Original Article

In silico Identification of Novel Compounds as Quorum-Sensing Inhibitors in Selected Waterborne Pathogens

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ABSTRACT

Quorum sensing (QS) is a process involved in producing, detecting, responding, and releasing signaling molecules to maintain physiological activities of most utilized by both gram-positive and gram-negative bacteria in various environmental conditions. This study aims to identify novel compounds that have potential QS inhibitory mechanisms against the gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*), and the gram-negative bacteria *Salmonella typhi* (*S. typhi*) and *Escherichia coli* (*E. coli*). Compounds that are structurally similar to the known QS inhibitors were identified using ligand-based screening. Candidate compounds with 40 to 80% similarity with the known QS inhibitors were further evaluated through molecular docking with the QS-associated enzymes, namely ComA, ComE, LsrF, LsrK, AgrC, AgrA, LsrB, and Hfq. The binding affinity was visualized to identify the different non-covalent binding interactions. Compounds with <-8.0 kcal/mol docking score were considered for evaluation for their distribution coefficient (LogD) at different optimal growth of the bacteria, such as pH 4, 6, 7.4, 7.8, 8, and 9. Out of the 63 compounds evaluated, only three compounds demonstrated a high binding affinity, namely 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl]urea and 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea with ComE of *S. pneumoniae* and 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one to AgrA of *S. aureus*. Their high binding affinity may be attributed to the numerous hydrogen bonds and hydrophobic interactions. However, only 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one has comparable LogD value with its QS inhibitor of AgrA, savarin, at the optimal growth pH for *S. aureus*. These findings suggest that the use of 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one may be effective in controlling *S. aureus* growth probably through inhibition of AgrA. However, further studies are needed to confirm these findings.

Key words: *In silico*, Ligand-based screening, Molecular docking, Quorum sensing, Waterborne pathogens.

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INTRODUCTION

In the Philippines, many are still at risk of contracting poorly managed water supply.[1] This problem introduces a significant risk of contracting waterborne diseases, despite great efforts to raise awareness. There were reported cases of acute bloody diarrhea, cholera, rotavirus, hepatitis A, and typhoid fever due to food and water contamination.[2] Acute bloody diarrhea caused by waterborne pathogens often implies urgent epidemic control in the community.[3] Waterborne typhoid fever outbreaks also indicate a devastating public health implication since it is associated with the consumption of contaminated groundwater and surface water supplies.[4] Moreover, the transmission of diseases such as cholera, diarrhea, dysentery, hepatitis A, typhoid, and

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polio are also linked to contaminated water and poor sanitation. Parasitic and bacterial infections associated with waterborne diseases pose a significant threat, especially in developing tropical or subtropical countries like the Philippines. These pathogens typically lead to various gastrointestinal diseases.[5]

There are numerous waterborne pathogens present in the environment. However, the study will focus on representative organisms common in the Philippines, namely *Salmonella typhi* (*S. typhi*), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Streptococcus pneumoniae* (*S. pneumoniae*). Both *S. aureus* and *S. pneumoniae* are considered gram-positive bacteria, whereas *S. typhi* and *E. coli* are gram-negative bacteria. These waterborne pathogens are associated with various diseases that could potentially lead to life-threatening conditions in humans.

Salmonella typhi is bacteria that only infect humans. They are primarily found in water primarily in bodies of water that are contaminated by human feces.[6] Ingesting water contaminated by *S. typhi* can affect the intestinal of an individual and can cause intestinal perforation.[7] The most common associated disease of *S. typhi* is the commonly known typhoid fever. Typhoid fever occurs most commonly in the Philippines and other countries with limited access to clean water and has poor water sanitation.[8] Infected individuals with typhoid fever would most likely experience diarrhea, nausea, abdominal pain, malaise, and enlarged liver.[8] Almost similar symptoms are observed after other bacterial infections like in *E. coli*.

E. coli is also a gram-negative bacterium that localizes in the intestine.[9] Most of the strains of *E. coli* which are part of our normal microflora, are harmless. However, there were those strains capable of releasing Shiga toxin.[10] This toxin leads to symptoms such as bloody diarrhea, vomiting and can severely lead to a life-threatening condition such as damage of the organs, which could lead to complications and death.[11] Bacterial infection is common in gram-negative bacteria and gram-positive bacteria, such as *S. aureus* and *S. pneumoniae*. *S. aureus* is a gram-positive present in the nasal microflora. However, sometimes it also causes skin infection.[12] Aside from this, this bacterium can also trigger pneumonia, heart valve infections, and bone infections. Usually, inhalation of the dispersed droplets of an infected person is sufficient to infect other individuals.[13] Meanwhile, *S. pneumoniae* is an opportunistic pathogen that resides and colonizes the upper respiratory tract of the host that can cause severe infection, damage, and severe conditions.[14] Its transmission increases with contact with the liquid or aerosol droplets, and fomites intermediates, and close contact with the infected individual.[15]

