1 (TSST-1) act as superantigens, activating enormously high numbers of T cells and inducing a severe immune-mediated cytokine eruption that manifests clinically as fever, rash, shock, and rapidly progressing multiple organ failure, frequently in young, previously healthy patients. Normal patient symptoms include fever, rash, and poisoning, which frequently lead to hypotension[2]. TSS may affect anyone at any age, however, it is most frequently seen in young, healthy adults and children[3]. TSS is primarily associated with menstruation, and recently, a considerable fraction of individuals with chronic rhino sinusitis were shown to carry TSST-1-producing *S. aureus* strains. Children are much less likely than adults to die from STSS (Streptococcal Toxic Shock Syndrome). Tissue necrosis and limb loss are two well-known side effects of shock and organ failure.

Corresponding Author: Muhammad Naveed, MD; e-mail: naveed.quaidian@gmail.com; Tariq Aziz, MD; e-mail: iwockd@gmail.com

Chromones belong to a class of organic compounds containing the benzo-γ-pyrone structure composed of a pyrone ring fused benzene ring. Due to their drug-like qualities, chromones have demonstrated widespread acceptance[4]. A vast family of chromones with interesting biological properties is both biosynthetic and synthetic in origin. Chromones are reported to have a variety of biological activities in traditional medicine, including antibacterial, anticancer, anti-inflammatory, anti-allergic, and anti-inflammatory. Due to their versatility in structural design and synthetic viability, chromones are a common structure for drug design and discovery in the field of medicinal chemistry[5].

Supportive treatments, specific antibiotic medication, and adjuvant immunomodulatory therapy are all used to treat staphylococcal TSS. There are also numerous potential treatments under development. Based on the available data, early antimicrobial regimens should be sufficiently comprehensive to address all potential diseases because therapy is frequently started before a firm diagnosis of TSS has been confirmed. TSS is commonly treated with the -lactam drugs flucloxacillin, cloxacillin, nafcillin, and clindamycin. Because of its high rates of constitutive and inducible resistance, especially among methicillin-resistant bacteria, clindamycin is not recommended for use as a single therapy[6].

The cost of conducting experiments and current ethical guidelines have made studies on living things much more difficult[2]. In this study, *in silico* methods have been successful and have grown into powerful tools for the search for disease remedies. Since conventional drug discovery is both expensive and time-consuming, computer-aided drug design (CADD) methodologies provide a way to increase drug development effectiveness while lowering both time and expense. There is a need for innovative tactics and ideas to address these urgent issues. Drug design for *Staphylococcus aureus* TSST-1, which causes toxic shock syndrome, has been proposed using *de novo* drug design, interaction analysis, pre-clinical testing, toxicity analysis, and its validation studies. The designed chromone would be cost-effective and provide a breakthrough step in the expansion of the pharmacology field.

Materials and Methods

Retrieval of Toxin Protein

The National Center for Biotechnology Information (NCBI, available at: https://www.ncbi.nlm.nih.gov/) facilitates access to biomedical and genomic data, advancing science and health[7]. The sequence of the targeted toxin *Staphylococcus aureus* protein of the toxic shock syndrome was accessed from NCBI inscribed in this study with accession number OHS90060.1.

Tertiary Structure of Toxin Protein

Swiss Model (available at: https://swissmodel.expasy.org) is used to convert the primary sequence obtained from NCBI into the tertiary structure. The 3D protein structure was visualized and purified by Discovery Studio Visualizer[8]. Discovery Studio was used to remove ligands and water molecules. The BIOVIA Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download) is a free, feature-rich molecular modeling tool for viewing, sharing, and analyzing data on proteins and small molecules.

Identifications of Chromones

20 chromones were selected which exhibit wide acceptability due to drug-like properties from the literature for screening purposes. From PubChem (available at: https://pubchem.ncbi.nlm.nih.gov), the 2D structures were obtained in Safety Data Sheet (SDS) format form and then using Discovery Studio saved in Protein Data Bank (PDB) format. A free chemistry database at the National Institutes of Health is called PubChem (NIH), which aids in drug development lead identification and optimization, compound-target profiling, polypharmacology research, and the clarification of unknown chemical identities.

Screening of Chromones

Chromones were screened through multiple ligands docking by using PyRx.(https://pyrx.sourceforge.io/). This is virtual screening software for computational drug development and discovery that searches libraries of antidotes against drug targets. The compound with the highest energy was selected for further optimization to increase its efficiency.

