Proceedings of Science and Mathematics
Faculty of science, universiti teknologi malaysia

Vol. 12, 2022, page 104-115

In silico Analysis of Anthraquinone Analogues against Penicillin-Binding Protein and Beta-Lactamase

Hue Seow Yie[1], Norazah Basar[2], Syazwani Itri Amran[1*]
[1]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia,
81310 UTM Skudai, Johor, Malaysia
[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,
81310 UTM Skudai, Johor, Malaysia
*Corresponding author: syazwaniitri@utm.my

Abstract
Antibacterial resistance has become a global healthcare problem. The widespread of antibacterial agents causes bacteria to evolve and develop resistance towards antibacterial agents. Beta-lactam antibiotic is the most used antibacterial agents in this world and resistance has been developed against beta-lactam antibiotics either through production of beta-lactamases or altered affinity of penicillin-binding protein to beta-lactam antibiotics. In this study, beta-lactamase and penicillin-binding protein were used to evaluate the binding affinity of 24 anthraquinone analogues using molecular docking approach and structural activity relationship analysis. Eight analogues that were tested against beta-lactamase and six analogues that were tested against penicillin-binding protein showed similar binding affinity when compared with the co-crystal ligand (reference compound). 1,5-dihydroxy-4,8-dinitroanthraquinone has the highest binding affinity with the beta-lactamase among the tested compounds in which nitro and hydroxyl group might help in improving the binding affinity. For penicillin-binding protein, 2-ethylanthraquinone had the highest binding affinity among the tested compounds in which ethyl group potentially improve their binding affinity. With the promising antibacterial activity, physicochemical and pharmacokinetic (ADMET) properties such as skin permeant and moderate toxicity, selected anthraquinone analogues were potentially to become transdermal antibacterial drugs and antibacterial colourants. This study revealed the potential of anthraquinone analogues as antibacterial agents.
Keywords Anthraquinone analogues; Antibacterial resistance; Antibacterial agent; Molecular docking; ADMET

Introduction

Antibacterial agents can kill or inhibit the growth of bacteria by inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis, inhibition of metabolic pathways and interference with cell membrane integrity, hence, preventing bacterial infection disease (Kwaśniewska *et al*., 2020). The extensive use of antibiotics in hospitals and community will exert evolutionary pressure on the bacteria which cause them to undergo the evolutionary process and selective pressure (Öztürk *et al*., 2015). As a result, there is the emergence of antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA). As the antibacterial resistance is increasing, it is important to screen new antibacterial agents to overcome the resistance of bacteria.

The natural compound has been a ready source for antimicrobial agents. It provides various structures that can assist in the design of compounds with good pharmacological activity (Lautié *et al*., 2020; Thomford *et al*., 2018). A study showed that anthraquinone become a new avenue for drug discovery of antimicrobial agents to solve the problem of drug-resistant bacterial diseases (Pollo *et al*., 2021). Reported antibacterial agents of anthraquinone are the emodin type of anthraquinone compounds such as aloe- emodin, 8–dihydroxyanthraquinone, hypericin and rhein (Malmir *et al*., 2017).

Penicillin-binding protein and beta-lactamase are both involved in the antibacterial activity and become target mechanisms for resistance to antibacterial agents, which is the resistance of beta-lactam antibiotics (Duraipandiyan *et al*., 2014). Penicillin-binding protein has been studied a lot for its resistance toward beta-lactams (Zapun *et al*., 2008). Resistance bacteria will produce beta-lactamase to cause the beta-lactam antibiotic ineffective toward its target, penicillin-binding protein. Therefore, beta-lactamase is the main cause of resistance to beta-lactam antibiotics (Wax *et al*., 2007). In the meantime, beta-lactam antibiotic is one of the most used antibiotics in this world, which accounts for 60% of the total usage of antibiotics in the world (Öztürk *et al*., 2015). Hence, the use of beta-lactamase and penicillin-binding protein as target bacterial proteins is crucial in screening the antibacterial agents. Oxacillin in penicillin-binding protein inhibits the transpeptidase activity of penicillin-binding protein which will block the usual transpeptidation reaction. This affected the last stage of the synthesis of peptidoglycan in the bacterial cell wall and eventually cause the death of bacteria (Öztürk *et al*., 2015; Turk *et al*., 2011). Beta-lactamase hydrolyses the beta-lactam ring and causes the beta-lactam antibiotic to become inactive toward its target which is the cell wall transpeptidase or penicillin-binding protein (Öztürk *et al*., 2015). Beta-lactamase inhibitor in beta-lactamase is used to overcome the activity of beta-lactamase.