Most bacteria use quorum sensing to maintain their physiological activities in various environmental settings.[16] This concept leads to the understanding of cell-to-cell communication in unicellular organisms and its importance for survival.[17] This process involves the production, detection, response, and release of chemical signal molecules called autoinducers (AIs) affected by different environmental conditions.[18] Moreover, the regulation of these signaling systems dictates bacterial growth and even motility.[19] This principle attracts various researchers to synthesize compounds that will influence the Quorum-sensing associated pathways.[19]

Different antibiotics target different signaling pathways, but one of the most common targets is the pathways associated with quorum sensing.[16] Antimicrobial agents have been widely used all over the world to prevent bacterial infections. However, most of these pathogens have developed resistance to a wide variety of commonly used antibiotics, such as daptomycin, vancomycin, penicillin, and methicillin.[17] Hence, the identification and synthesis of novel compounds will aid in the screening method during drug development.

With various infections and drug resistance problems currently observed worldwide, chemical interference with bacterial cell-to-cell communication is potentially an effective way to control these infections.[20] With the use of virtual screening techniques, such as QSAR modeling, to predict the physicochemical properties of the novel compound; and molecular docking to determine the binding affinity and interactions of the novel compounds, this study would be able to determine a novel compound that can inhibit the quorum sensing activity of the selected waterborne pathogens.[21]

This study aims to identify novel compounds that have potential quorum sensing inhibitory mechanisms against selected waterborne pathogens. With ligand-based screening techniques, compounds with high structural similarity with the known QS inhibitors were identified. Moreover, the molecular docking experiment predicted the binding interaction of these compounds to determine the solubility coefficient of the candidate compounds with the QS-associated enzymes in the selected waterborne pathogens.[22] Lastly, assessing the LogD of the compounds can be associated with the solubility and distribution efficiency of the compound in a particular environmental pH.[22]

MATERIALS AND METHODS

Ligand-based Screening of Candidate Compounds

The known inhibitory ligands of the quorum-sensing associated enzymes AgrC and AgrA of *Staphylococcus aureus* are AIP-III (CID: 102228828) and savirin (CID: 3243271) respectively, while for the enzymes ComA and ComE of *Streptococcus pneumoniae*, the known inhibitory compounds are 1,3-disubstituted ureas (CID: 9509) and fluoride (CID: 28179), respectively. For the enzymes of *Salmonella typhi*, namely LrsB and Hfq, the known inhibitory ligand is rifampicin (CID: 135398735). Lastly, for the enzymes LsrF and LsrK of *Escherichia coli*, the known inhibitory ligands are (3-hydroxy-2-oxopropyl) dihydrogen phosphate (CID: 77620531) and celastrol (CID: 122724), respectively. The 3D structure of these compounds was exported from PubChem (www.pubchem.ncbi.nlm.nih.gov) and imported in Ligand-Based Virtual Screen-Workflow Builder (Mcule, USA). The similarity threshold was adjusted until at least 1 compound is detected.

Virtual Molecular Docking of the Candidate Compounds

Using the identified active and binding sites for each enzyme, the candidate compounds were docked to their respective enzymes using Mcule. Before docking, the preparation of the enzymes was followed based on a previous study.[23] The 3D structure of the candidate compounds was retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov). The researchers downloaded the crystal structures of the QS-associated enzymes ComA (PBD ID: 5XE8), ComE (PBD ID: 4CBV), LsrF (PBD ID: 3GLC), LsrK (PBD ID: 5YA0), AgrC (PBD ID: 4BXI), AgrA (PBD ID: 4G4K), LsrB (PBD ID: 5GTA), and Hfq (PBD ID: 2YLC) from Protein Database (https://www.rcsb.org/). The predicted docking score with <-8.0kcal/mol was considered a high binding affinity.[23] The binding interactions of the compounds were visualized using JSMol (JMol Development Team, USA).

Evaluation of the LogD values of the Candidate Compounds

The canonical smiles of the compounds were exported from PubChem and imported in ChemAxon (USA) to calculate their LogD value at different pH. The different pH considered pertains to the optimal growth rate pH of the pathogens, as follows: *E. coli* (pH 6.5 and 7.5); *S. typhi* (pH 4.0 and 9.0); *S. aureus* (pH 4.0 and 9.8); and *S. pneumoniae* (pH 6.5 and 8.3).

RESULTS

Ligand-based Screening of Candidate Compounds

Compounds that were considered to be similar to the known inhibitors have a threshold value of 0.4 to 0.8, as shown in Table 1. In *S. typhi*, the known inhibitor of *LsrB*, rifampicin, has three structurally similar compounds at the threshold value of 0.4. The Hfq inhibitor, rifampicin, has three similar compounds at the threshold value of 0.4. In *E. coli*, the known inhibitor of LsrF, (3-hydroxy-2-oxopropyl) dihydrogen phosphate, has three structurally similar compounds at the threshold value of 0.6. In contrast, LsrK inhibitor celastrol has three similar compounds at a threshold value of 0.8. In *S. aureus*, the known inhibitor of AgrC, Autoinducing peptide (AIP) III, has two structurally similar compounds at a threshold value of 0.7. In contrast, AgrA inhibitor savirin only has one similar compound at a threshold value of 0.7. In *S. pnuemoniae*, the known inhibitor of ComA, fluoride, has two structurally similar compounds at the threshold value of 0.8; whereas, ComE inhibitor, 1,3-Disubstituted Urea, has 49 similar compounds at a threshold value of 0.7.

