MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Fadhlina *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 19 (2023) 553-562

RESEARCH ARTICLE

Molecular Docking Studies of an Isolated Angucycline of *Stereospermum fimbriatum*, a Novel Anti-MRSA Agent

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Abstract A novel angucycline, C1 isolated from *Stereospermum fimbriatum* stem bark was subjected to molecular docking studies on five main targets of penicillin-binding protein 2a (PBP2a), β-lactamase, DNA topoisomerase IV, dehydrosqualene synthase (CrtM) and sortase A (SrtA) for anti-MRSA activity. The binding sites and docking scores of known inhibitors (positive control) were compared with C1. Docking analysis was carried out by AutoDock 4.0 package. The binding site of C1 closely resembled the positive control in all screened receptors. Inhibition constant of C1 was lower than the positive control tested for PBP2a, β-lactamase, dehydrosqualene synthase and sortase A except against DNA Topoisomerase IV. Structure-activity relationship (SAR) analysis of C1 showed that 7-CO was the most significant contributor to its activity since it formed hydrogen bonds with four of the five screened receptors. Molecular docking of C1 indicated that C1 can be a good candidate for new anti-MRSA drug development.

Keywords: *Stereospermum fimbriatum*, molecular docking, Methicillin-resistant *Staphylococcus aureus* (MRSA), anti-MRSA plant, C1.

Introduction

The urge to look for new effective drugs to treat infectious diseases has risen as microorganisms are getting more resistance to the available antimicrobial drugs [1-2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections are a significant concern in both healthcare and community settings. MRSA is a type of bacteria that has developed resistance to many commonly used antibiotics, including methicillin and other beta-lactam antibiotics. Structurally modified penicillin-binding proteins such as PBP2a has a reduced binding affinity to β-lactams antibiotics, hence are responsible for the rise of this resistant bacteria [3-5]. The second main mechanism of resistance is via the production of a β-lactamase enzyme which can hydrolyse and destroy the structure of β-lactam antibiotic [6]. Therefore, penicillin-binding proteins and β-lactamase enzymes are the major targets during antibacterial drug discovery. Other important targets include DNA topoisomerase IV which is crucial for RNA transcription and DNA replication [5] as well as virulence factors such as staphyloxanthin and sortase A that contribute to the pathogenicity of various G+ and G- bacteria [7-9].

In recent years, there has been growing interest in exploring natural resources for potential antimicrobial properties against MRSA. Plants have served as a valuable source of medicinal remedies for humanity. Despite this, many ethno-medicinal plants have not undergone thorough scientific validation to confirm their therapeutic values [10]. *Stereospermum fimbriatum*, a member of the Bignoniaceae family, is one such unexplored species. Locally known as “Chicha”, *S. fimbriatum* has been traditionally utilized for treating stomachaches (shoot), and postpartum recovery (root). In Malaysia, the juice derived from *S. fimbriatum’s* leaves has been traditionally used to alleviate earaches, and itching [10]. However, there is a lack of comprehensive scientific studies that have been conducted to substantiate these traditional claims. Previous studies on the stem bark of *S. fimbriatum* had been reported on the antimicrobial

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Received: 16 March 2023
Accepted: 27 July 2023

activities and its bioactive compounds [10-12]. A previously isolated novel angucycline, coded as C1, from the stem bark of *S. fimbriatum* [1] was further analysed by molecular docking in the present study. In this study, docking analysis of a novel angucycline of *S. fimbriatum* might reveal the possible mechanism of action as well as highlights important structural features.

Materials and Methods

AutoDock 4.0 was used to perform docking calculations based on Lamarkian Genetic Algorithm (LGA). The research was conducted using the method described previously [5]. The Protein Data Bank (PDB) http://www.pdb.org was used to get three-dimensional (3D) receptor structures. Using Discovery Studio Visualizer v17.2.0.16349, water molecules were removed from the PDB files (PDB 1VQQ, PDB 1BLM, PDB ID 4EMV, PDB 2ZY1, PDB ID 1T2P). AutoDock tools Auto 1.5.6. was used to add polar hydrogen atoms and Gasteiger charges to the receptor. ChemDraw Ultra 12.0 was used to create the ligand structure, which was subsequently optimised using MMFF94 force field model by OpenBabel 2.4.1.

AutoDock tools 1.5.6. were used to assign Gasteiger partial charges to the ligand. During the docking computation, all single bonds in the evaluated ligands were considered rotatable. The center of the grid box was positioned at the center of the co-crystallized ligand in the receptor, and the box was sized 60 x 60 x 60 with a grid spacing of 0.375 Å in each dimension. Lamarkian Genetic Algorithm (LGA) was used for docking. Each docking computation produced 20 structures, which were assessed using default settings such as short-range Van der Waals contacts, electrostatic interactions, and hydrogen bonding. Discovery Studio Visualizer v17.2.0.1634 was used to create the images.