In this study, anthraquinone analogues were screened against bacterial proteins, beta-lactamase and penicillin-binding protein through *in silico* molecular docking. Docking analysis was done based on free binding energy and binding profiles and followed by Structure-Activity Relationship (SAR) analysis. Lastly, the selected anthraquinone analogues were assessed for their adsorption, distribution, metabolism, excretion and toxicity (ADMET) properties to find out the potential antibacterial agent.

Materials and methods

The structure of the anthraquinone analogues was obtained from Mohammed (2016). The 2D structure of the compound library was prepared by using ChemSketch (*ACD/ChemSketch*, 2012). 2D structures acted as a reference for drawing 3D structures The 3D structure of the compound library was drawn by using Avogadro (Hanwell *et al*., 2012). Geometry optimization was performed to get the stabilized geometry for all the atoms which were represented by the lowest energy value given by the Avogadro program. A molecule with the lowest energy or local energy minimum is closest to the structure of the starting molecular arrangement and more stable (Hongmao, 2016; Roy *et al*., 2015). The 3D structures were saved in the PDB format.

For protein structure preparation, protein crystal structure of beta-lactamase (PDB ID: 3S1Y) and penicillin-binding protein (PDB ID: 5OJ1) were downloaded from the PDB database (http://www.rcsb.org/pdb/) (Berman *et al*., 2000). The structure of the beta-lactamase and penicillin-binding protein were studied by identifying the active sites. Besides, the separate log files for the protein and its original ligand were prepared and saved in PDB format files. The PDB format file of protein was uploaded into the AutoDock Tools (ADT) 1.5.6 (Morris *et al*., 2009) to remove the co-crystal ligand and the water molecules, followed by adding the hydrogen atoms.

AutoDock Tools (ADT) 1.5.6 (Morris *et al*., 2009) and AutoDock 4.2.6 program from the Scripps Research Institute (http://www.scripps.edu/mb/olson/doc/autodockAutoDock) were used for molecular docking of bacterial proteins, beta-lactamase and penicillin-binding protein with the compound library of anthraquinone

analogues. Control docking was performed using the original ligand from the crystal structure. For beta-lactamase, ligand S1Y was used while for penicillin-binding protein, ligand 1S6 was used. Both ligand and protein PDB format files were uploaded in the AutoDock Tools 1.5.6. After that, the ligand and protein were in PDBQT format files to perform docking in the AutoDock Tools 1.5.6. The binding site grid coordinates were determined. The search for docking was carried out by using the Genetic Algorithm with 10 runs and population size of 150. The output of docking was using Lamarckian GA (4.2). For control, the docking log (DLG) file produced after docking was opened by using Notepad and the RMSD value of the docking model with the lowest free binding energy was identified to validate the docking procedure. The binding site grid coordinates were obtained from control docking and the same docking procedures were used to dock anthraquinone analogues into the beta-lactamase and penicillin-binding protein.

To analyse the docking results, the docking log (DLG) file produced after docking was loaded into AutoDock Tools 1.5.6 again to analyse the energies and docking pose of all 10 conformations. The docking model with the lowest free binding energy was selected and its estimated inhibition constant was recorded from the DLG file. To know the binding profiles, the PDB format of the protein was opened by using Notepad. Then, the DLG file produced after docking was opened by using another Notepad and ligand scripts of the docking model with the lowest free binding energy was copied and pasted into the PDB file of the protein in the Notepad. The file was saved in PDB format and sent to Protein-Ligand Interaction Profiler (PLIP) (Adasme *et al*., 2021) to analyse the ligand conformation and interactions between protein and ligand. Besides, Structure-Activity Relationship (SAR) studies were carried out. The DLG file of the docking model and the PDBQT file of the protein were loaded into AutoDock Tools 1.5.6 to select the best docking pose with the lowest free binding energy and write complex to export the conformation. PyMOL (Schrödinger & DeLano, 2020) was used to visualise the post docking model. The docking model was superimposed with the crystal structure and co-crystal ligand of control to identify the substituents or functional groups that improve the binding affinity with the target protein.