Functional Group Optimization of Chromones

Mcule tool (available at: https://mcule.com/) was used for the functional group optimization of chromones. Mcule Tool is an online drug discovery platform. The functional groups were optimized to enhance the effectiveness of the

chromones. In the case of chromones, amide groups and 2 cycloheptanes were added for prominent efficiency and targeting, and the one with the maximum energy was selected for further analysis.

Interaction Analysis

A molecular docking software called Autodock Vina (available at: http://vina.scripps.edu/) is free and open source[9]. The docking analysis of a best-screened chromone was revealed by Autodock Vina. The most stable posture of docking was the root mean square interactions (RMSD) values and the lowest binding energy of the conformations. After the protein and the ligand was prepared, the binding sites of the protein were adjusted with x, y, and z values and centers as active sites, and the grid was set. Then Docking was performed between the targeted toxin protein and the best-screened chromone.

ADMET Analysis

Pre-clinical drug candidate testing can be done online using SwissADME (available at: http://www.swissadme.ch/). It predicted the key characteristics of the potential medicine, including absorption, distribution, metabolism, excretion, and toxicity. The input had the Protein Data Bank (PDB) structure of the best functional group-optimized chromone, and the drug's attributes for drug likeliness were attained.

MD Simulations

The IMods server enabled simulations of molecular dynamics (available at: http://imods.chaconlab.org/). It provides details regarding the verification of the interaction analysis and forecasts how the protein will behave while interacting with the molecule, allowing simulation of the interaction in the host's body. The docked complex was used as input for this tool, and the outcomes from the simulations were investigated[10].

Results

Retrieval of Toxin Protein Structure

The toxin protein of *Staphylococcus aureus* with the accession number OHS90060.1 was retrieved from National Center for Biotechnology Information (NCBI) and converted into the 3D structure using the Swiss model as shown in Figure 1.

Identification of Chromones

For screening purposes, 20 chromones were chosen from the literature based on the inhibition factor. The 2D structures were downloaded from PubChem in SDS format and saved in PDB format using Discovery Studio. The 3D structures of chromones are depicted in **Supplementary Table I**.

Screening of Ligands

To filter out the best candidate, 20 chromones were docked against the toxin protein using PyRx. Based on their binding affinities with the toxin protein, the 20 compounds were examined. Energy levels range from -4.7 to -7.7 Kcal/mol. The chromone 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone was elected as the best candidate since it had the lowest binding affinity with the toxin protein, which was -7.7 Kcal/mol. The list of binding affinities of chromones with the targeted toxin protein of toxic shock syndrome is shown in Table I. The docking complex of toxin protein and selected chromone is depicted in Figure 2A.

Functional Group Optimization of Chromones

Mcule was used to add 2 cycloheptane and amide groups to stabilize the structure and raise the score and energy of the chosen 7-Glucosyloxy-5-hydroxy-2-[2-(4 hydroxyphenyl) ethyl] chromone and the effectiveness of the selected chromone was enhanced as shown in Figure 2B.

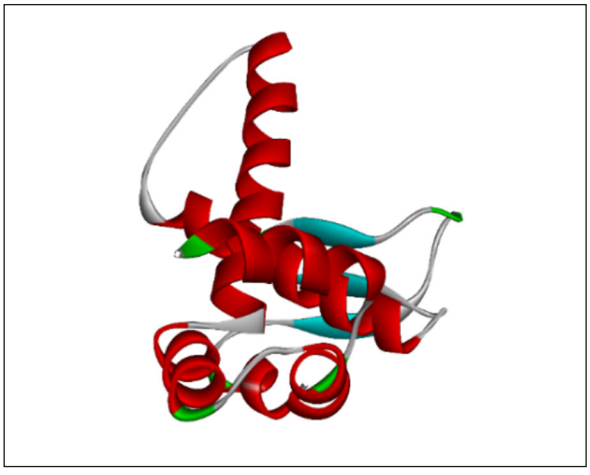

Figure 1. Toxin Protein of *Staphylococcus aureus*.

Table I. List of binding affinities of chromones with targeted toxin protein.