Results and Discussion

Molecular docking plays a crucial role in the virtual screening of promising ligands as part of the drug discovery process. In this study, a molecular docking analysis was conducted to determine the binding sites and affinity of C1 towards the five key proteins associated with anti-MRSA activity (penicillin binding proteins 2a, β-lactamase, DNA topoisomerase IV, dehydrosqualene synthase and sortase A). Additionally, this analysis identified significant structural characteristics of the compound that could be linked to its observed activity.

Penicillin Binding Proteins 2a (PBP2a)

Vanillic acid, a recognised MRSA inhibitor with a minimum inhibitory concentration (MIC) of 500 g/mL, was employed as a positive control [3]. C1 was found to bind strongly to the PBP2a receptor, with a minimum binding energy of -5.63 kcal/mol compared to -1.69 kcal/mol for the positive control. This affinity disparity was also seen in other metrics. The positive control and C1 had ligand efficiency of -0.47 and -0.07, respectively. The positive control and C1 had inhibition constants of 75.21 and 0.06 M, respectively (Table 1). The positive control and C1 had van der Waals energies of -5.09 and -2.17, respectively, while for electrostatic energy, the value was -1.13 and 0.18 kcal/mol, respectively. In comparison to the positive control, C1 had distinct interactions with the receptor (Figure 1A-E). A superimposed structure of co-crystalised ligand, C1 and positive control in docked conformations are illustrated in Figure 1C.

C1 established ten hydrogen bonds with various amino acids in the receptor, including Lys 406 with 7-CO and 8-OCH_3, Glu 447 with 11-OH and 12-CO, Tyr 446 and the A-ring, Thr 600 with 7-CO and 8-OCH_3, Ser 403, Ser 462, and Gly 599 with 7-CO. Furthermore, C1 exhibited van der Waals interactions with Asn 464, Ser 462, Thr 582, Ala 601, Asp 463, and Ser 598. Alkyl interactions were observed with Ala 642, Arg 445, and His 583. Regarding its structure, 8-OCH3, 11-OH, 12-CO, and 7-CO were found to contribute to the anti-MRSA activity of C1 by establishing hydrogen bonds with the receptor. Additionally, the alkyl interactions formed by 4-CH_3 and 5-CH_3 might have played a role in stabilizing the bonds between ligand and receptor.

Ceftobiprole is a fifth-generation cephalosporin-lactam antibiotic and approved by FDA. Previous studies [4, 13] reported that ceftobiprole established hydrogen bonds with Tyr 446, Lys 406, Ser 403, Ser 462, Asn 464, and Glu 447. In the present study, C1 was also observed to form hydrogen bonds with the active site. C1 also showed a lower MIC value at 6.25 µg/mL against MRSA in comparison to vanillic acid which was 500 µg/mL [1, 3]. C1 may be able to block cell wall formation in MRSA based on the formation of multiple hydrogen bonds with the active site, particularly Ser 403.