Virtual Molecular Docking of the Candidate Compounds

The 3D structure of the novel compounds was docked in the crystal structure of the different quorum sensing associated proteins on the selected bacteria, namely *S. typhi*, *E. coli*, *S. pneumoniae*, and *S. aureus*. The binding affinity of the novel compounds with the different quorum sensing enzymes is shown in Table 1. Compounds with the most negative docking scores (<-8 kcal/mol) were considered high binding affinities.[24] In *S. typhi*, all the novel compounds have a low binding affinity to LsrB and Hfq. Meanwhile, in *S. aureus*, only 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one, has a high binding affinity to AgrA, while the rest of the compounds has a low binding affinity to AgrC. In *S. pneumoniae*, two compounds, namely 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl]urea, have a high binding affinity to ComE, while the rest of the compounds has a low binding affinity to ComA. In *E. coli*, the novel compounds 2-(1H-imidazol-5-yl) butanedioic acid, 2-(1H-imidazol-5-yl)butanoic acid, and 2-(1H-imidazol-5-yl)-3-methylbutanoic acid has a low binding affinity to LsrF. Meanwhile, the visual structure for LsrK cannot be generated.

The main non-covalent interactions between the novel compounds and the different quorum sensing enzymes were hydrophobic interactions and hydrogen bonds,

Table 1: Novel compounds similar with the known inhibitors of the selected waterborne pathogens' QS Enzyme and their corresponding threshold and docking score.

Organism	QS Enzyme	Known Inhibitor	Chemical ID (CID)	Novel Compound (IUPAC Name)	Threshold	Docking score
S. typhi	LsrB	rifampicin	135398735	N-(5-Ethoxy-2-methyl-2,3-dihydro-1-benzofuran-6-yl)cyclohex-3-ene-1-carboxamide	0.4	-0.5
	Hfq	rifampicin	135398735	2-(Cyclopent-2-en-1-yl)-N-(5-ethoxy-2-methyl-2,3-dihydro-1-benzofuran-6-yl) acetamide	0.4	-2.4
				N-(5-Ethoxy-2-methyl-2,3-dihydro-1-benzofuran-6-yl)-2-methoxy-5-methylbenzamide	0.4	-2.9
E. coli	LsrF	(3-hydroxy-2-oxopropyl) dihydrogen phosphate	77620531	2-(1*H*-imidazol-5-yl)butanedioic acid	0.6	-5.35
				2-(1H-imidazol-5-yl)butanoic acid	0.6	-4.87
				2-(1*H*-imidazol-5-yl)-3-methylbutanoic acid	0.6	-5.12
	LsrK	celastrol	122724	(2R,4aS,6aR,6aS,14aR,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylic acid	0.8	cannot be generated
				(2S,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylic acid	0.8	cannot be generated
				10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylic acid	0.8	cannot be generated
S. aureus	AgrC	autoinducing peptide (AIP) III	102228828	(3R,4R)-1-(7a-Methyl-5-oxo-2,3,6,7-tetrahydropyrrolo[2,1-b][1,3]thiazole-3-carbonyl)-4-phenylpyrrolidine-3-carboxylic acid	0.7	-4.05
				(3R,4R)-1-(3-Pentanoyl-1,3-thiazolidine-4-carbonyl)-4-phenylpyrrolidine-3-carboxylic acid	0.7	-3.92
	AgrA	savirin	3243271	3-[(4-Methylphenyl)sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H)-one	0.7	-8.85
S. pneumoniae	ComA	fluoride	28179	hydron;fluoride	0.8	-0.8
				fluorane	0.8	-0.8
	ComE	1,3-Disubstituted Urea	9509	1,3-bis[4-(dimethylamino)phenyl]urea	0.7	-5.825
				1-(4-aminophenyl)-3-[4-(dimethylamino) phenyl]urea	0.7	-6.125
				1-[4-(methylamino)phenyl]-3-phenylurea	0.7	-6.5
				N'-[4-(methylamino)phenyl]-N-phenylcarbamimidate	0.7	-6.375
				1-phenyl-3-[4-(phenylcarbamoylamino) phenyl]urea	0.7	-7.7
				1,3-bis(4-aminophenyl)urea	0.7	-6.1
				1-(4-aminophenyl)-3-phenylurea	0.7	-6.775
				1-[4-(dimethylamino)phenyl]-3-phenylurea	0.7	-6.0
				1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea	0.7	-8.55
				1-[4-(dimethylamino)phenyl]-3-naphthalen-1-ylurea	0.7	-7.15

Continued...

Table 1: Cont'd.

