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In Silico Analysis of Anthraquinone Analogues against Bacterial Proteins

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Abstract
The increasing number of multidrug-resistant bacteria has resulted in the emergence of new diseases, which lead to life-threatening infections in hospitalized patients worldwide. Beta-lactam antibiotics are still the most commonly used antibacterial agents in the present chemotherapeutic armamentarium. Thus, beta-lactamase and penicillin-binding protein (PBP) were chosen as the two targeted bacterial proteins because they promote antibacterial resistance specifically toward beta-lactam antibiotics.. In this study, natural compounds, anthraquinone analogues were studied for antibacterial activity by looking at their affinity towards beta lactamase and penicillin binding protein (PBP). using molecular docking method, structural-activity relationship (SAR) approach and Adsorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) prediction. 11 out of 21 anthraquinone analogues exhibited higher binding affinity towards beta-lactamase and PBP than the co-crystal ligands as control. These analogues were selected as hit compounds to predict ADMET and drug-likeness properties. Anthraquinone-2-sulfonic acid and physicon-1-O-glucoside showed the highest binding affinity towards beta-lactamase recorded free binding energy value of -8.83 kcal/mol and PBP recorded free binding energy value of -8.44 kcal/mol, respectively how good these compounds against the control which recorded free binding energy value of -8.60 kcal/mol and -7.95 towards beta-lactamase and PBP respectively. In terms of physicochemical and pharmacokinetic parameters, both of these compounds satisfied drug-likeness properties as anthraquinone-2-sulfonic acid showed molecular weight of 288.28 g/mol, log P value of 1.81, the ratio of hydrogen acceptor to hydrogen donor is 5 to 1 and only 1 rotatable bond while physicon-1-O-glucoside showed molecular weight of 428.39 g/mol, log P value of 0.82, the ratio of hydrogen acceptor to hydrogen donor is 9 to 3 and only 3 rotatable bond. Their promising antibacterial activities may be developed into different types of antibacterial agents such as oral drugs, transdermal drugs, colourants and also pesticides.

Keywords beta-lactamase; penicillin-binding protein (PBP); docking; structural-activity relationship; ADME-toxicity assessment

Introduction

Antibacterial agent is most commonly described as the agent that disinfects surfaces and eliminates potentially harmful bacteria. It can be classified into 5 major groups according to (i) type of action, (ii) sources, (iii) spectrum of activity, (iv) chemical structure and (iv) function. Its prophylactic function is to prevent the growth of bacteria causing diseases. However, resistant strains may develop

naturally due to the overuse of antibiotics. Thus, antibiotic resistance issues become a global public crisis for human health as bacteria develop the ability to defeat the drugs designed to kill them.

Beta-lactam antibiotics are still the most commonly used antibacterial agents in the present chemotherapeutic armamentarium. Beta-lactamase (β-lactamase), the enzyme that hydrolyzes beta-lactam antibiotics is the main factor contributing to antibacterial resistance. This is the most common mechanism of antibacterial resistance that happened in gram-negative bacteria. Besides, β-lactamases also evolved from PBPs, which also encounter covalent modification by penicillin and other beta-lactams. The amplification of PBPs and formation of PBPs that exhibited a low affinity for beta-lactam antibiotics contributed to antibiotic resistance. Thus, the development of new antibiotics is needed to increase the effectiveness to combat the development of antibacterial resistance. Natural compounds are found to exhibit better antibacterial activities compared to the marketed antibiotics to fight against multidrug-resistant bacteria.

Therefore, this encourages us to initiate the study of anthraquinone analogues to find the best candidate that can act as an antibacterial agent. Anthraquinone is a natural compound, which is commonly found in plants. Anthraquinone exhibited promising antimicrobial activities. In this study, different types of anthraquinone analogues are screened through in silico screening approach for their antibacterial activities. The candidate which consists of the best antibacterial properties is determined by analysing the affinity of the anthraquinone analogues toward the targeted protein. Next, the analysis of the structure-activity relationship is also performed to predict the best pharmacophore that poses antibacterial activity. Thus, it may guide the modification or synthesis of desirable new compounds to achieve the best antibacterial performance.