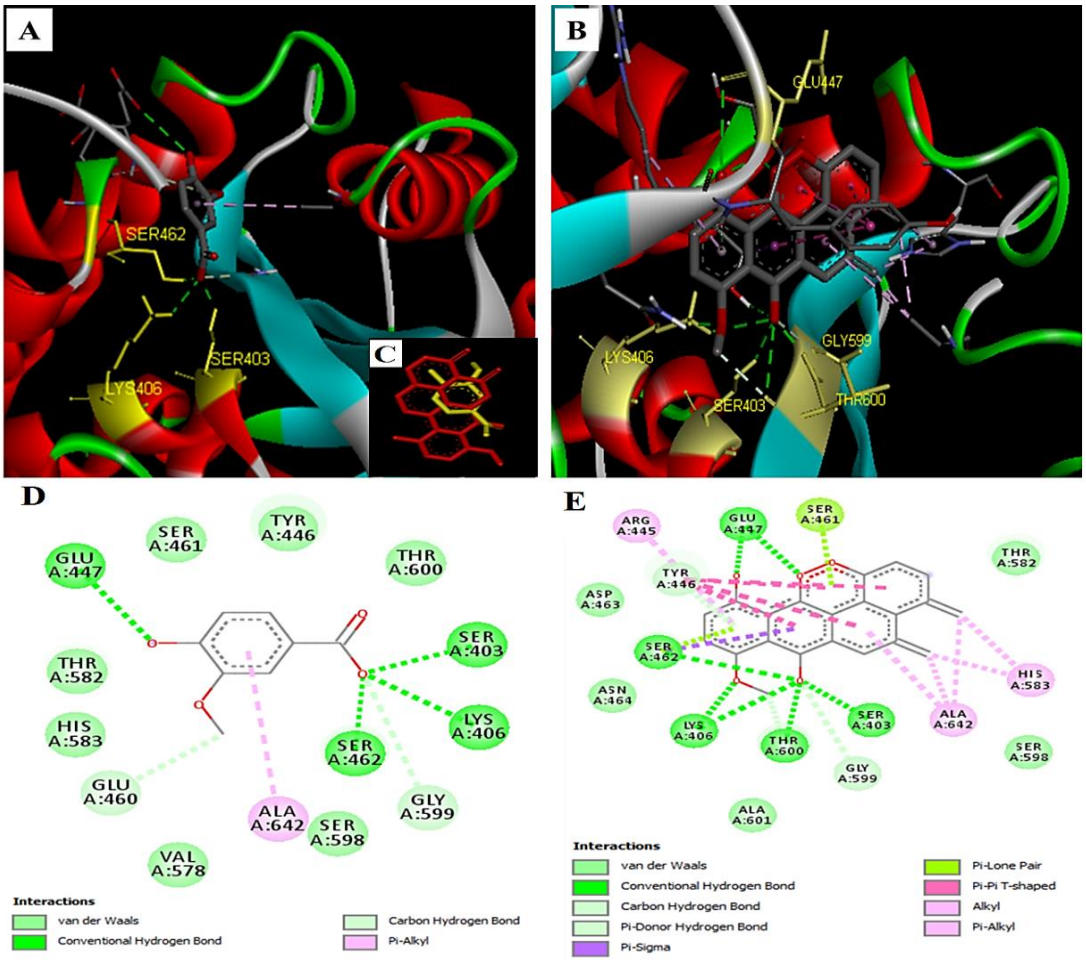

Figure 1. Binding site of vanillic acid (A) and C1 (B). Vanillic acid and C1 are presented as sticks while receptors are presented as cartoon style. The superimposed structure of vanillic acid (yellow) and C1 (red) in docked conformations (C). Interactions of vanillic acid (D) and C1 (E) in the binding site

β-Lactamase

Kaempferol was employed as a positive control as it has previously been shown to have a high affinity for the 3BLM receptor. The binding site of C1 was identical to kaempferol. C1 bound to the β-lactamase receptor with a least binding energy of -8.11 kcal/mol, while the binding energy of kaempferol was -6.77 kcal/mol. Kaempferol and C1 had ligand efficiency of -0.32 and -0.31, respectively. Kaempferol and C1 had inhibition constants of 10.88 and 1.14 μM, respectively (Table 1). Kaempferol and C1 had Van der Waals energy of -6.91 and -8.21, respectively. Meanwhile, for electrostatic energy, the value was -0.16 and 0.19 kcal/mol for Kaempferol and C1, respectively. In comparison to Kaempferol, C1 had slightly different interactions with the receptor (Figure 2A-E). Van der Waals interactions were formed between C1 and Glu 166, Ser 216, Asn 170, Ser 235. As for hydrogen bonds, the interactions were observed between C1 and Ser 70, Asn 132, Ser 130, Gln 237, and Tyr 105, while for alkyl, interaction formed between C1 and Ala 69, and C1 with Lys 234 and Lys 73 for pi-cation interaction. Pi-donor hydrogen bonds were also observed, for example, ring B and C with Ser 130, and ring A and B with Ser 70.

The anti-MRSA activity of the compound was enhanced through the formation of hydrogen bonds between specific amino acid residues in the receptor and certain functional groups in the compound's structure. More specifically, 7-CO (Tyr 105), 8-OCH3 (Asn 132), 11-OH (Gln 237 and Ser 70), and 12-CO (Gln 237) contributed to this activity by forming hydrogen bonds with the receptor, while the alkyl interactions of 5-CH3 may have served to stabilize the bonds between the compound and the receptor. Additionally, C1 formed hydrogen bonds with Ser 70 and Ser 130, a pi-anion bond with Lys 73, and van der Waals interactions with Glu 166, all of which contributed to its strong interaction with the catalytic residues in the active site. Thus, C1 might have the ability to suppress the β-lactamase and restore the activity of existing β-lactam antibiotics by combination therapy.