ADMET assessment was carried out to know the absorption, distribution, metabolism, excretion, and toxicity of the selected potential anthraquinone analogues. The SMILES format of the anthraquinone analogue was converted from PDB format through the Open Babel web server (http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html) (O'Boyle *et al*., 2011). The SMILES codes were inserted into SwissADME (http://www.swissadme.ch/) (Daina *et al*., 2017) to generate pharmacokinetic profiles, physicochemical properties and drug-likeness. Lastly, the potential toxic properties of the compound were predicted with eMolTox web server (https://xundrug.cn/moltox) (Ji *et al*., 2018) by using SMILES format of the anthraquinone analogue.

Results and discussion

The structure of both bacterial proteins used in this study was retrieved from the Protein Data Bank (PDB) database where the PDB ID for the beta-lactamase structure is 3S1Y originated from *Pseudomonas aeruginosa* (Chen *et al*., 2011) while the penicillin-binding protein with PDB ID of 5OJ1 was selected and this protein belongs to *Streptococcus pneumoniae* R6 (Bernardo-García *et al*., 2018). The beta-lactamase structure with PDB ID of 3S1Y was selected because it is in complex with a beta-lactamase inhibitor, S1Y. Therefore, the mechanism of action of the docked compounds is potentially similar to this beta-lactamase inhibitor (Elfaky *et al*., 2020). Besides, the penicillin-binding protein with PDB ID of 5OJ1 was chosen because the protein is in complex with a beta-lactam antibiotic, Oxacillin which has an ID of 1S6.

Control docking was conducted to determine the binding site coordinates and to ensure the docking procedure is accurate. The co-crystal ligand, S1Y was re-docked into the beta-lactamase (PDB ID: 3S1Y) while the co-crystal ligand, 1S6 was re-docked into the penicillin-binding protein (PDB ID: 5OJ1). The binding site grid coordinates for beta-lactamase and penicillin-binding protein were determined and their *x*, *y* and *z* value were presented in Table 1. These coordinates were used to conduct the docking

procedure by using the library compound of anthraquinone analogues. This can ensure all the library compounds of anthraquinone analogues will be docked into the same binding site. Table 2 showed the docking results of the control compound with respective bacterial proteins. The docking conformation with the lowest free binding energy value was chosen. Superimpositions of the control docking with the original protein crystal structure for beta-lactamase had an RMSD value of 2.0Å while penicillin-binding protein had an RMSD value of 1.607Å. Both have an RMSD value equal to or smaller than 2Å, indicating there is a high similarity or minimum deviation between the docked ligands and originally embedded ligand in the crystal structure, therefore validating the docking procedure (Almutairi *et al*., 2014; Carpio Arévalo & Amorim, 2021; Elfaky *et al*., 2020). The binding site grid coordinates obtained from control docking and the same docking procedures were used to dock the compound library of anthraquinone analogues into the beta-lactamase (PDB ID: 3S1Y) and penicillin-binding protein (PDB ID: 5OJ1).

Table 1: Binding site grid coordinates for beta-lactamase and penicillin-binding protein

Protein	Coordinate *x*	Coordinate *y*	Coordinate *z*
Beta-lactamase (PDB ID: 3S1Y)	7.385	12.895	17.472
Penicillin-binding protein (PDB ID: 5OJ1)	33.353	-17.859	53.231

Table 2: Estimated free binding energy and inhibition constant of control compound with bacterial protein

Control compound	Free binding energy (kcal/mol)	Inhibition constant, K_i (μM)
S1Y of Beta-lactamase (PDB ID: 3S1Y)	-8.67	0.44
1S6 of Penicillin-binding protein (PDB ID: 5OJ1)	-7.56	2.88

24 anthraquinone analogues and co-crystal ligand, S1Y that acted as control were docked with beta-lactamase (PDB ID: 3S1Y). Eight compounds have free binding energy lower than -7.0 kcal/mol as shown in Table 3. These compounds showed similar binding affinity with the beta-lactamase when compared with the control, S1Y which had free binding energy of -8.67 kcal/mol. S1Y also showed an inhibition constant of 0.44 μM. The compound, 1,5-dihydroxy-4,8-dinitroanthraquinone has the lowest free binding energy (-9.03 kcal/mol) that indicated the highest binding affinity, and the lowest inhibition constant (0.24 μM) that showed the highest potency with the beta-lactamase among all the 24 anthraquinone analogues. It also showed higher binding affinity and potency than the control, S1Y.