Organism	QS Enzyme	Known Inhibitor	Chemical ID (CID)	Novel Compound (IUPAC Name)	Threshold	Docking score
				1-phenyl-3-[5-(phenylcarbamoylamino) naphthalen-1-yl]urea	0.7	-8.125
				1,1-dimethyl-3-[4-(methylamino)phenyl]urea	0.7	-5.125
				1-(4-aminophenyl)-1,3-dimethylurea	0.7	-5.2
				3-[4-(dimethylamino)phenyl]-1,1-dimethylurea	0.7	-5.325
				1-methyl-3-[4-(methylcarbamoylamino) phenyl]urea	0.7	-5.25
				3-[4-(dimethylcarbamoylamino)phenyl]-1,1-dimethylurea	0.7	-5.2
				1-(4-aminophenyl)-3-methylurea	0.7	-5.2
				1-methyl-3-[5-(methylcarbamoylamino) naphthalen-1-yl]urea	0.7	-6.375
				1,3-dimethyl-1,3-diphenylurea	0.7	-6.8
				1-methyl-1,3-diphenylurea	0.7	-6.7
				1,3-diphenylurea	0.7	-6.625
				1,3-dinaphthalen-1-ylurea	0.7	-7.5
				1-methyl-3-naphthalen-1-yl-1-phenylurea	0.7	-7.5
				1-naphthalen-1-yl-3-phenylurea	0.7	-7.3
				1-naphthalen-2-yl-3-phenylurea	0.7	-7.275
				1,3-diphenyl-1-[4-(phenylcarbamoylamino) phenyl]urea	0.7	-7.725
				1,3-bis(4-anilinophenyl)urea	0.7	-7.9
				1-(4-anilinophenyl)-3-phenylurea	0.7	-7.35
				1,3-dimethyl-1-phenylurea	0.7	-5.6
				1,1,3-trimethyl-3-phenylurea	0.7	-5.625
				1-methyl-3-phenylurea	0.7	-5.05
				1,1-dimethyl-3-phenylurea	0.7	-5.5
				1,1-dimethyl-3-naphthalen-2-ylurea	0.7	-6.8
				1-methyl-3-naphthalen-2-ylurea	0.7	-6.725
				1-methyl-3-naphthalen-1-ylurea	0.7	-6.875
				1,1-dimethyl-3-naphthalen-1-ylurea	0.7	-6.725
				1-(4-anilinophenyl)-3-methylurea	0.7	-6.35
				[4-(dimethylamino)phenyl]urea	0.7	-5.3
				[4-(carbamoylamino)phenyl]urea	0.7	-5.125
				(4-aminophenyl)urea	0.7	0.15
				1-(4-aminophenyl)-3-[3-(dimethylamino) phenyl]urea	0.7	-6.225
				N-[4-[[4-(dimethylamino)phenyl] carbamoylamino]phenyl]acetamide	0.7	0.3
				N-methyl-N-[4-(phenylcarbamoylamino) phenyl]acetamide	0.7	-6.775
				1,3-bis[4-(diethylamino)phenyl]urea	0.7	0.25
				N-[4-[(4-acetamidophenyl)carbamoylamino] phenyl]acetamide	0.7	-6.5
				N-[4-[[4-[acetyl(methyl)amino]phenyl] carbamoylamino]phenyl]-N-methylacetamide	0.7	0.1625
				1-[4-(diethylamino)phenyl]-3-phenylurea	0.7	-6.55
				N-[4-(phenylcarbamoylamino)phenyl] acetamide	0.7	0.1
				1-[4-(ethylamino)phenyl]-3-methylurea	0.7	-5

as shown in Table 2. Out of all the novel compounds found to be structurally similar with the known inhibitors, only three novel compounds are found to have high binding affinity; namely, 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one from *S. aureus*, and 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl]urea from *S. pneumoniae*. The binding interactions of these quorum sensing-associated enzymes and novel compounds with various amino acids are shown in Table 2.

In *S. typhi*, most novel compounds are docked in chain A of LsrB; wherein there are more hydrophobic interactions than hydrogen bonds present. Similarly, there are more hydrophobic interactions in Hfq than hydrogen bonds.

In *E. coli*, most of the novel compounds docked in chain E of LsrF exhibited more hydrogen bond formation than hydrophobic interactions. However, the crystal structure for LsrK cannot be generated.

In *S. aureus*, the amino acids where most of the novel compounds were docked in chains A and B of AgrC. Both novel compounds (3R,4R)-1-(7a-Methyl-5-oxo-2,

Table 2: Binding interactions of the novel compounds with high affinity to various quorum sensing enzyme and their corresponding amino acid residue.