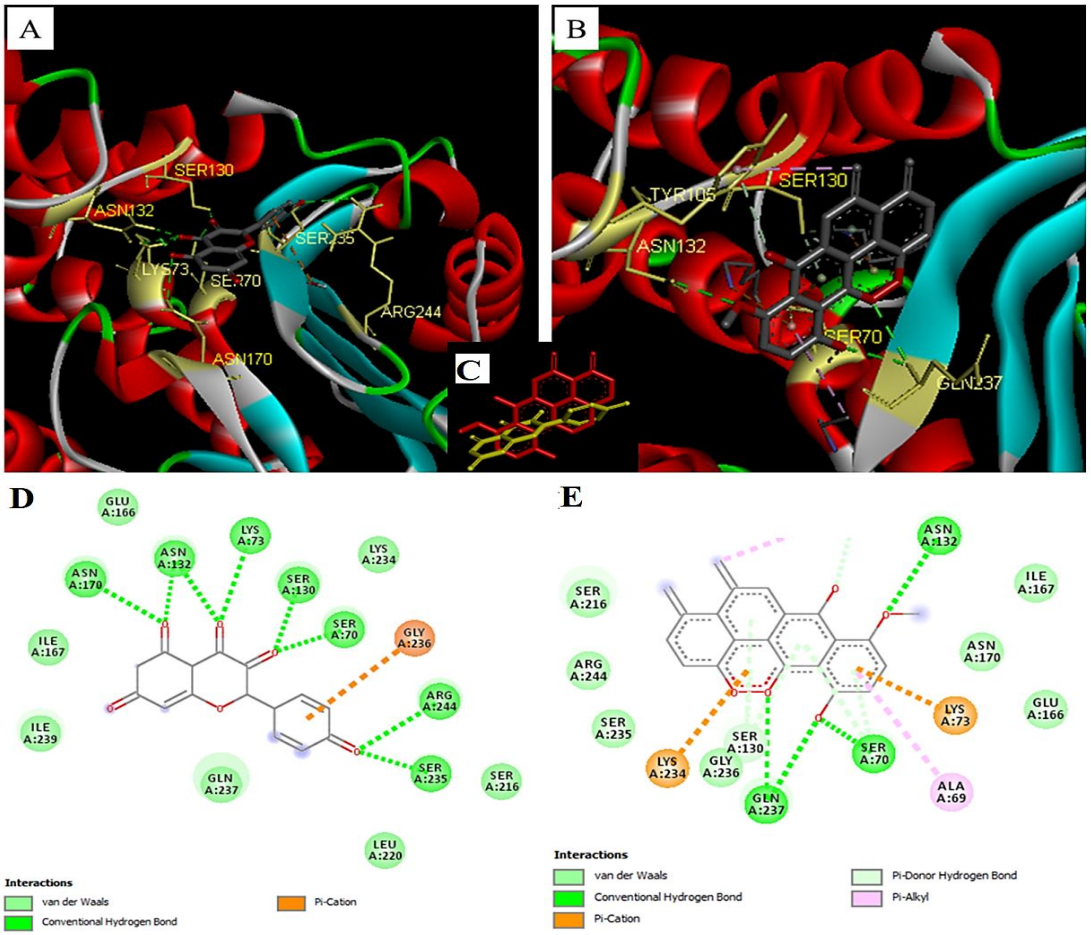

Figure 2. Binding site of kaempferol (A) and C1 (B). Kaempferol and C1 are presented as sticks while receptors are presented as cartoon style. The superimposed structure of kaempferol (yellow) and C1 (red) in docked conformations (C). Interactions of kaempferol (D) and C1 (E) in the binding site

DNA Topoisomerase IV

The positive control used for this analysis was co-crystallized ligand (5-(2-(ethylcarbamoyl)-4-(3-(trifluoromethyl)-1H-pyrazole-1-yl)-1H-pyrrolo[2,3-b]pyridin-5-yl)nicotinic acid). C1 exhibited a binding energy of -8.98 kcal/mol upon binding to the DNA topoisomerase IV receptor, which is comparable to the binding energy of the positive control co-crystallized ligand (-9.07 kcal/mol). The ligand-receptor parameters were also found to be similar. The positive control and C1 had ligand efficiency of -0.28 and -0.35, respectively. The positive control and C1 had inhibition constants of 0.22 and 0.26 µM, respectively (Table 1). Additionally, the van der Waals energy for the C1 and positive control were -9.54 and -10.05 kcal/mol, while the electrostatic energy values were 0.26 and -0.82 kcal/mol, respectively. C1 was found to interact differently with the receptor in comparison to the positive control despite having similar ligand-receptor docking scores (Figure 3A-E).

Hydrogen bonds were formed between the co-crystallized ligand and Asp 78, Asn 51, as well as Thr 172. There were also carbon-hydrogen interactions between the ligand and Met 83 as well as Pro 84. Hydrophobic interactions of the ligand with residues such as Arg 81, Glu 55, Ile 98, Met 83, Arg 140 and Val 122 further strengthened the ligand's binding to the receptor. C1, on the other hand, formed a hydrogen bond between its 11-OH group and Ser 124, as well as a carbon-hydrogen bond between its 7-CO group and Ala 52, and a hydrogen bond with Asp 78. Van der Waals interactions between C1 and the surrounding Val 76, Glu 55, Arg 81, Thr 172, Phe 99, His 120, Ile 48, and Ile 98 residues further stabilized its binding to the receptor. Additionally, pi-sigma interactions with Val 122 and Asn 51, as well as pi-alkyl interactions with Val 174 and Met 83 residues, were observed. There was no interaction observed at the lowest binding energy between C1 and residues such as Arg 140 and Pro 84.