No.	Chromones	Binding affinity (Kcal/mol)
1	7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl)ethyl]chromones	-7.7
2	2H,6H-Benzo(1,2-b5,4-b)dipyran-8-carboxylic acid, 3,4-dihydro-2,2,4,10-tetramethyl-6-oxo-	-7.4
3	5,6,7,8-Tetrafluoro-2-(2,3,4,5,6-pentafluorophenyl)chromen-4-one	-7.1
4	Ophiopogonone	-6.9
5	6-Ethyl-3-(1H-tetrazole-5-yl)chromone	-6.7
6	5,8-Dihydroxy-2-(2-phenyl ethyl)chromone	-6.6
7	5,8-Dihydroxy-2-(2-(4-methoxyphenyl)ethyl)chromone	-6.5
8	5,7-Dihydroxy-2-[2-(4-hydroxyphenyl)ethyl] chromone	-6.5
9	6-Hydroxy-2-[2-(4-methoxyphenyl)ethyl]chromen-4-one	-6.5
10	2-Phenethyl-5-hydroxy chromone	-6.5
11	6,7-Dimethoxy-2-(2-(4-methoxyphenyl)ethyl)chromone	-6.2
12	Chromone-2-carbo hydroxamic acid	-6.2
13	Granulosin	-6
14	Bucromarone	-6
15	6-(2'-Hydroxypropyl)chromone-2-carboxylate	-6
16	Bis(acetoxymethyl)cromoglycate	-5.7
17	7-Hydroxy-4-oxo-4H-chromen-2-carboxylic acid	-5.6
18	Fusarochromanone	-5.6
19	Chromocarb	-5.4
20	4-chromone	-4.7

Interaction Analysis

Based on binding energy, the functional group optimized chromone with the lowest binding energy was 7-glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromones was selected. Using Autodock Vina, docking was done between the toxin protein and the functional group-optimized chromone. The chosen docked model had a binding energy of -10.0 Kcal/mol which was more than the -7.7 Kcal/mol without functional group optimization as shown in Figure 3.

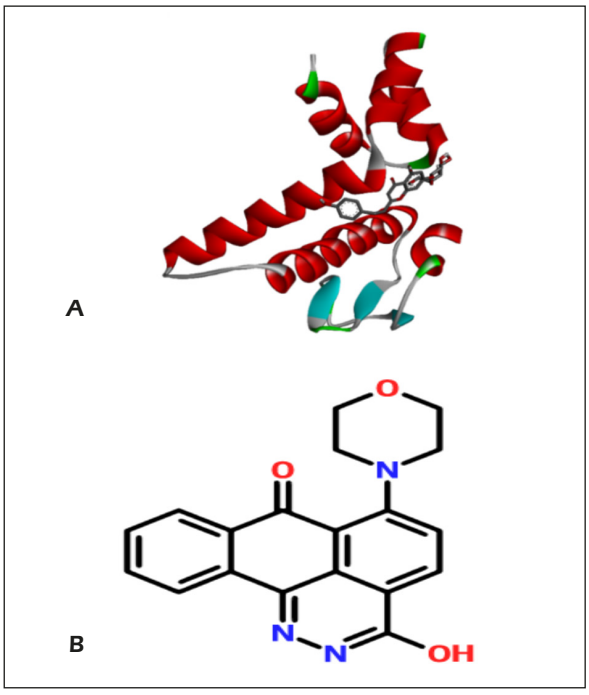

Figure 2. A, Docking complex of toxin protein with 7-Glucosyloxy-5-hydroxy-2- [2-(4 hydroxyphenyl) ethyl] chromone; **B,** 7-Glucosyloxy-5-hydroxy-2- [2-(4 hydroxyphenyl) ethyl] chromone with the addition of cycloheptane and amide groups.

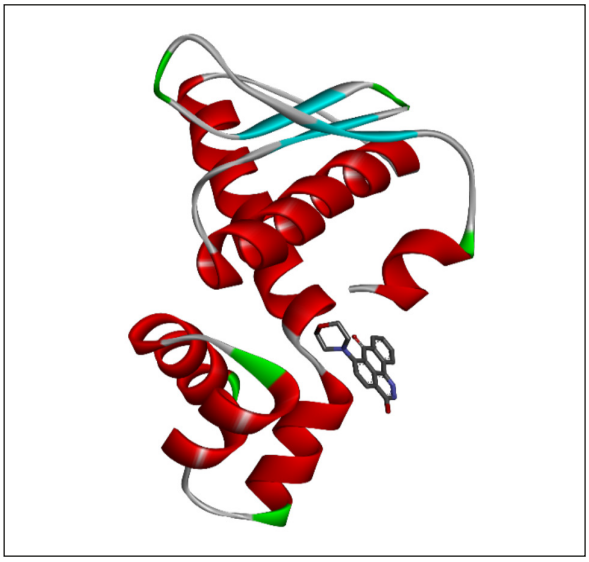

Figure 3. The docking complex of toxin protein from *Staphylococcus aureus* with functional group optimized 7 Glucosyloxy-5-hydroxy-2-[2-(4 hydroxyphenyl) ethyl] chromone.