Organism	QS enzyme	Known Inhibitor	Novel Compound	CID	Binding Interaction	Amino Acid
S. aureus	AgrA	Savirin		3243271	6 hydrophobic interactions	phe 161A asn 177B arg 78B arg 178B tyr 229A tyr 229A
					1 hydrogen bond	glu 163A
			3-[(4-Methylphenyl) sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H)-one	3244855	6 hydrophobic interactions	tyr 153B asp158B leu 175B (2) asp 176B glu 226A
					2 hydrogen bonds	asp 158B tyr 229A
S. pnuemoniae	ComE	1,3-Disubstituted Urea		9509	5 hydrophobic interactions	ile 29A ile 31A thr 128A lys 129A leu 133A
			1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino) naphthalen-1-yl]urea	27190755	12 hydrophobic interactions	val 3A (2) ile 29A pro 30A ile 31A leu 54A ile 125A (2) thr 128A lys 129A (2) leu 133A
					2 hydrogen bonds	lys 2A (2)
			1-phenyl-3-[5-(phenylcarbamoylamino) naphthalen-1-yl]urea	4469005	10 hydrophobic interactions	val 3A (2) ile 29A pro 30A ile 31A leu 54A ile 125A leu 133A asn 145A asp 150A
					3 hydrogen bonds	pro 30A asp 150A

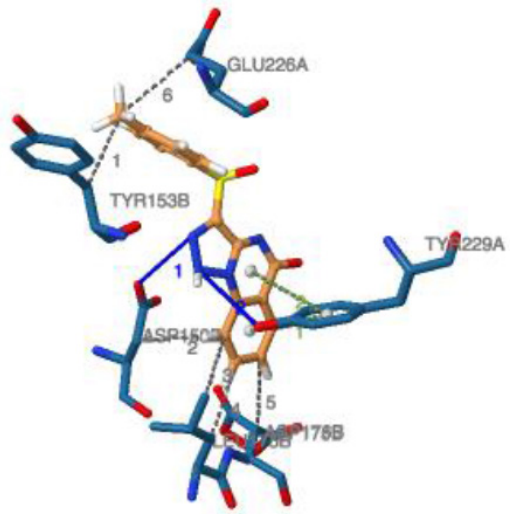

Figure 1: Crystal Structure of the (A) known inhibitor Savirin and the (B) ligand 3-[(4-Methylphenyl)sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H) with AgrA.

3,6,7-tetrahydropyrrolo[2,1-b][1,3]thiazole-3-carbonyl)-4-phenylpyrrolidine-3-carboxylic acid and (3R,4R)-1-(3-Pentanoyl-1,3-thiazolidine-4-carbonyl)-4-phenylpyrrolidine-3-carboxylic acid have 2 hydrophobic interactions and 2 hydrogen bonding. In AgrA, the novel compound 3-[(4-Methylphenyl)sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H)-one has 6 hydrophobic interactions and 2 hydrogen bonding in two distinct amino acids. 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo [1,5-a]quinazolin-5(4H)-one docked on AgrA of *S. aureus*, showed to be one among the three novel compounds which have the highest binding affinity out of all the novel compounds in this study, as shown in Figure 1. Savirin, the known inhibitor for *S. aureus*, was docked on AgrA and showed six hydrophobic interactions and one hydrogen bond in chain A, as shown in Figure 1. Among the compounds, the known inhibitor savirin has the least hydrogen bonding but showed to have the most number of hydrophobic interactions along with 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one, which showed to have the most number of non-covalent interactions.

In *S. pneumoniae*, most novel compounds were docked in chain A of isoleucine, leucine, and lysine. In ComA, no hydrophobic interactions and hydrogen bonding were noted. In ComE, there are more hydrophobic interactions present than the number of hydrogen bonds since most of the novel compounds have 1-12 hydrophobic interactions compared to the 1-6 hydrogen bond present various amino acids.

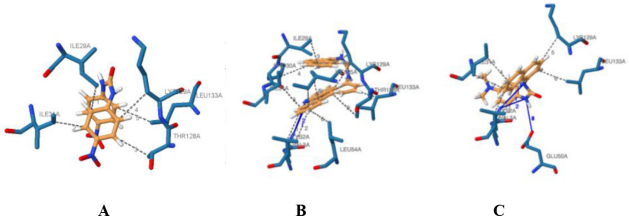

Figure 2: Crystal Structure of the A: known inhibitor (A) 1,3-Disubstituted Urea, and ligands (B) 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and (C) 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl] urea with Com E.

Notably, among the novel compounds for ComE of *S. pneumoniae*, 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl]urea, as shown in Figure 2 , are found to have the highest binding affinity out of all 49 novel compounds. Furthermore, a known inhibitor for *S. pneumoniae*, 1,3-disubstituted urea, was docked to ComE and showed five hydrophobic interactions and no hydrogen bonding, as shown in Figure 2.

Assessment of the LogD values of the candidate compounds

The known inhibitors whose corresponding novel compounds scored <-8 kcal/mol in the docking experiment were evaluated for their LogD, as shown in Table 3. These known inhibitors were Savirin docked on AgrA of *S. aureus* and 1,3-Disubstituted Urea docked on ComE of *S. pneumoniae.* The Log D of the counterpart novel compounds, namely 3-[(4-Methylphenyl)sulfonyl] [1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one that was docked on AgrA of *S. aureus*, 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl] urea, and 1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl] urea that was docked on ComE of *S. pneumoniae* were compared with that of the known inhibitors.