Materials and methods

Firstly, a list of anthraquinone analogues is retrieved from Mohammed (2016). 2D structure of anthraquinone analogues is sketched by using the Chemdraw sketching tool available at https://chemdrawdirect.perkinelmer.cloud/js/sample/index.html. The 3D structure of the ligands is drawn by Avogadro software (Hanwell et al., 2012). The chemical structure of each anthraquinone analogue is studied to recognize the location of functional groups. There are different functional groups joined to different carbon atoms. After that, the geometry of every anthraquinone analogue was optimized until the lowest energy is achieved. Then, each of these anthraquinone analogues is saved in .pdb files for molecular docking in the next step. The PDB files of the anthraquinone analogues are prepared for this study.

The molecular docking approach performed in this study is as followed by the methodology described by Ahmed & Shohael (2019). The 3D structure of beta-lactamase (β-lactamase) and penicillin-binding protein was retrieved from the online database called Protein Data Bank (PDB), with PDB ID: 3S1Y and 5OJ1 respectively (Berman et al., 2003). The website is available at https://www.rcsb.org/. The separate log files for the protein and the original ligand bound to each crystal structure were prepared and saved in PDB format. The PDB file of the target protein is loaded into AutoDock Tools version 1.5.6 (Morris et al., 2009). Docking poses generated by the AutoDock Tools are directly loaded into PyMOL. Superimposition between the docking model and crystal structure is performed in PyMOL to compare the protein-ligand interactions. By this step, the RMSD value is identified to determine the docked conformation between the scaffold with the original ligand and control. Therefore, this procedure was repeated using the anthraquinone library compounds. A total of 20 runs were set to perform the molecular docking of library compounds. The estimated free binding energy and estimated inhibition constant of each library compounds that were bound to beta-lactamase and PBP were calculated using AutoDock Tools to determine the binding affinity.

To determine the substituents that contribute to the significant binding affinity towards the targeted protein, Protein-Ligand Interaction Profiler (PLIP) was used to identify the interaction between protein and their ligands (Adasme et al., 2021). PDB file of the targeted protein is prepared by finding the docking models with the lowest free binding energy in the .dlg file. The ligand scripts of the lowest

free binding energy docking model are pasted into the protein PDB file and saved as complex.pdb format. The complex.pdb file is uploaded to PLIP to run the analysis.

The absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis is performed at the website available at http://www.swissadme.ch/. (Daina et al., 2017). Anthraquinone analogues were evaluated based on the ADMET parameters such as drug likeness and solubility. Firstly, the SMILE code of docking libraries is generated by drawing out their structure in the free online program called OpenBabel (O'Boyle et al., 2011). This program is freely accessed and available at the website:http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html. Next, the SwissADME tool is used to evaluate their ADME profile by pasting the SMILE codes in the text box provided to run the compounds. The result generated shows the information of the molecule which includes the physicochemical properties, lipophilicity, water-solubility, pharmacokinetics, drug-likeness and medicinal chemistry. These parameters are then analysed to determine the drug qualification of the docking library compounds. Apart from that, the target molecules of the docking libraries compounds are verified using SWISSTargetPrediction Tool. The toxicity assessment is run by eMolTox which is also a free online program available at https://xundrug.cn/moltox (Ji et al., 2018).

Results and discussion

2D structure of anthraquinone analogues were drawn using the Chemdraw sketching tool (Mills, 2006). The 2D structure drawing was aided in generating the 3D structure of the compound library. 3D structures of anthraquinone analogue were prepared using the Avogadro program based on the list retrieved from Mohammed (2016). The geometry of the chemical structure was optimized until it achieves the lowest optimization energy (kJ/mol). Geometry optimization was performed to stabilize the geometry of compounds achieved by the lowest optimization energy as the sketched chemical structures are not energetically favourable. The interactions and energies are determined by bond stretching, torsional energy, angle bending and other nonbonded attributes (Roy et al., 2015). The lowest optimization energy ensured the accurate molecular mechanics parameters of ligands to avoid distorted results.