Table 3: The docking results of eight anthraquinone analogues and control with beta-lactamase

No.	Anthraquinone Analogues	Free binding energy (kcal/mol)	Inhibition constant, K_i (μM)
-	S1Y (control)	-8.67	0.44
1	1,5-dihydroxy-4,8-dinitroanthraquinone	-9.03	0.24
2	Lucidin ω-methylether	-7.51	3.10
3	Nordamnacanthal	-7.37	3.97

No.	Anthraquinone Analogues	Free binding energy (kcal/mol)	Inhibition constant, K_i (μM)
4	Damnacanthal	-7.37	3.93
5	Rubiadin	-7.25	4.86
6	Morindone	-7.24	4.92
7	1,4-diamino-2,3-dichloroanthraquinone	-7.15	5.75
8	Morindone-5-methyl-ether	-7.14	5.87

For penicillin-binding protein (PDB ID: 5OJ1), there were six out of 24 anthraquinone analogues that have free binding energy equivalent to or lower than -6.60 kcal/mol as shown in Table 4. These compounds showed a similar binding affinity with the penicillin-binding protein when compared with the control, 1S6 which had free binding energy of -7.56 kcal/mol. The control also exhibited an inhibition constant of 2.88 μM. The compound, 2-Ethylanthraquinone had the lowest free binding energy (-6.68 kcal/mol) that indicated the highest binding affinity and the lowest inhibition constant (12.70 μM) which showed the highest potency with the penicillin-binding protein among all the 24 anthraquinone analogues.

Table 4: The docking results of six anthraquinone analogues and control with penicillin-binding protein

No.	Anthraquinone Analogues	Free binding energy (kcal/mol)	Inhibition constant, K_i (μM)
-	1S6 (control)	-7.56	2.88
1	2-Ethylanthraquinone	-6.68	12.70
2	2-formyl-1-hydroxy-anthraquinone	-6.66	13.19
3	Damnacanthal	-6.66	13.20
4	2,3-dimethyl-anthraquinone	-6.66	13.13
5	Rubiadin-1-methyether	-6.61	14.38
6	Anthraflafic acid	-6.60	14.65

The compound 1,5-dihydroxy-4,8-dinitroanthraquinone was the best-docked compound against the beta-lactamase with the free binding energy of -9.03 kcal/mol which showed higher binding affinity and stronger interactions than the co-crystal ligand. The compound interacted with the protein through four hydrogen bonds with SER90, ASN179, SER345 and ASN370. It exhibited the same hydrogen bonds with the co-crystal ligand at SER90, ASN179 and SER345. It formed an additional hydrogen bond at ASN370 with hydroxyl group of the compound. The compound was well-fitted into the binding site of beta-lactamase same as the control (S1Y) but the compound was shorter than the control (Figure 1). The extended side of the control (S1Y) consisted of s-azepine and amino alkyl chain that made more interactions with the protein and contributed to the binding affinity (Chen *et al*., 2011). However, the binding affinity of 1,5-dihydroxy- 4,8-dinitroanthraquinone was higher than the co-crystal ligand. The hydroxyl and nitro group in the compound might help in improving the binding affinity between the compound and the protein. The presence of polar functional groups can improve the antibacterial activity of the anthraquinone analogues (Malmir *et al*., 2017). Hydroxyl groups at the aromatic ring of anthraquinone seemed important to produce antibacterial compounds, especially for gram-negative

bacteria (Alhadrami *et al*., 2022).

According to Duraipandiyan *et al*. (2014), with the hydroxyl groups in the compounds, 1,5,7-trihydroxy-3-hydroxy methyl anthraquinone exhibited good binding energy and antibacterial activity when docked against beta- lactamase. It formed hydrogen bonds with the hydroxyl group at positions C-1 and C-5 of the compound which is similar to the compound 1,5-dihydroxy-4,8-dinitroanthraquinone.