DISCUSSION

Virtual screening method has been used in the drug discovery process for lead detection, lead optimization, and scaffold hopping.[25] *In silico* screening method offers an affordable and accessible alternative to high-throughput screening for discovering new drugs.[26] It can also assess the potential toxicity of the compounds and predict the binding interaction of the drugs to vulnerable protein targets.[27] The researchers found novel compounds with structures similar to that of the known inhibitors of the four selected waterborne pathogens, and these findings were used in molecular docking to test whether the novel compounds have high binding affinities.

Table 3: LogD value of the known inhibitors and novel compounds that has binding affinity with the respective QS enzymes at the optimal pH of its corresponding bacteria.

Organism	Compounds	pH					
		4	6	7.4	7.8	8	9
S. aureus (AgrA)	Savirin	2.56	2.56	2.56	-	2.56	2.56
	3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one	1.83	1.82	1.82	-	1.82	1.82
	1,3-Disubstituted Urea	-	3	-	3	3	3
S. pneumoniae (ComE)	1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea	-	7.23	-	7.23	7.23	7.23
	1-phenyl-3-[5-(phenylcarbamoylamino)naphthalen-1-yl]urea	-	5.25	-	5.25	5.25	5.25

Recent studies regarding the novel compounds against *S. typhi* have not been associated with quorum sensing and antimicrobial properties. However, the following compounds derived from the same functional group had shown quorum sensing properties towards the different pathogen. Phenazine-1carboxamide had shown inhibition towards *Pseudomonas*.[28] N-(4-chloro-phenyl)-2-[5-[4-(pyrrolidone-1-solfonyl)-phenyl]-[1,3,4] oxadiazol-2-yl-sulfanyl]-acetamide inhibits the adherence of *E. coli* to the human ladder cells.[29] N,N-Diethyl-m-methylbenzamide (DEET)-based repellent for *Culex pipiens pallens* is primarily a vector of *Wuchereria bancrofti*.[30]

Likewise, the novel compounds against *E. coli* showed no studies on their quorum-sensing nor antimicrobial properties in recent years. However, other butanoic acid derivatives have shown quorum sensing and antimicrobial activity. *Melia dubia* extract containing myristic acid methyl ester has potential quorum quenching properties against *E. coli*.[31] 4-(4,5-dibromo-1-methyl-1H-pyrrole-2-carboxamido) butanoic acid isolated from *Agelas* sp. inhibited QS of *C. violaceum*.[32] Additionally, 3-methylbutanoic acid and 2-methylbutyric acid had shown antibacterial activities is produced by *Psuedomonas*.[33]

Furthermore, two more compounds of *E. coli* show modulation of quorum sensing activities in *Pseudomonas spp*. 1H-pyrrole-2-carboxylic acid and Phenazine carboxylic acid inhibit quorum sensing and related virulence factors of *Pseudomonas aeruginosa*.[34,35] However, these mechanism is not yet demonstrated in *E. coli*.

The novel compound against *S. aureus* does not show significant findings associated with quorum sensing and antimicrobial properties. A similar compound from the family of one of the novel compounds, 2-(4-methylphenyl)-1,3-thiazole-4-carboxylic acid and 9H-xanthene-9-carboxylic acid, inhibits *S. aureus* infections by binding C terminus of AgrA and disrupt AgrA-DNA binding.[36] This mechanism is similar with its known inhibitor, Savirin, which had shown to block autoinduction in *S. aureus*.[37]

In this study, the researchers considered -8 to -11 kcal/mol as those with high binding affinities, following studies stating that those with higher binding affinity tend to have more unbound molecules than smaller ones with more negligible binding affinity.[38] The principle of Gibbs free energy (ΔG) explains this occurrence, hence act as the basis of changes and stability in protein-ligand binding. The more there is free energy, the more negative the docking scores are, which equates to higher binding affinity. Such change only occurs when Gibbs free energy is negative due to solvent-entropy gain and enthalpy decrease overcompensating the unfavorable contributions of enthalpy increase and entropy decrease. Thus, achieve the state of equilibrium at constant pressure and temperature.[25] Scores of <-15 kcal/mol tend to be too tight for the average half-life of human proteins, and thus, were not considered in this study.[39] The docking score generated through molecular docking reflects the binding affinity of the ligands to their receptors, which means that the more negative the docking score, the higher the binding affinity. Compounds that demonstrated high binding affinities are 1-phenyl-3-[5-(phenylcarbamoylamino) naphthalen-1-yl]urea, with a docking score of -8.125 kcal/mol, and 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea, with a docking score of 8.55 kcal/mol, that were both docked on ComE of *S. pneumoniae*; and 3-[(4-Methylphenyl)sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H)-one, with a docking score of -8.85 kcal/mol, that was docked on AgrA of *S. aureus*.