Two bacterial proteins were selected in this study, which are beta-lactamase (β-lactamase) and penicillin-binding protein. The structure of both proteins was retrieved from Protein Data Bank. The PDB ID of β-lactamase is 3S1Y with resolution value of 1.40 Å originated from Pseudomonas aeruginosa (Chen et al., 2011). This protein structure was selected because it is in complex with a β-lactamase inhibitor with ID: S1Y as shown in Figure 1 (a).

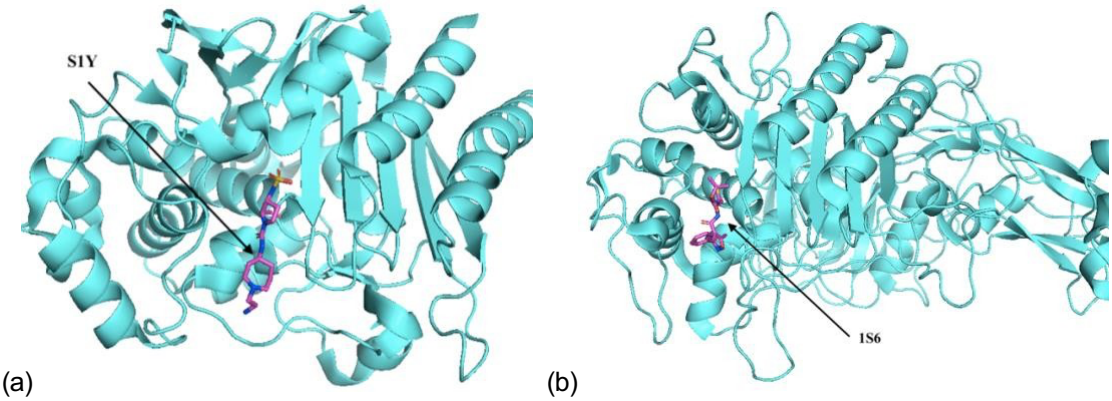

Figure 1 Protein crystal structure of (a) β-lactamase (PDB ID: 3S1Y) in complex coloured in cyan with its co-crystallized ligand (ID: S1Y) coloured in magenta and (b) PBP (PDB ID: 5OJ1) in complex coloured in cyan with its co-crystallized ligand (ID: 1S6) coloured in magenta.

Besides, *Pseudomonas aeruginosa* produce AmpC type beta-lactamase, which present the pathogens generating wide range of life-threatening chronic infections in hospitalized patients globally (Glen & Lamont, 2021; Moya *et al*., 2009). AmpC type of beta-lactamase is the major reason that causes the intrinsic resistance to many penicillin as high levels of *amp*C expression is induced when the screening of antibiotic-resistant *P. aeruginosa* clinical isolates were performed (Bonilla & Muniz, 2010; Glen & Lamont, 2021). This protein has only one chain with 371 amino acid length. The catalytic function of beta-lactamases contributes to the resistance to beta-lactam antibiotics by hydrolysing the beta-lactam ring of these versatile antibiotics (Wax *et al*., 2007). The generation of beta-lactamase which causes resistance to beta-lactam antibiotics is commonly occurred in gram-negative bacteria (Worthington & Melander, 2013).

Next, penicillin-binding protein (PBP) with PDB ID: 5OJ1 was also selected in this study. The resolution value of 5OJ1 is 2.85 Å and originated from *Streptococcus pneumoniae R6* (Bernardo-García *et al*., 2018). This protein has only one chain with an amino acid length of 702. PBP inaugurates the transglycosylases and transpeptidases that catalyze the insertion and crosslinking of newly synthesized peptidoglycan precursors in to the wall, which makes it a good target for beta-lactam antibiotics (Meszaros & Balogh, 2010). Transpeptidases will be inhibited by the beta-lactam antibiotics in manifesting their activity resulted in the inhibition of cell-wall crosslinking and bacterial death. However, resistance towards beta-lactam antibiotics in *S. pneumoniae* is contributed by the mutant variants of PBP2x. The reason why this protein structure is selected as it is in complex with oxacillin (ID: 1S6) as shown in Figure 1 (b).

In this complex, the crystal structures of protein showed that the active site of PBP was covalently modified by oxacillin, which resulted in conformational change of PBP and develop the resistance towards beta-lactam antibiotics (Bernardo-García *et al*., 2018).