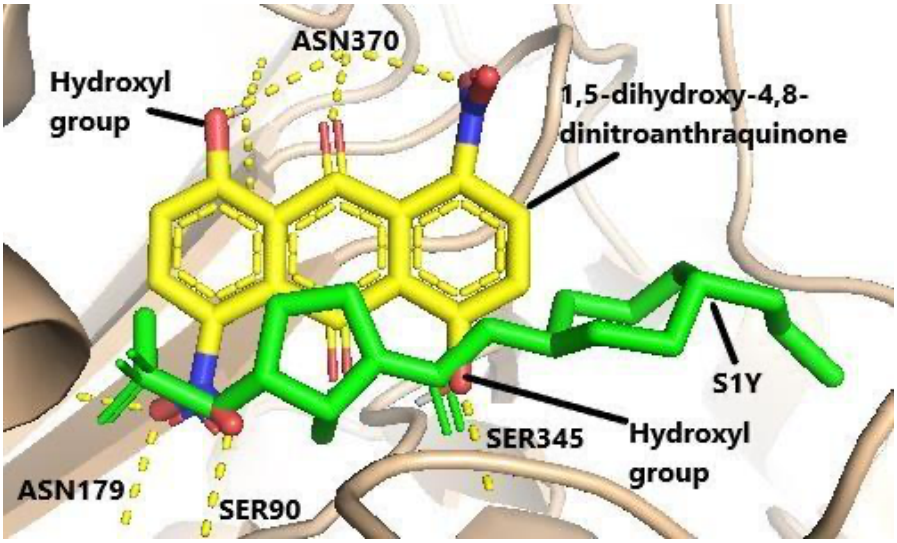

Figure 1 Binding mode of co-crystal ligand S1Y (Green) in the active site of beta-lactamase (PDB ID: 3S1Y) with the compound (Yellow), 1,5-dihydroxy-4,8-dinitroanthraquinone. Yellow- dotted line represents hydrogen bond

The compound, 2-Ethylanthraquinone showed a similar binding affinity as the control with the free binding energy of -6.68 kcal/mol which was the highest binding affinity and strongest interactions with the protein among all the compounds. However, the binding affinity of 2-Ethylanthraquinone was slightly lower than the co-crystal ligand. 2-Ethylanthraquinone interacted with the protein through two hydrogen bonds with ASN397 and GLN552, and hydrophobic interactions with ARG372, ASN377, PHE450 and GLN452. It formed the same hydrogen bonds with the control at ASN397 and GLN552, and the same hydrophobic interactions at ASN377, PHE450 and GLN452. 2-Ethylanthraquinone formed extra hydrophobic interaction between ARG372 and ethyl group of the compound. 2-Ethylanthraquinone was well-fitted into the binding site of penicillin-binding protein same as the control (1S6) and superimposed with the side chain of the control, but the compound was shorter than the control (Figure 2). The extended side of the control (1S6) consisted of 6-aminopenicillanic acid nucleus that made more interactions with the protein and contributed to the binding affinity (Bernardo-García *et al*., 2018). Hence, 2-Ethylanthraquinone showed a slightly lower binding affinity than the control. Since the free binding energy of 2-Ethylanthraquinone was slightly higher than the co-crystal ligand, this compound did not have a higher binding affinity than the co-crystal ligand. However, the additional hydrophobic interaction between ARG372 and ethyl group of the compound may potentially increase the binding affinity of the compound to the protein slightly when compared with other compounds. It is reported that the addition of alkyl group such as methyl group in the anthraquinone ring can improve the antibacterial activity of gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (Chalothorn *et al*., 2019).