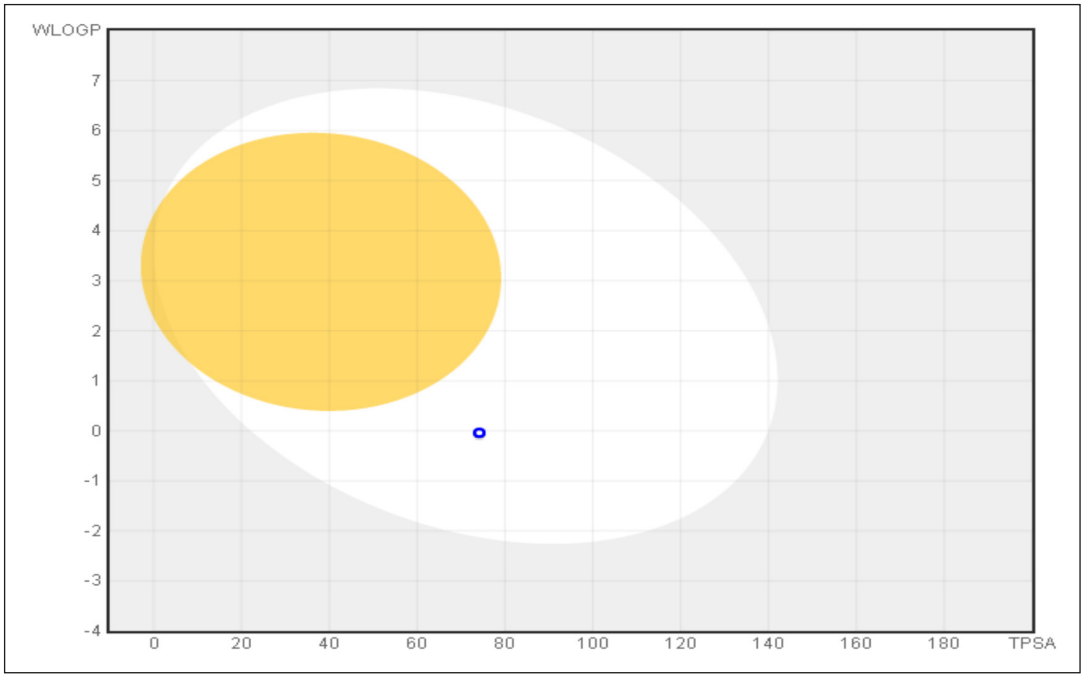

Figure 4. Boiled egg analysis of 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone by SwissADME; Drug (blue dot), blood-brain barrier (*yellow*), gastrointestinal area (*white*).

ADMET Analysis

SwissADME was used to examine physiochemical parameters, water solubility, gastrointestinal (GI) absorption, skin permeability, and bioavailability score. The drug-likeness properties depicted no violations at all as shown in the given Table II.

Table II. ADME Parameters of pre-clinical testing of functional group optimized 7-Glucosyloxy-5-hydroxy-2-[2-(4 hydroxyphenyl) ethyl] chromone.

ADMET parameters	Parametric values
Formula	C19H23N3O3
Molecular weight	341.40 g/mol
Number of heavy atoms	25
Number of aromatic heavy atoms	0
Fraction Csp3	0.58
Num. rotatable bonds	1
Num. H-bond acceptors	4
Num. H-bond donors	2
Molar Refractivity	104.44
TPSA	74.16 Å²
ESOL	-2.67
GI absorption	High
Skin Permeation (Log Kp)	-7.50 cm/s
Bioavailability Score	0.55
Synthetic Accessibility	5.14
Drug likeness	0 violation of rules

TPSA: Topological Polar Surface Area; ESOL: Water Solubility Log S.

The boiled egg model prophesies a quick, intuitive, simple-to-repeat, remarkably robust way to study the entry of tiny compounds into the brain and gastrointestinal absorption, which is extremely helpful for the creation and administration of drugs. When a molecule is present in the boiled egg model's yellow area, it is predicted that it can cross the blood-brain barrier, but when it is present in the white area, it is assumed that the molecule will be absorbed through the gastrointestinal tract. As shown in Figure 4, the molecule is present in a white area which predicts there is gastrointestinal absorption.