Before performing the docking procedure, the ligand or the small molecules in the protein structure need to be removed. Therefore, the co-crystallized ligands from both proteins with ligands ID: S1Y and 1S6 are removed from their protein structure respectively using PyMOL. Different or separated log files for the ligand and protein were produced for both the protein and co-crystallized ligands from each protein as an input file for docking procedure later. The purpose of preparing a separated log file for protein and ligands is to have an empty binding site so that it allows docking of the library compounds into the same binding site.

Control docking was performed using the co-crystallized ligands (ligands ID: S1Y and 1S6) from the crystal structure to validate the docking procedure and determine the ligand-binding site coordinate, also known as grid coordinate. Performing control docking allows the confirmation of accurate docking procedures. This can be accomplished by redocking the co-crystallized ligands into the protein crystal structure to determine the binding site coordinates (Hosseini *et al*., 2021). The binding site coordinates were determined and the *x*, *y* and *z* values are presented in Table 1. These coordinates were be used to perform docking procedures using the library compounds. This procedure ensures the library compounds that will be docked are positioned in the same binding site.

Table 1 Coordinates of ligand binding site for β-lactamase and PBP determined from control docking procedure.

Coordinates	**Beta-Lactamase**	**PBP**
x	7.385	33.353
y	12.895	-17.859
z	17.472	53.231

In this analysis, S1Y and 1S6 were docked into the β-lactamase and PBP structure respectively resulting in 20 docking models. The most favoured docking model for β-lactamase showed a lower binding energy of -8.60 kcal/mol while for PBP it showed a lower binding energy of -7.95 kcal/mol. These two docking models were chosen to undergo the superimposition with the original crystal complex retrieved from the Protein Data Bank. The significant difference in the orientation can be referred to as the RMSD value. RMSD value is the quantitative measure that is commonly used to compare the docked conformation with the reference conformation or with other docked conformation (Kufareva & Abagyan, 2012). The superimposition of the control docking model with the original protein-ligand complex was visualized using PyMOL. The RMSD value was also calculated using PyMOL. RMSD value <2 Å indicates the docking models have high similarity from the crystal structure. This step justifies that the docking procedure is correct. The RMSD value of this superimposition of control docking model represented the Figure 2 was 0.338 Å. This RMSD value indicates the similarity of the control docking model with the original crystal structure of β-lactamase since the RMSD value is lesser than 2.00 (Ramírez & Caballero, 2018). The figure exhibited that both of the proteins and ligands are overlapped. Although the heads and tails of the ligands are twisted to each other, both of the ligands located at the same binding site. It explained that this docking solution deviate from the position of control but it still located at the desired orientation (Ramírez & Caballero, 2018). Therefore, the docking procedure is validated.

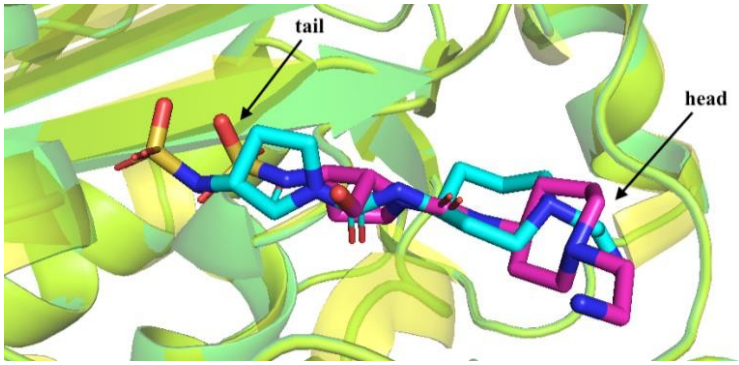

Figure 2 Superimposition of control docking model for β-lactamase with original protein-ligand complex

Besides, the calculated RMSD value of this superimposition of control docking model was 0.313 Å. In Figure 3, both ligands are overlapped and located at the same binding site although the docking model deviates from the reference model as the head and tail of both ligands were twisted to each other. This RMSD value also indicated that the control docking model is very close to the original crystal structure of PBP as the RMSD value is lesser than 2.00. These few steps validated that the docking procedures were accurate. Therefore, this docking procedure will be performed for the library compound later.