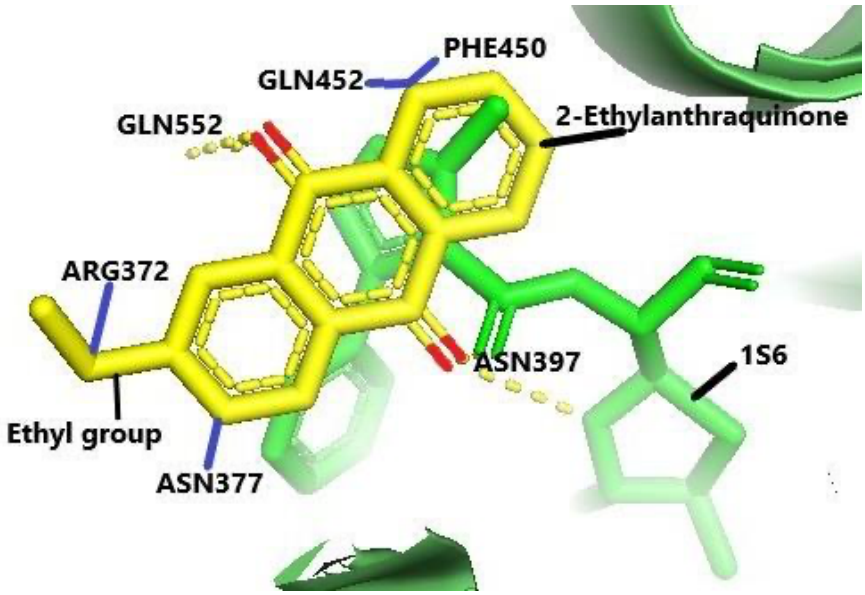

Figure 2 Binding mode of co-crystal ligand 1S6 (Green) in the active site of penicillin-binding protein (PDB ID: 5OJ1) with the compound (Yellow), 2-Ethylanthraquinone. Yellow-dotted line represents hydrogen bond and indigo line represents hydrophobic interaction

Co-crystal ligand in penicillin-binding protein inhibits the transpeptidase activity of penicillin-binding protein which will block the usual transpeptidation reaction. This affected the last stage of the synthesis of peptidoglycan in the bacterial cell wall and eventually cause the death of bacteria (Öztürk *et al*., 2015; Turk *et al*., 2011). Some bacteria developed beta-lactamase that can destroy the co-crystal ligand or antibacterial agent before it can inactivate the penicillin-binding protein which results in the survival of bacteria even in the presence of antibacterial agents (Duraipandiyan *et al*., 2014). Beta-lactamase hydrolyses the beta- lactam ring and causes the beta-lactam antibiotic to become inactive toward its target which is the cell wall transpeptidase or penicillin-binding protein (Öztürk *et al*., 2015). Co-crystal ligand in the beta-lactamase act as a beta-lactamase inhibitor to overcome the activity of beta-lactamase. Beta-lactamase inhibitor acts as a substrate that binds with beta-lactamase to produce sterically unfavourable interactions or inactivate beta- lactamase permanently through a secondary chemical reaction in the binding site (Khanna & Gerriets, 2021). Anthraquinone analogues that bind with the penicillin-binding protein and beta-lactamase in the same way as their co-crystal ligand were expected to undergo the same mechanism.

Absorption, distribution, metabolism, excretion (ADME) and toxicity assessment of selected potential antibacterial compounds of anthraquinone analogue were performed to know their drug-likeness, pharmacokinetics and potential toxicity (Kulanthaivel *et al*., 2018). They give indications about the safety and efficiency of the selected potential antibacterial compound after being administrated into a human body. 1,5-dihydroxy-4,8-dinitroanthraquinone showed the highest binding affinity with beta-lactamase with acceptable oral bioavailability, good pharmacokinetic properties, not blood-brain barrier (BBB) permeant, not a substrate for P-glycoprotein (P-gp), skin permeant, only one CYP inhibitor out of five and moderate potential toxicity. However, it had a topological polar surface area that was out of the acceptable range and low gastrointestinal (GI) absorption. 1,5-dihydroxy-4,8-dinitroanthraquinone did not obey the Lipinski rule- of-five as its topological polar surface area was out of the acceptable range but according to Gurung *et al*., (2016), it was still acceptable for oral availability with only one violation. In spite of the fact that it was acceptable for oral availability, the compound showed low gastrointestinal (GI) absorption which

indicated there is only a low probability for the compound to undergo passive absorption by the gastrointestinal tract (Bojarska *et al*., 2020). The compound may not be suitable for oral administration.

Anthraquinone derivatives were used in applications such as active ingredients of an anti-allergic cream and antibacterial gel wound dressing (Park *et al*., 2010; Watroly *et al*., 2021; Xi *et al*., 2018). Transdermal dosage forms such as cream, gel, ointment, lotion and spray can exert a local effect at the active site through steady drug delivery after topical application. The compound that undergoes a topical route of administration required a certain degree of lipophilicity. As 1,5-dihydroxy-4,8-dinitroanthraquinone was predicted with low lipophilicity, techniques such as chemical enhancers, biochemical enhancers, electroporation and others can be applied to enable steady drug concentration during administration. The topical route of administration also can reduce systemic exposure and eventually decrease the undesired side effects (Maharao *et al*., 2020). Hence, with the predictions that 1,5-dihydroxy-4,8-dinitroanthraquinone was skin permeant together with other favourable properties, a topical route of administration for local action may be used for this compound to produce antibacterial cream or antibacterial gel wound dressing that aimed at bacteria which released beta-lactamase. Table 5 presented absorption, distribution, metabolism, excretion (ADME) and toxicity assessment of 1,5-dihydroxy-4,8-dinitroanthraquinone.