MD Simulations

IMods modified the structure's force field concerning various time intervals to be utilized for critical analysis of the structure. Less deformation is visible in the chosen structure at each level of residue capacity. The docking complex structure's Eigen value is 1.305321e-04. Better interactions between the various residues are shown by the heat map's high co-related regions and low RMSD value. Figure 5A shows the given protein structure predicted the Mini-Nutritional Assessment (MNA) mobility; image B displays deformability, indicating little deformations at

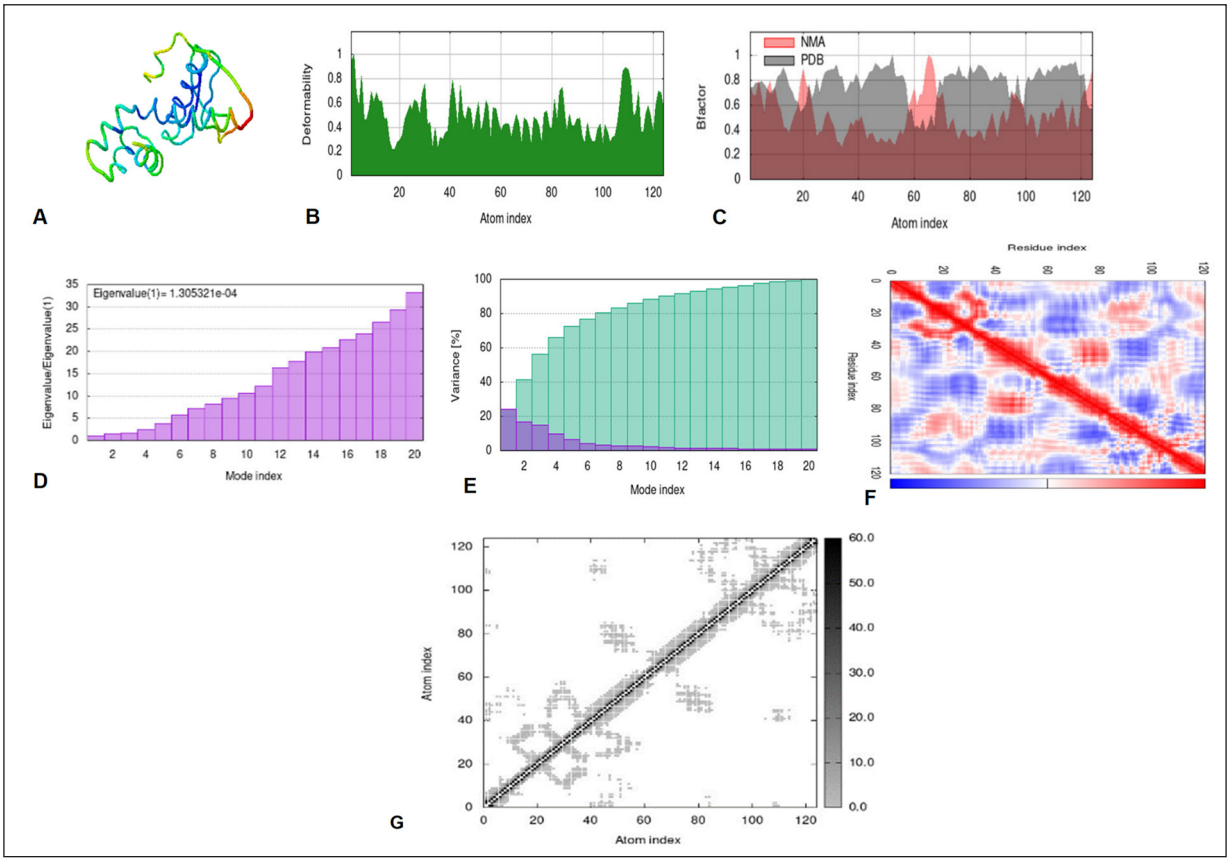

Figure 5. The results of molecular dynamic simulation simulated drug complex (**A**) Tertiary structure of a protein based on MNA mobility (**B**) Deformability (**C**) B-factor (**D**) Eigon values (**E**) Variance (individual variations are indicated by the purple color, and cumulative variances are indicated by the green color), correlated (*red*) and anti-correlated (*blue*) motions are shown on the covariance map in (**F**), and (**G**) elastic network (*darker grey region indicated stiffer regions*).

the residue level; image C indicates B-factor; image D prediction of Eigenvalue as 1.305321e-04; image E demonstrates the variation, which is further elaborated in both green and red; image F shows covariance; while image G depicts the elastic network, with the stiffer portions indicated by darker grey areas.

Discussion

Staphylococcus aureus is the bacteria causing toxic shock syndrome which seems to affect individuals with older age. The symptoms are acute fever, hypotension, rashes, peeling of the skin from hands and feet, diarrhea, vomiting, sore throat, and water imbalance in the body. Over time, crucial conditions lead to kidney failure, lung swelling respiratory failure along with different organ dysfunction[2]. In 1978 earliest discovery of toxic shock syndrome appeared, and reports were released in the 1980s. The TSS was first reported in seven children in the age group ranging from 8-17 years old[1].