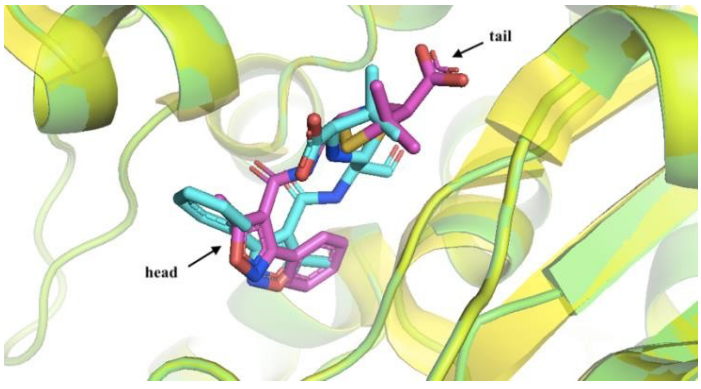

Figure 3 Superimposition of control docking model for PBP with original protein-ligand complex

A total of 20 runs were set to perform the molecular docking of library compounds. The estimated free binding energy and estimated inhibition constant of each library compounds that were bound to β-lactamase and penicillin-binding protein (PBP) are presented in Table 2 and Table 3 respectively.

Table 2 Estimated free binding energy (kcal/mol) and estimated inhibition constant, Ki (μM) of anthraquinone analogues for β-lactamase.

No	Anthraquinone analogues	Free binding energy (kcal/mol)	Inhibition constants, Ki (μM)
1	Frangulin (A)	-8.20	0.97
2	Frangulin (B)	-7.67	2.40
3	Emodin-8-O-glucoside	-7.00	7.42
4	1,8-dimethoxy-6-hydroxy-3-methyl-anthraquinone	-7.07	6.62
5	1,6,8-trimethoxy-3-methylanthraquinone	-7.46	3.39
6	Aloe-emodin-2-acetate	-7.59	2.72
7	Questin	-7.26	4.73
8	Physicon-1-O-glucoside	-8.36	0.74
9	Anthraquinone-2-carboxylic acid	-7.95	1.49
10	Anthraquinone-2-sulfonic acid	-8.83	0.34
11	Rhein	-7.53	3.00

Table 3 Estimated free binding energy (kcal/mol) and estimated inhibition constant, Ki (μM) of anthraquinone analogues for PBP.

No	Anthraquinone analogues	Free binding energy (kcal/mol)	Inhibition constants, Ki (μM)
1	Frangulin (A)	-8.24	0.91
2	Frangulin (B)	-7.08	6.47
3	Emodin-8-O-glucoside	-7.85	1.75
4	Physicon-1-O-glucoside	-8.44	0.65
5	Anthraquinone-2-sulfonic acid	-7.06	6.69

The binding affinity of potential compounds was studied by comparing the protein-ligand binding interactions with the control. Protein-ligand interactions was analysed by the protein-ligand interaction profiler (PLIP). PLIP visualized and detected the interactions such as hydrogen bonds, hydrophobic interactions, pi-stack interactions salt bridges (Adasme *et al*., 2021). Anthraquinone-2-sufonic acid is chosen to analyze the binding profile as it showed the lowest binding energy with -8.83 kcal/mol. Anthraquinone-2-sulfonic acid with beta-lactamase model was overlapped with the control model as shown in Figure.4. From Figure 4, it indicates that Anthraquinone-2-sulfonic acid shared a higher similar interactions with the compound as both compounds formed hydrogen bonds at SER90, ASN179, THR343 and ASN373 as well as salt bridges at LYS342. It also formed pi-stacking at TYR249 and salt bridges at LYS342.