Understanding the mechanisms of quorum sensing inhibition in designing drugs is critical to analyze the protein-ligand interactions. The intermolecular binding

interactions present in the novel compounds and the different quorum sensing enzymes, such as hydrogen bonding and hydrophobic interactions, are critical factors in stabilizing the favored ligands of the compound.[40] Hydrophobic interactions are essential for folding proteins, keeping them stable, biologically active, and decreasing undesirable interactions with water.[41] This type of interaction is also considered the main driving force in drug-receptor interactions.[42] Likewise, hydrogen bonds also contribute to the stability of the protein-ligand complex, taking into consideration the H-bond donor and acceptor present, which can indicate whether the protein-ligand complex has a weak or strong interaction. H-boding pairing is a practical design for ligands with high binding affinity because it is considered the facilitator for binding ligands to specific proteins. However, H-bond donors and acceptors can affect the binding affinity results when establishing a robust protein-ligand interaction since it causes the pairings to have synergistic strong-strong or weak-weak H-bonding capacity. A mixed strong-weak H-bond pairing decreases the binding affinity of the compound.[43]

The Agr quorum-sensing system in *S. aureus* is essential for virulence regulation by increasing the expression of toxins and degradative exoenzymes.[44] The Agr system coordinates the transition to an invasive mode, which involves increased virulence factor development and decreased surface proteins. AgrA directly controls the expression of many genes associated with virulence regulation. It induces the gene transcription of the phenol-soluble modulin (PSM) α and β proteins30 and the AgrBDCA operon at the P2 promoter and the regulatory RNA, RNAIII, at the P3 promoter.[45] Inhibition of the Agr system entails the downregulation of the virulence factors that are often necessary for the progression of diseases such as infective endocarditis, skin and soft tissue infections, pneumonia, and septic arthritis, and osteomyelitis.[44] Impingement or complete obstruction of such a system is an effective means to weaken the virulence of staphylococcal pathogens and control the staphylococcal disease.[37]

S. pneumoniae's capsular polysaccharide (CPS) is a key virulence factor necessary for effective colonization of the host's nasopharyngeal tract and invasive infections in the blood and lungs.[46] Peptide pheromone, competence-stimulating peptide (CSP), regulates the acquisition of antibiotic resistance genes in *S. pneumoniae*. CSP binds to the ComD receptor, which activates the ComE transcription factor, tagged as the "master regulator of competence"[47] to initiate DNA uptake and integration into the *S. pneumoniae* genome. CSP-ComD/E also controls the expression of virulence factors needed for infection.[48] The attenuation of *S. pnuemoniae* infectivity may be made possible through the inhibition CSP, along with the ComE transcription factor, deeming it a therapeutic approach to counter clinical conditions caused by chronic biofilm, more specifically pneumococcal infections.[49,50]

Binding affinity is also influenced by various non-covalent intermolecular interactions such as hydrogen bonds and hydrophobic interactions.[51] Notably, understanding the concept of hydrogen bonding is also essential for predicting accurate protein-ligand binding since its contribution is considered to have significance in protein-ligand interactions. A strong hydrogen bonding is required for most ligands to have a high binding affinity.[52] However, the ability of the protein-ligand interactions to exhibit high binding affinities is attributed to the general non-covalent interactions present,[53] as in the case of this study, more hydrophobic interactions are seen in comparison to the number of hydrogen bonds. These hydrophobic interactions often contribute significantly to the binding affinity in ligands with large lipophilic groups.[53]

In *S. typhi*, novel compounds for both LsrB and LsrK have more hydrophobic interactions than hydrogen bonds present, moderately increasing the binding affinity of the QS enzymes with the novel compounds. However, compared to the other novel compounds in this study, the total non-covalent interactions present in LsrB and LsrK are lesser, hence lower binding affinities. Conversely, there are more hydrogen bonds than hydrophobic interactions present in the novel compounds for LsrF in *E. coli*. In contrast, the novel compounds for LsrK neither showed hydrogen bonds nor hydrophobic interactions as its crystal structure could not be generated. Likewise, the novel compounds for LsrF have lesser total non-covalent interactions that contribute to its low binding affinity.

In *S. aureus*, there is an equal number of hydrogen bonds and hydrophobic interactions present in the novel compounds for AgrC. At the same time, there are more hydrophobic interactions than hydrogen bonds present in 3-[(4-Methylphenyl) sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one, the only novel compound for AgrA. Of the two QS enzymes, the novel compound for AgrA has a more significant number of hydrophobic interactions. However, it also has a more significant total number of non-covalent interactions present, thus, increasing its binding affinity.

In *S. pneumoniae*, hydrophobic interactions and hydrogen bonds were not present in the novel compounds for ComA. On the other hand, there are more hydrophobic interactions than hydrogen bonds in the novel

compounds for ComE. Notably, the two novel compounds of ComE, namely, 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl] urea and 3-[(4-Methylphenyl)sulfonyl] [1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one, despite having a greater number of hydrophobic interactions, the total number of non-covalent interactions present for both is high, thus, increasing its binding affinity.