Antibiotics and immunoglobins were first used to cure TSS. Clindamycin was the first-line drug used for the cure of TSS, but the death rate was still high. In the future, such strategies should be adopted which disturb the interaction between the bacterial toxins and T cells[11]. Penicillin or amino penicillin is usually combined with Clindamycin to block the 50S sub-unit of the bacterial ribosomes and inhibits protein synthesis[12].

The antibiotic resistance of *Staphylococcus aureus* made treatment difficult. The -lactam drugs flucloxacillin, cloxacillin, nafcillin, and clindamycin were commonly used to treat TSS. As it had high rates of constitutive and inducible resistance, especially among methicillin-resistant bacteria, clindamycin is not recommended for use as a single therapy[6]. For the treatment of

TSS firstly the necrotic activity of cells is analyzed through biopsy and histopathology. Patients with serious conditions are cured when inotropic drugs use crystalloids and vasopressors in large amounts. Further, this syndrome leads to kidney failure which is cured through kidney replacement therapy[13].

Chromones were widely used as a secondary metabolite in the cure of a variety of diseases, and they are integral structures in medicinal chemistry that also acts as a phytonutrient. Chromones can act as antiviral, antioxidant, antibacterial, anti-inflammatory, and anticancer[14-23]. Recent chromones-based drugs are reported as anti-cancerous drugs that have previously been designed using several varieties of chromones[24-29]. The chromones family exhibits biological features with both synthetic and biosynthetic origins[30].

In this study, docking analysis using computational analysis was used to examine the screening of chromones that result in potential and attachments to Toxin protein. As chromones have a higher potential to make effective drugs against diseases. In this case, we have used 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromone for drug designing purposes against toxic shock syndrome. A drug designed for toxic shock syndrome cannot bind strongly to the target while the functional group optimization of the chromones screened in this study, 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromones had proved strong binding energies and can lead to the eradication of bacteria causing toxic shock syndrome.

To overcome multiple organ damage and deaths more potent and focused drug has been designed and thus proposed in this paper stage. Many computer-aided drugs have been synthesized against diseases that have great toxicity and high score[31-35]. Conclusively, the studies that were presented in this study contain sufficient computational information to allow for the propagation of a specifically targeted toxic shock syndrome. It would be helpful if *in vitro* research could use this drug for an indication of the reliability of the proposed drug[36].

Conclusions

Staphylococcus aureus causes toxic shock syndrome, which seems to affect people world widely. Fever, hypotension, rashes, peeling skin on the hands and feet, diarrhea, vomiting, sore throats, and an imbalance in bodily fluids are all signs of toxic shock syndrome. Because *Staphylococcus aureus* is resistant to antibiotics, treatment is difficult. Since constitutive and inducible resistance to clindamycin occurs at substantial rates, it is not recommended to use it as the sole treatment. Because traditional drug discovery is both expensive and time-consuming, this study makes use of computer-aided drug design (CADD) methodologies to increase drug development efficiency while decreasing both time and cost.

To resolve the issue of antibiotic resistance and make effective drugs against toxic shock syndrome a variety of chromones were used. In this study, the best-selected chromones which have high binding affinity were selected. The old antibiotics treatment for TSS was less effective towards toxin protein of *Staphylococcus aureus* and this bacterium also became resistant to clindamycin. While optimized 7-Glucosyloxy-5-hydroxy-2-[2-(4-hydroxyphenyl) ethyl] chromones showed substantial binding energies and had the potential to eradicate the bacteria that cause toxic shock syndrome. All analyses have shown that a chosen, optimized chromone with no violations is effective against the TSS toxin protein.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

The authors greatly acknowledge and express their gratitude to the Researchers Supporting Project number (RSP2023R462), King Saud University, Riyadh, Saudi Arabia.

Ethics Approval

Not applicable, as no humans or animals are involved in this study.

Informed Consent

Not applicable as no humans or any organizations were involved in this study.

Availability of Data and Materials

All the data generated in this study is available on request from the corresponding author.

Funding

This study is self-supported and no external funding has been received in this study.

Authors' Contribution

Conceptualization, M.N, N.U, I.A, K.J and M.A.S.; methodology, T.A..A.S and M.A; software, N.A.; valida-tion, A.S.; formal analysis, M.A., A.A.S, T.A, and M.N.; investigation, M.N, N.U, I.A, K.J, and M.A.S; re-sources, T.A and M.A.; data curation, Z.D, and M.N.; writing—original draft preparation, A.A.S, and M.A writing—review and editing, T.A, and M.A.; visualization, F.A supervision, M.N, and T.A.; project admin-istration, M.N.; funding acquisition, T.A.