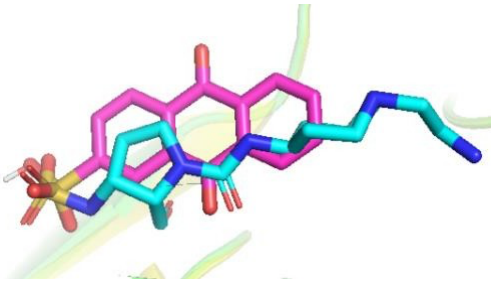

Figure 4 Superimposition of anthraquinone-2-sulfonic acid docking model for beta-lactamase with original protein-ligand complex

Besides, physicon-1-O-glucoside is also chosen to analyze the binding profile as it showed the lowest binding energy with -8.44 kcal/mol. physicon-1-O-glucoside with PBP model was overlapped with the control model as shown in Figure 4.5. From Figure 5, It indicates that physicon-1-O-glucoside shares similar interaction with 1S6 as both formed hydrogen bonds to the binding site of PBP at ASN377, THR550 and GLN552. Both also formed hydrophobic interactions at ASN377.

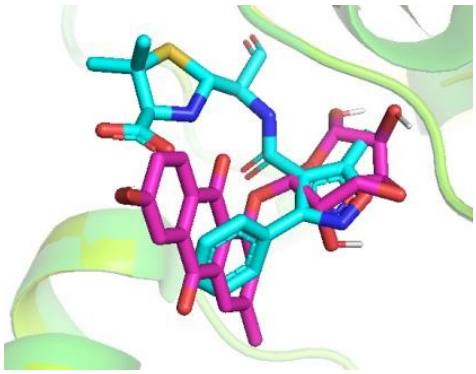

Figure 5 Superimposition of physicon-1-O-glucoside docking model for PBP with original protein-ligand complex

Absorption, distribution, metabolism, excretion and toxicity (ADMET) assessment is conducted to investigate the safety and efficacy of drug compounds based on four properties which include absorption, distribution, metabolism and excretion. A qualified lead compound should have high potency, efficacy and safety. Before starting the assessment, a process called 'hit to lead' was performed to select the potential lead compounds in the study. The lead compounds are chosen from a collection of hits by refining the criteria to qualify as the most promising molecules for further development (Hughes *et al*., 2011). Therefore, anthraquinone analogues were chosen as hits to detect the interaction with the two targeted bacterial proteins based on their antibacterial activities in different aspects. Calculations of binding free energy help in the lead optimization stage to identify the most potent drug candidate (Mondal *et al*., 2019). Free binding energy also predicted the binding affinity of the compound towards the targeted protein. Therefore, the chosen 11 library compounds as shown in Table 4.2 manifested significant comparable free binding energy with S1Y. There were 5 library compounds that showed comparable free binding energy with 1S6 as shown in Table 4.3.

Anthraquinone-2-sulfonic acid exhibited the lowest free binding energy with a value of -8.83 kcal/mol, which is even lower than S1Y. Physicon-1-O-glucoside has the lowest free binding energy of -8.44 kcal/mol, which is also lower than the control, 1S6. Thus, both of these compounds have the highest potency as both exhibited the highest binding affinity towards beta-lactamase and penicillin-binding protein (PBP). However, several rules suggested by Lipinski, Veber, Muegge, Ghose and Egan need to be considered if these anthraquinone analogues are modified into antibacterial agents. Therefore, several parameters are examined for the potential antibacterial activities of anthraquinone analogues in the development of antibacterial agents such as oral drugs, transdermal drugs, antibacterial colourants and pesticides as shown in Table 4.

Table 4 ADMET assessment of selected potential anthraquinone analogues

Com-pound	MW	HBA : HBD	TP-SA	NR-BO	Log K_P	Log P	Log S	GI absor-ption	BBB perme-ant	P-gp subst-rate	CYP inhi-bitor
Anthra-quinone -2-sulfonic acid	288.28	5:1	96.89	1	-6.11	1.81	-3.73	High	No	No	No
Physi-con-1-O-gluco-side	428.39	9:3	135.1	3	-8.22	0.82	-3.2	High	No	No	No

Abbreviation: Molecular weight (MW, g/mol); number of hydrogen bonds acceptors (HBA) and number of hydrogen bonds donors (HBD); Topological polar surface area (TPSA, Å²); number of rotatable bonds (NRBO); lipophilicity (Log P), skin permeability (Log K_p, cm/s), solubility (Log S), gastrointestinal absorption (GI absorption), blood-brain barrier permeant (BBB permeant), P-glycoprotein substrate (P-gp substrate), Cytochromes P450 inhibitor (CYP inhibitor)