The activity of the compound was contributed by the strong hydrogen bonds formed by 7-CO and 11-

OH with the receptor, while hydrophobic interactions may have stabilized the ligand-receptor bonds. In addition, docking interactions with DNA topoisomerase IV showed similarities between C1 and 2-(dimethoxymethyl)-1-hydroxyanthracene-9,10-dione, which has a similar backbone to C1 [5]. The MIC value of 2-(dimethoxymethyl)-1-hydroxyanthracene-9,10-dione against MRSA (two species) was found to be 3.9 and 7.8 µg/mL, whereas C1's MIC value against MRSA was 6.25 µg/mL [1, 5]. These results suggest that C1 acts as a DNA topoisomerase IV inhibitor. Furthermore, a synthesized azaindole's MIC value against MRSA was reported as 1.56 µM, while C1's MIC value was 35.7 µM, showing a 23-fold difference [14]. Interestingly, the docking scores of the azaindole and C1 were comparable despite the large difference in their MIC values.

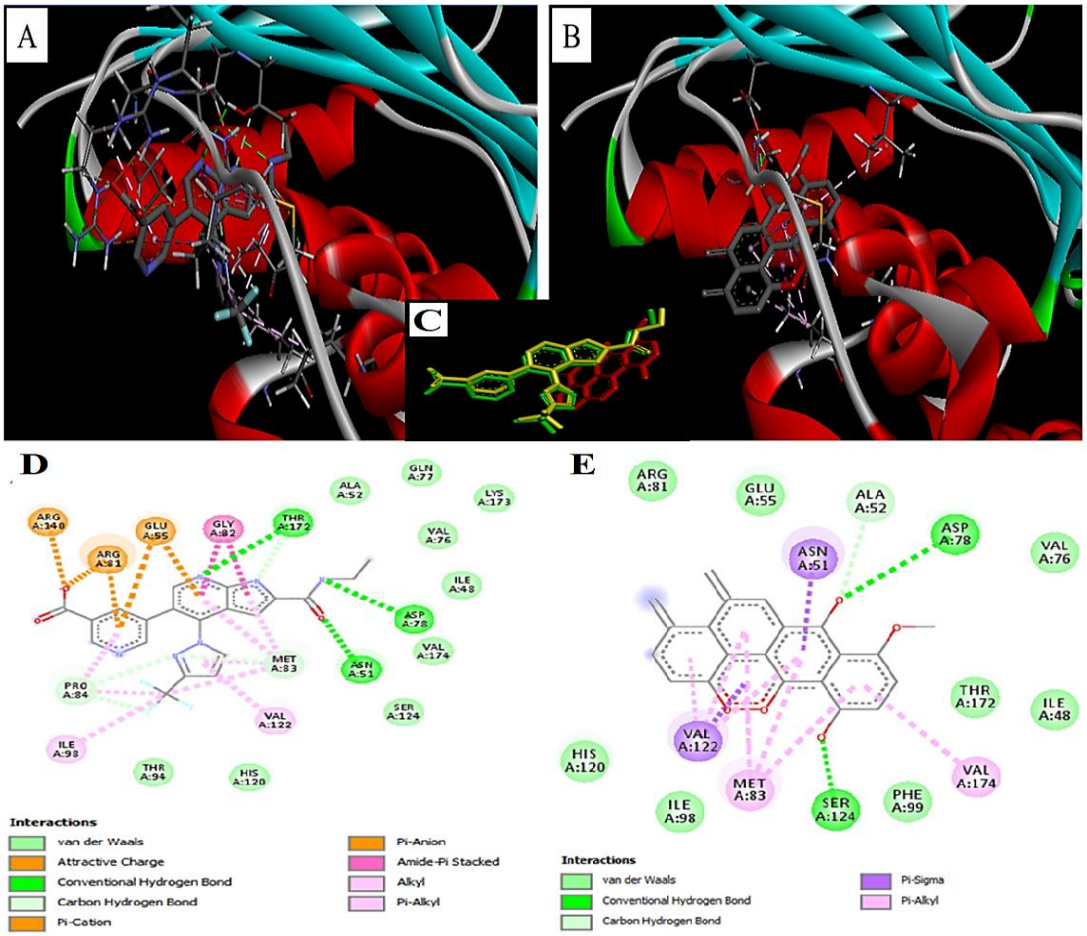

Figure 3. Binding site of co-crystalized ligand (A) and C1 (B). The co-crystalized ligand and C1 are presented as sticks while receptors are presented as cartoon style. The superimposed structure of C1 (red) and co-crystalized ligand (yellow), as well as co-crystalized ligand with its original X-ray coordinates (green) in docked conformations (C). Interactions of co-crystallized ligand (D) and C1 (E) in the binding site.

Dehydrosqualene synthase (CrtM)

In this study, a known CrtM inhibitor (Inhibitor 830) [9], was used as a positive control. The positive control was observed to dock with a least binding energy of -7.15 kcal/mol at the expected binding site, forming hydrogen bonds with Arg 45 and Gln 165 via amide CO and amide NH, respectively. Additionally, important interactions were noted, including van der Waals interactions with Phe 26, Tyr 41, Val 133, Ala 134, Gly 138, Leu 145, Gly 161, Leu 164, Asn 168, Ile 241, and Tyr 248. Pi-sigma interactions with Val 137 and Leu 141, and pi-alkyl interactions with Ala 157 and Leu 160 were also observed. Furthermore, a pi-pi-T shaped interaction with Phe 22 was noted.