References

1) Todd, J, Fishaut, M, Kapral, F, Welch, T. Toxic shock syndrome associated with phage-group-I staphylo-cocci. Lancet 1978; 2: 1116-1118.
2) Chesney PJ, Davis JP, Purdy WK, Wand PJ, Chesney RW. Clinical manifestations of toxic shock syndrome. J Am Med Assoc 1981; 246: 741-748.
3) Todd J K, Weisenthal AM, Ressman M, Caston SA, Hopkins RS. Toxic shock syndrome II: Estimated occurrence in Colorado as influenced by case ascertainment methods. Am J Epidemiol 1985; 122: 857-867.
4) Emami S, Ghanbarimasir Z. Recent advances of chroman-4-one derivatives: Synthetic approaches and bi-oactivities. Eur J Med Chem 2015; 93: 539-563.
5) Gaspar A, Matos MJ, Garrido J, Uriarte E, Borges F. Chromone: a valid scaffold in medicinal chemistry. Chem Rev 2014; 114: 4960-4992.
6) Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in Staphylococcus aureus: a study from North India. J Postgrad Med 2009; 55: 176-179.
7) Kuznetsov A, Bollin CJ. NCBI Genome Workbench: Desktop Software for Comparative Genomics Visu-alization and GenBank Data Submission. Methods Mol Biol 2021; 2231: 261-295.
8) Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 2018; 46: W296-W303.
9) Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock Vina with AutoDockTools: a tutorial. J Comput Chem 2012; 33: 845-850.
10) López-Blanco JR, Garzón JI, Chacón P. iMod: multipurpose normal mode analysis in internal coordinates. Bioinformatics 2011; 27: 2843-2850.
11) Annane D, Clair B, Salomon J. Managing toxic shock syndrome with antibiotics. Expert Opin Pharmacother 2004; 5: 1701-1710.
12) Stevens DL, Bryant AE, Yan S. Invasive group A streptococcal infection: New concepts in antibiotic treatment. Int J Antimicrob Agents 1994; 4: 297-301.
13) Schmitz M, Roux X, Huttner B, Pugin J. Streptococcal toxic shock syndrome in the intensive care unit. Ann Intensive Care 2018; 8: 1-10.
14) Awasthi A, Devi U. Synthesis of some substituted chromones. Prog Agric 2021; 21: 272-282.
15) Ouyang Y, Li J, Chen X, Fu X, Sun S, Wu Q. Chalcone Derivatives: Role in Anticancer Therapy. Bio-molecules 2021; 11: 894.
16) Winter E, Devantier Neuenfeldt P, Chiaradia-Delatorre LD, Gauthier C, Yunes RA, Nunes RJ, Creczyn-ski-Pasa TB, Di Pietro A. Symmetric bis-chalcones as a new type of breast cancer resistance protein inhibitors with a mechanism different from that of chromones. J Med Chem 2014; 57: 2930-2941.
17) Berczyński P, Kładna A, Kruk I, Piechowska T, Aboul-Enein HY, Bozdağ-Dündar O, Ceylan-Unlusoy M. Antioxidant activities of some new chromonyl-2,4-thiazolidinediones and chro-monyl-2,4-imidazolidinediones having chromone cores. J Fluoresc 2013; 23: 1319-1327.
18) Kim MK, Yoon H, Barnard DL, Chong Y. Design synthesis and antiviral activity of 2-(3-amino-4-piperazinylphenyl) chromone derivatives. Chem Pharm Bull 2013; 61: 486-488.
19) Shaveta, Singh A, Kaur M, Sharma S, Bhatti R, Singh P. Rational design synthesis and evaluation of chromone-indole and chromone-pyrazole based conjugates: identification of a lead for anti-inflammatory drug. Eur J Med Chem 2014; 77: 185-192.
20) Liu Q, Qiang X, Li Y, Sang Z, Li Y, Tan Z, Deng Y. Design synthesis and evaluation of chro-mone-2-carboxamido-alkylbenzylamines as multifunctional agents for the treatment of Alzheimer's disease. Bioorg Med Chem 2015; 23: 911-923.
21) Fernández-Bachiller MI, Pérez C, Monjas L, Rademann J, Rodríguez-Franco MI. New tacrine-4-oxo-4H-chromene hybrids as multifunctional agents for the treatment of Alzheimer's disease with cholinergic antioxidant and β-amyloid-reducing properties. J Med Chem 2012; 55: 1303-1317.
22) Abid SMA, Younus HA, Al-Rashida M, Arshad Z, Maryum T, Gilani MA, Alharthi AI, Iqbal J. Sulfonyl hydrazones derived from 3-formylchromone as non-selective inhibitors of MAO-A and MAO-B: Synthesis, molecular modelling and in-silico ADME evaluation. Bioorg Chem 2017; 75: 291-302.
23) Demetgül C, Beyazit N Synthesis. Characterization and antioxidant activity of chitosan-chromone derivatives. Carbohydr Polym 2018; 181: 812-817.
24) Patil VM, Masand N, Verma S, Masand V. Chromones: Privileged scaffold in anticancer drug discovery. Chem Bio Drug Des 2021; 98: 943-953.
25) Mohsin NUA, Irfan M, Hassan SU, Saleem U. Current Strategies in Development of New Chromone De-rivatives with Diversified Pharmacological Activities: A Review. Pharm Chem J 2020; 54: 241-257.