From Table 4, both analogues fulfilled very pharmacokinetic and pharmacodynamics parameters assessed using SwissADME. These two analogues fulfilled Lipinski's rule of five as Lipinski's rule of five predicted that poor absorption or permeation happens when there are (i) more than 5 hydrogen donors, which are expressed as the sum of all OH and NH groups, (ii) molecular weight exceeds 500 Dalton, (iii) log P greater than 5, (iv) more than 10 hydrogen bond acceptors as it is expressed as the sum of all Ns and Os (Choy & Prausnitz, 2011; Lipinski *et al*., 2001). Besides, they also fulfilled several rules suggested by Veber, Ghose, Egan and Muegge. Veber's rule stated that the number of rotatable bonds (NRBO) may determine the flexibility of compounds. It allows increasing affinity between the compound and its targeted protein. The number of rotatable bonds should be 10 or fewer (Veber *et al*., 2002). Egan rule also suggested that the compounds with good bioavailability should possess not more than 5.88 log P and not more than 131 Å of total polar surface area (Egan et al., 2000). Ghose's rule demonstrated that the molecular weight of a good bioavailability compound should lies in between 160 to 480. The lipophilicity, log P of a compound should also lies within a range of -0.4 to 5.6 while the number of atoms should be in the range of 20 to 70 (Ghose et al., 1999). Moreover, Muegge's rule advocated that the ideal molecular weight of a compound should be in between 200 to 600 while log P value is suggested to lie in a range of -2 to 5. Muegge also suggested that the total polar surface area ≤ 150 Å, the number of rings ≤ 7, the number of carbon > 4, the number of heteroatoms > 1, the number of rotatable bonds ≤15, the hydrogen bond acceptors ≤ 10, and the hydrogen bond donors ≤ 5 (Pathania & Singh, 2021). These few parameters may examined the potential of both analogues to be modified as antibacterial agent such as oral drugs, transdermal drugs,

antibacterial colourant and pesticides. In addition, another important parameter that need to be considered for the development of transdermal drugs is skin permeability coefficient (K_p). Potts and Guy adapted that K_p is linearly correlated with molecular size and lipophilicity. Thus, the lower the skin permeability of the molecule is induced by a more negative log K_p (Daina et al., 2017). The standard range of log K_p for skin permeability should be lied in between -8.0 to -1.0 (Ya'u Ibrahim et al., 2020). The log K_p value of chosen compounds fall within the standard range except physicon-1-O-glucoside, which has a log K_p value of -8.22. Therefore, only anthraquinone-2-sulfonic acid is likely to permeate the skin.

Conclusion

In this study, 21 anthraquinone analogues were evaluated for their antibacterial activities against beta-lactamase and penicillin-binding protein (PBP). Anthraquinone-2-sulfonic acid exhibited the potential to be modified as a compound in antibacterial agents to fight against beta-lactamase. It was the best-docked compound with beta-lactamase as it has the highest potency as well as good physicochemical and pharmacokinetic parameters. physicon-1-O-glucoside is the best-docked compound with PBP. It also showed the highest potency as well as fulfilled every physicochemical and pharmacokinetic parameter. Therefore, this study revealed that natural compounds indicate potential antibacterial activities towards bacterial proteins that contribute to the development of beta-lactam antibiotic resistances. In order to innovate prominent antibacterial agents in future, more works such as structural modification and in vitro testing are recommended. The simplest method of structural modification is to optimize the functional groups, which is based on the chemical similarity principle. It indicates that bioactivity will be similar if the chemical structure is similar. Various laboratory methods such as disk-diffusion and broth or agar dilution are commonly used to examine the in vitro antibacterial activity of a test compound. Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are the significant parameters that evaluate in vitro levels of the resistance developed by the specific bacterial strains to the applied antibiotic. The MIC evaluates the lowest concentration of antibacterial agents that are capable of preventing the bacteria from growing. MBC evaluates for the lowest concentration of antibacterial drugs that can kill all the bacteria. These further evaluation methods may modify potential lead compounds to effectively fight against bacterial proteins that generate antibacterial resistance to beta-lactam antibiotics.

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