C1 exhibited a least binding energy of -8.85 kcal/mol, which was lower than the positive control's binding energy of -7.15 kcal/mol, and similar ligand-receptor parameters. The Ligand efficiency of C1 and the positive control were -0.34 and -0.3, respectively. Additionally, the inhibition constant (Table 1) of C1 and

the positive control were found to be 0.32 µM and 4.98 µM, respectively. Van der Waals energy of C1 and the positive control were -9.11 and -9.79, respectively, meanwhile, for electrostatic energy, the values were -0.05 and 0.17 kcal/mol, respectively.

Hydrogen bonds of C1 were detected between 11-OH and Gln 165, as well as 8-OCH_3 with residues such as Arg 45 and Tyr 41. Van der Waals interactions of C1 were observed to have similar binding residues as the positive control such as Gly 161, Asn 168, and Tyr 248. The 4-CH_3 and 5-CH_3 of C1 formed alkyl interactions with Ala 134, Val 137, Leu 164, and Leu 141. The presence of 8-OCH_3 and 11-OH in the structure played a role in the anti-MRSA activity by forming strong hydrogen bonds, whereas 4-CH_3 and 5-CH_3 contributed to stabilizing the ligand-receptor bonds.

The CrtM enzyme plays a crucial role in the pathogenesis of methicillin-resistant S. aureus as a virulence factor [9]. Previous research has identified Arg 45 as the critical amino acid in the binding site, along with other notable residues such as Val 137, Leu 164, Gln 165 and Asn 168 [7, 9]. Hydrogen bonds of C1 were formed with residues such as Tyr 41, Arg 45, and Gln 165, as well as interaction through van der Waals with Asn 168, and alkyl interactions with Val 137 and Leu 164. The strong binding affinity of C1 to the binding site (Figure 4A-E), along with its interaction with all significant residues, suggests that its mechanism of action against MRSA may involve inhibiting the CrtM enzyme and staphyloxanthin production.

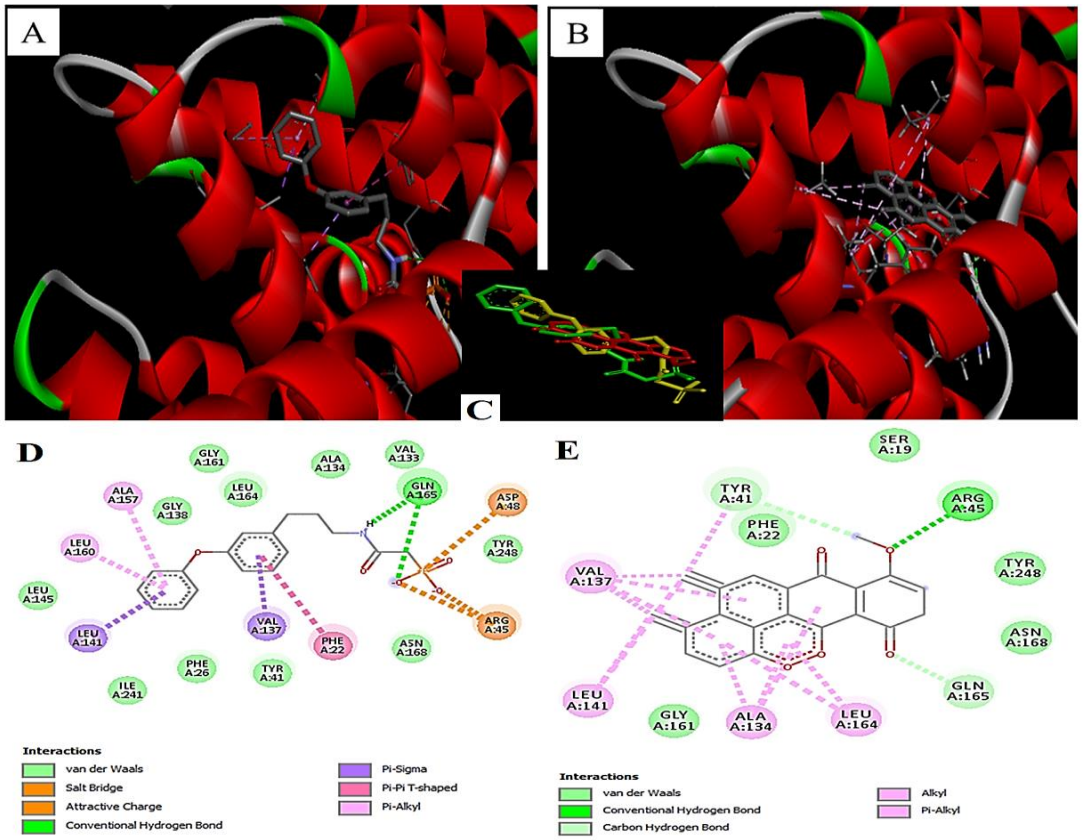

Figure 4. Binding site of Inhibitor 830 (A) and C1 (B). Inhibitor 830 and C1 are presented as sticks while receptors are presented as cartoon style. The superimposed structure of C1 (red) and Inhibitor 830 (yellow), as well as co-crystalized ligand with its original X-ray coordinates (green) in docked conformations (C). Interactions of Inhibitor 830 (D) and C1 (E) in the binding site