26) Li F, Wu JJ, Wang J, Yang XL, Cai P, Liu QH, Kong LY, Wang XB. Synthesis and pharmacological evaluation of novel chromone derivatives as balanced multifunctional agents against Alzheimer's disease. Bioorg Med Chem 2017; 25: 3815-3826.

27) Reis J, Gaspar A, Milhazes N, Borges F. Chromone as a Privileged Scaffold in Drug Discovery: Recent Advances. J Med Chem 2017; 60: 7941-7957.

28) Benny AT, Arikkatt SD, Vazhappilly CG, Kannadasan S, Thomas R, Leelabaiamma MSN, Radhakrishnan EK, Shanmugam P. Chromone: A Privileged Scaffold in Drug Discovery: Developments in the Synthesis and Bioactivity. Mini Rev Med Chem 2022; 22: 1030-1063.

29) Keri RS, Budagumpi S, Pai RK, Balakrishna, RG. Chromones as a privileged scaffold in drug discovery a review. Eur J Med Chem 2014; 78: 340-374.

30) Silva CF, Pinto DC, Silva AM. Chromones: A Promising Ring System for New Anti-inflammatory Drugs. Chem Med Chem 2016; 11: 2252-2260.

31) Naveed M, Waseem M, Aziz T, Hassan JU, Makhdoom SI, Ali U, Alharbi M, Alsahammari A. Identifi-cation of Bacterial Strains and Development of anmRNA-Based Vaccine to Combat Antibiotic Resistance in Staphylococcus aureus via In Vitro and In Silico Approaches. Biomedicines 2023; 11: 1039.

32) Naveed M, Ain NU, Aziz T, Javed K, Shabbir MA, Alharbi M, Alsahammari A, Alasmari AF. Artificial Intelligence Assisted Pharmacophore Design for Philadelphia Chromosome-Positive Leukemia with Gamma-Tocotrienol: A Toxicity Comparison Approach with Asciminib. Biomedicines 2023; 11: 1041.

33) Muhammad N, Makhdoom SI, Ali U, Jabeen K, Aziz T, Khan AA, Jamil S, Shahzad M, Alharbi M, Al-shammari A. Immunoinformatics Approach to Design Multi-Epitope-Based Vaccine against Machupo Virus Taking Viral Nucleocapsid as a Potential Candidate. Vaccines 2022, 10, 1732.

34) Naveed M, Sheraz M, Amin A, Waseem M, Aziz T, Khan AA, Ghani M, Shahzad M, Alruways MW, Dablool AS, Elazzazy AM, Almalki AA, Alamri AS, Alhomrani M. Designing a Novel Peptide-Based Multi-Epitope Vaccine to Evoke a Robust Immune Response against Pathogenic Multidrug-Resistant Providencia heimbachae. Vaccines (Basel) 2022; 10: 1300.

35) Naveed M, Shabbir MA, Ain NU, Javed K, Mahmood S, Aziz T, Khan AA, Nabi G, Shahzad M, Alharbi ME, Alharbi M, Alshammari A. Chain-Engineering-Based De Novo Drug Design against MPXVgp169 Virulent Protein of Monkeypox Virus: A Molecular Modification Approach. Bioengineering (Basel) 2022; 10: 11.

36) Chen G, Jin HZ, Li XF, Zhang Q, Shen YH, Yan SK, Zhang WD. A new chromone glycoside from Rhododendron spinuliferum. Arch Pharm Res 2008; 31: 970-972.