2-Ethylanthraquinone exhibited the highest binding affinity with penicillin-binding protein with acceptable oral bioavailability, good pharmacokinetic properties, skin permeant, not a substrate for P-glycoprotein (P-gp), high gastrointestinal (GI) absorption, only two CYP inhibitor out of five and less potential toxicity. However, it showed moderate soluble in water and permeant to the blood-brain barrier (BBB). The moderate water solubility of 2-Ethylanthraquinone may cause some difficulties for the compound to be distributed and reach the target site through the bloodstream which consists of plasma. The ability of the compound to permeant through the blood-brain barrier (BBB) may also cause adverse effects on the brain which will be dangerous if it is taken as an oral drug. Hence, 2-Ethylanthraquinone might not be suitable to be an oral drug. Anthraquinone derivatives had been made into antibacterial colourants before. With the promising antibacterial properties of 2-Ethylanthraquinone, it might be used as antibacterial colourants for textiles. Antibacterial colourants are functional colourants that allow the combination of the dyeing process with the functional finishing process and lead to a more environment-friendly textile manufacturing process with less energy and water usage. When the textile fabrics are dyed with antibacterial colourants, it can inactivate the bacteria on the dyed fabrics (Alihosseini & Sun, 2011). Table 5 showed absorption, distribution, metabolism, excretion (ADME) and toxicity assessment of 2-Ethylanthraquinone.

Conclusion

There were eight anthraquinone analogues out of all 24 anthraquinone analogues that showed higher or similar binding affinity with the beta-lactamase when compared with the co-crystal ligand. 1,5-dihydroxy-4,8-dinitroanthraquinone has the highest binding affinity and the highest potency with the beta-lactamase. Nitro and hydroxyl group in the compound, 1,5-dihydroxy-4,8-dinitroanthraquinone might help in improving the binding affinity with beta-lactamase. For penicillin-binding protein, there were six anthraquinone analogues from 24 anthraquinone analogues that showed a similar binding affinity when compared with the co-crystal ligand. 2-Ethylanthraquinone had the highest binding affinity and the highest potency with the penicillin-binding protein. Ethyl group in 2-Ethylanthraquinone might help in improving the binding affinity with penicillin-binding protein. The anthraquinone analogues that showed promising antibacterial activity were further studied to predict physicochemical and pharmacokinetic (ADMET) properties of tested anthraquinone analogues. With the predictions that 1,5-dihydroxy-4,8-dinitroanthraquinone was skin permeant together with other favourable properties, a topical route of administration for local action may be used for this compound to produce antibacterial transdermal drug. Besides, the promising antibacterial properties of 2-ethylanthraquinone showed it was potentially to form antibacterial colourants for textiles.

Table 5: Absorption, distribution, metabolism, excretion (ADME) and toxicity assessment of selected compounds

Compound	MW	HBA : HBD	TPSA	*n*Rot	*M*Log *P*	Log K_p	Log *S*	GI absorption	BBB permeant	P-gp substrate	CYP inhibitor	Bioavai-lability	Potential toxicity
1,5-dihydroxy-4,8-dinitro-anthraquinone	330.21	8:2	166.24	2	-1.29	-6.24	Soluble -3.96	Low	No	No	1/5	0.55	Liver, Geno-toxicity, CNS, DNA, Immune
2-Ethyl-anthraquinone	236.27	2:1	34.14	1	2.37	-4.64	Moderate soluble -4.49	High	Yes	No	2/5	0.55	Geno-toxicity, Liver

Abbreviations: Molecular weight (MV, g/mol), number of hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), topological polar surface area (TPSA, Å²), number of rotatable bonds (*n*Rot), lipophilicity (*M*Log *P*), skin permeability (Log *K*p, cm/s), solubility (Log *S*), gastrointestinal absorption (GI absorption), blood-brain barrier permeability (BBB permeant), P-glycoprotein substrate (P-gp substrate), number of Cytochromes P450 inhibitor out of total 5 inhibitors (CYP inhibitor) and central nervous system (CNS)

References

ACD/ChemSketch. (2012). Advanced Chemistry Development, Inc. www.acdlabs.com

Adasme, M. F., Linnemann, K. L., Bolz, S. N., Kaiser, F., Salentin, S., Haupt, V. J., & Schroeder, M. (2021). PLIP 2021: expanding the scope of the protein–ligand interaction profiler to DNA and RNA. *Nucleic Acids Research*, *49*(W1), W530–W534.