Sortase A (SrtA)

In this study, Myricetin, a well-known inhibitor of SrtA, was used as a positive control due to its strong binding affinity and ability to inhibit SrtA [15]. Myricetin had a binding energy of -6.27 kcal/mol. Interestingly, C1, showed a similar binding location and had higher binding energies of -9.06 kcal/mol. In terms of ligand efficiency, C1 and Myricetin had values of -0.35 and -0.27, respectively, and inhibition constants of 0.00023 µM and 0.025 µM respectively (Table 1). Furthermore, van der Waals energies for

C1 and Myricetin were -9.21 and -7.9 kcal/mol, respectively, while for electrostatic energies, the values were -0.15 and -0.45 kcal/mol, respectively.

Although the C1 binding site was similar to that of myricetin, there were slight differences in the way C1 interacted with the receptor (Figure 5A-E). C1 formed hydrogen bonds through the 8-OCH_3 and 7-CO groups with Arg 197, 11-OH with Glu 105 and Ser 116, 1-CO with Asn 114, and 5-CH3 with Val 168. There was no hydrogen bond formed with Gln 172. Additionally, van der Waals interactions were observed with Gly 167, Pro 163, Leu 169 and Ile 182, alkyl interactions with Ile 158, Val 166, Ile 199 and Val 201, and pi-sigma bond with Thr 180. As for the structural relationship, the 8-OCH_3, 7-CO, 11-OH, and 1-CO groups contributed to C1's activity by forming strong hydrogen bonds with important residues such as Glu 105, Ser 116, and Arg 197 while the 4-CH_3 and 5-CH_3 groups may have played a critical contribution in stabilizing the ligand-receptor bonds through their hydrophobic interactions.

According to previous research, the active site of transpeptidase enzymatic involved residues such as Glu 105, Ser 116, Thr 180, Thr 183, Asp 185, Try 187, Lys 196 and Arg 197 [16]. C1 was found to form hydrogen bonds with Glu 105, Ser 116, and Arg 197 as well as with Thr 180 through pi-sigma interactions. There was no interaction found with residues such as Thr 183, Asp 185, Try 187 and Lys 196. Nonetheless, owing to the establishment of multiple hydrogen bonds between C1 and SrtA's active side, C1 could potentially impede substrate binding to the SrtA catalytic site, thereby hindering the emergence of resistance.

In addition, a previous study on structure-activity relationship analysis identified important structural properties for potent SrtA inhibitors, and C1 met many of these requirements, including a minimum rotatable bonds' number, nitrogen, oxygen, and hydrogen atoms, unsaturation degree and other structural properties [8]. While myricetin's MIC value was reported to be 128 µg/mL [17], C1's MIC value was significantly lower at 6.25 µg/mL. Despite the 20-fold difference in MIC values, the docking scores suggested that myricetin and C1 have distinct mechanisms of action.