The LogD value measures the pH-dependent differential solubility of all species in the octanol and water system. Therefore it is considered a suitable descriptor for the lipophilicity of ionizable compounds.[54] LogD appears to be important for analyzing properties of candidate drugs in various biologic conditions with varying pH and a key factor that can determine binding affinity to target proteins.[55] It is used to measure the lipophilicity of candidate drugs, which contributes to its ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties and its solubility, permeability, potency, and selectivity. It can foresee the success or failure rate of drug discovery and development since it is used *in vitro* and *in silico* evaluation.[56] High lipophilicity, with a logD value of greater than 5, affects the properties as mentioned earlier as it tends to bind to hydrophobic targets rather than the target protein. At the same time, low lipophilicity can also affect the permeability and potency that can result in low efficacy of the compound. Generally, logD values ranging from 1-3 are considered to have optimal physicochemical and ADME properties for oral drugs with optimal bioavailability.[57,58]

The pH 4, 6, 7.4, 7.8, 8, and 9 were considered in the study to account for the environmental pH of the pathogens' optimal growth. Also, the physiologically relevant pH of the compound is in was considered, as well.[54] With the logD values of the known inhibitors set as the reference values, the compound with logD values near this is considered soluble in that specific environmental pH wherein the growth of the bacteria is optimal.

In *S. aureus*, the logD value of the novel compound for AgrA, 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one, is 1.84 at pH 4 and 1.82 at pH 6-9, which is considerably near the reference logD value of savirin, the known inhibitor for AgrA, which is 2.56 at pH 4-9, indicating that this novel compound has an optimal solubility in that specific pH.

Conversely, the log D values of the two novel compounds for ComE of *S. pnuemoniae*, namely, 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 3-[(4-Methylphenyl)sulfonyl][1,2,3] triazolo[1,5-a]quinazolin-5(4H)-one, is 7.23 and 5.25 respectively at pH 6-9. These logD values are considerably high in reference to the logD value of 1,3-disubstituted urea, the known inhibitor for ComE, three at pH 6-8 and 2.99 at pH 9. The predicted logD values of the novel compounds indicate that these are highly lipophilic compounds, which are more likely to permeate biological membranes, and can lead to undesired events *in vivo*.

CONCLUSION

In this study, the researchers gathered 63 structurally similar compounds (40-80%) to the known quorum sensing inhibitors of the various waterborne pathogens. LsrB and Hfq, QS enzymes for *S. typhi*, have one and two novel compounds, respectively. For *E. coli*, LsrF and LsrK QS enzymes have three novel compounds each. *S. aureus* QS enzymes AgrC have two novel compounds, and AgrA have one novel compound. Lastly, for *S. pneumoniae*, ComA enzyme has two novel compounds while ComE has 49 novel compounds. Out of these 63 compounds, only 3 compounds have demonstrated high binding affinities, namely 3-[(4-Methylphenyl)sulfonyl] [1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one of AgrA, the QS enzyme of *S. typhi* and the two novel compounds for ComE of *S. pnuemoniae*, namely, 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one. The high binding affinities of these compounds may be attributed to the high number of binding interactions, specifically the hydrogen bonds and hydrophobic interactions present. Apparently, the logD values of the novel compound 3-[(4-Methylphenyl) sulfonyl][1,2,3]triazolo[1,5-a]quinazolin-5(4H)-one is near the reference logD values of savirin, the known inhibitor for AgrA, indicating that this compound is soluble in the specific environmental pH wherein the growth of *S. aureus* is optimal. However, the logD values of the novel compound 1-naphthalen-1-yl-3-[5-(naphthalen-1-ylcarbamoylamino)naphthalen-1-yl]urea and 3-[(4-Methylphenyl)sulfonyl][1,2,3]triazolo[1,5-a] quinazolin-5(4H)-one are greater than the logD values of 1,3-disubstituted urea, the known inhibitor for ComE, indicating that these are highly lipophilic compounds in the specific environmental pH wherein the growth of *S. pneumoniae* is optimal. These findings may suggest the target enzyme of these compounds during QS. However, these findings are not yet conclusive and need to be verified further by *in vivo* and *in vitro* investigations.

Contribution Details

JS Nas formulated the concept and design of the study. All authors contributed with the literature search, experimental studies, data acquisition, and data analysis. K Bawar spearheaded the writing of the manuscript. JS Nas edited the manuscript. All authors read and approved the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

QS: Quorum-Sensing; **LogD:** Distribution coefficient; **AI:** Autoinducers; ΔG: Gibbs free energy; **CPS:** Capsular polysaccharide; **CSP:** compe-tence-stimulating peptide; **ADMET:** Absorption, Distribution, Metabolism, Excretion, and Toxicity.

SUMMARY

The alarming number of multidrug resistant bacteria motivates the scientific community to identify novel compounds and target proteins. The QS activity of the bacteria is the typical target of various drugs. In this study, through in silico experiment, different lead compounds were identified, which may modulate the QS activity in various waterborne pathogens.

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