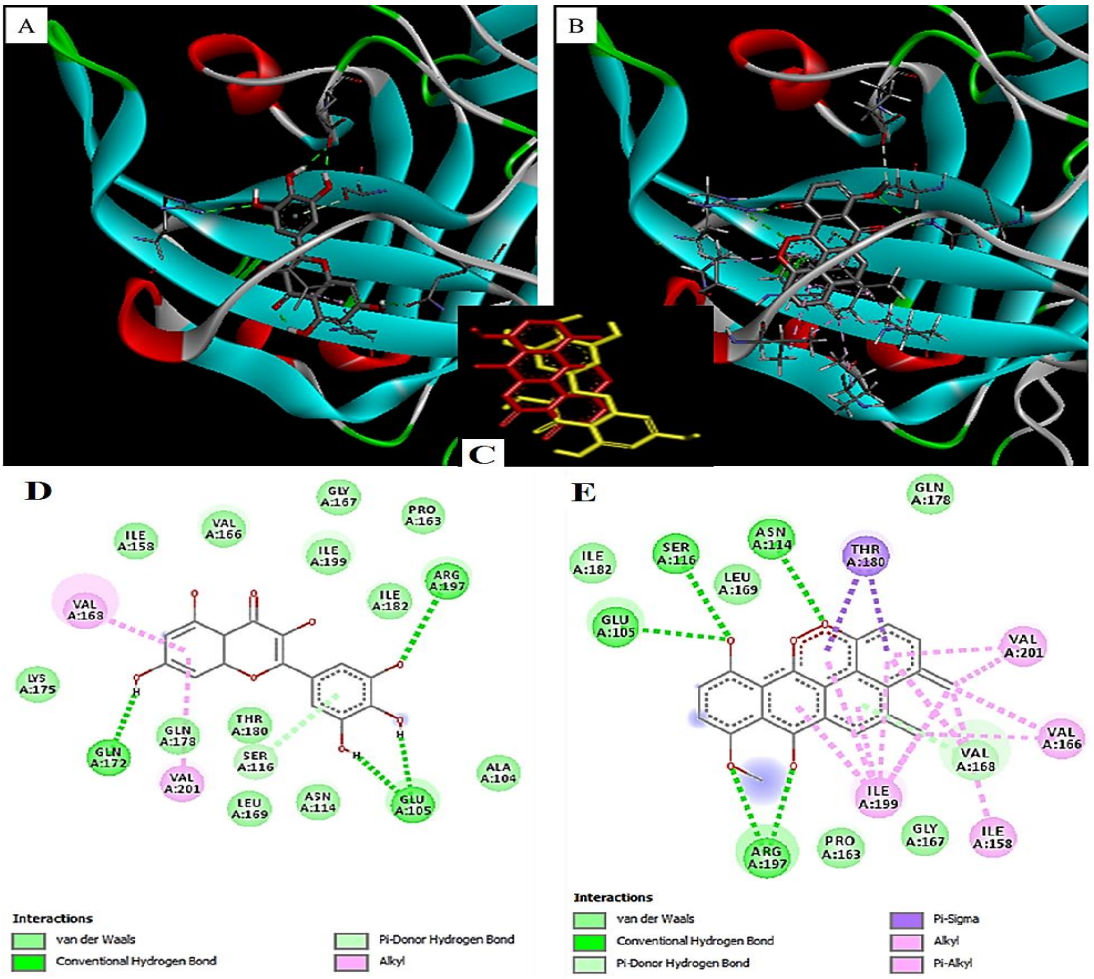

Figure 5. Binding site of myricetin (A) and C1 (B). Myricetin and C1 are presented as sticks while receptors are presented as cartoon style. The superimposed structure of C1 (red) and myricetin (yellow) in docked conformations (C). Interactions of myricetin (D) and C1 (E) in the binding site

Table 1. Summary of interacting active residues and main parameters for positive controls and C1

Receptors	Interacting active sites		No. of H-bond for C1	Inhibition constant (μM)		Binding energy (kcal/mol)	
	Positive control	C1		Positive control	C1	Positive control	C1
PB2a	Ser 403	Ser 403	1	75.21	0.06	-1.69	-5.63
β-lactamase	Glu 166, Lys 73, Ser 130, Ser 70	Glu 166, Ser 130, Ser 70	2	10.88	1.14	-6.77	-8.11
DNA Topoisomerase IV	Arg 140, Asn 51, Asp 78	Asn 51, Asp 78	1	0.22	0.26	-9.07	-8.98
Dehydrosqualene syntanse	Asn 168, Gln 165	Asn 168, Gln 165, Arg 45	2	4.98	0.32	-7.15	-8.85
Sortase A	Glu 105, Arg 197, Ser 116, Thr 180	Glu 105, Arg 197, Ser 116	3	0.025	0.00023	-6.27	-9.06

Conclusions

Structure-activity relationship (SAR) of C1 varied between various receptors. The functional groups of C1 were 1-CO, 4-CH_3, 5-CH_3, 7-CO, 8-OCH_3, 11-OH and 12-CO. Among these, 7-CO contributed the most to activity as it formed hydrogen bonds with 4 out of the 5 screened receptors such as PBP2a, β-lactamase, DNA Topoisomerase IV and Srt A. It was followed by 8-OCH_3 and 11-OH which formed hydrogen bonds with 3 receptors. The 8-OCH_3 formed hydrogen bonds with PBP2a, CrtM and Srt A, while 11-OH formed hydrogen bond with β-lactamase, CrtM and Srt A. Other functional groups contributed significantly to the anti-MRSA activity in only one receptor, where 12-CO and 5-CH_3 formed hydrogen bonds with β-lactamase only, while 1-CO formed hydrogen bonds with Srt A only. The 5-CH_3 and 4-CH_3 formed alkyl interactions with PBP2a, β-lactamase, CrtM and Srt A which might have contributed to stabilizing C1 in the active site. In conclusion, the novel compound C1, derived from *S. fimbriatum*, exhibited promising anti-MRSA activity, suggesting its potential as an alternative treatment for MRSA infections. However, it is crucial to emphasize that further comprehensive scientific research and clinical trials are necessary to evaluate the effectiveness and safety of C1 as an anti-MRSA agent.

Conflicts of Interest

The author(s) confirm that this article's content has no conflict of interest.

Acknowledgment

The authors would like to acknowledge the International Islamic University Malaysia (IIUM) for providing necessary resources and support in conducting this study. This work was supported by Postgraduate Research Grant (PGRG) No. 55209 from University Malaysia Terengganu.

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