Alhadrami, H. A., Abdulaal, W. H., Hassan, H. M., Alhakamy, N. A., & Sayed, A. M. (2022). In Silico-Based Discovery of Natural Anthraquinones with Potential against Multidrug-Resistant *E. coli*. *Pharmaceuticals 2022, 15*(1), 86.

Alihosseini, F., & Sun, G. (2011). Antibacterial colorants for textiles. *Functional Textiles for Improved Performance, Protection and Health*, 376–403.

Almutairi, M. S., Hegazy, G. H., Haiba, M. E., Ali, H. I., Khalifa, N. M., & Soliman, A. E. M. M. (2014). Synthesis, Docking and Biological Activities of Novel Hybrids Celecoxib and Anthraquinone Analogs as Potent Cytotoxic Agents. *International Journal of Molecular Sciences*, *15*(12), 22580.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research*, *28*(1), 235–242.

Bernardo-García, N., Mahasenan, K. V., Batuecas, M. T., Lee, M., Hesek, D., Petráčková, D., Doubravová, L., Branny, P., Mobashery, S., & Hermoso, J. A. (2018). Allostery, Recognition of Nascent Peptidoglycan, and Cross-linking of the Cell Wall by the Essential Penicillin-Binding Protein 2x of *Streptococcus pneumoniae*. *ACS Chemical Biology*, *13*(3), 694–702.

Bojarska, J., Remko, M., Breza, M., Madura, I. D., Kaczmarek, K., Zabrocki, J., & Wolf, W. M. (2020). A Supramolecular Approach to Structure-Based Design with A Focus on Synthons Hierarchy in Ornithine-Derived Ligands: Review, Synthesis, Experimental and in Silico Studies. *Molecules 2020, 25*(5), 1135.

Carpio Arévalo, J. M., & Amorim, J. C. (2021). An in-silico analysis reveals 7,7′-bializarin as a promising DNA gyrase B inhibitor on Gram-positive and Gram-negative bacteria. *Computers in Biology and Medicine*, *135*, 104626.

Chalothorn, T., Rukachaisirikul, V., Phongpaichit, S., Pannara, S., & Tansakul, C. (2019). Synthesis and antibacterial activity of emodin and its derivatives against methicillin-resistant *Staphylococcus aureus*. *Tetrahedron Letters*, *60*(35), 151004.

Chen, H., Blizzard, T. A., Kim, S., Wu, J., Young, K., Park, Y. W., Ogawa, A. M., Raghoobar, S., Painter, R. E., Wisniewski, D., Hairston, N., Fitzgerald, P., Sharma, N., Scapin, G., Lu, J., Hermes, J., & Hammond, M. L. (2011). Side chain SAR of bicyclic β-lactamase inhibitors (BLIs). 2. N-Alkylated and open chain analogs of MK-8712. *Bioorganic & Medicinal Chemistry Letters*, *21*(14), 4267–4270.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*(1), 1–13.

Duraipandiyan, V., AL-Dhabi, N. A., Balachandran, C., Raj, M. K., Arasu, M. V., & Ignacimuthu, S. (2014). Novel 1,5,7-Trihydroxy-3-Hydroxy Methyl Anthraquinone Isolated from Terrestrial *Streptomyces* sp. (eri-26) with Antimicrobial and Molecular Docking Studies. *Applied Biochemistry and Biotechnology, 174*(5), 1784–1794.

Elfaky, M. A., El-Halawany, A. M., Koshak, A. E., Alshali, K. Z., El-Araby, M. E., Khayat, M. T., & Abdallah, H. M. (2020). Bioassay Guided Isolation and Docking Studies of a Potential β-Lactamase Inhibitor from *Clutia myricoides*. *Molecules, 25*(11), 2566.

Gurung, A. B., Bhattacharjee, A., & Ali, M. A. (2016). Exploring the physicochemical profile and the binding patterns of selected novel anticancer Himalayan plant derived active compounds with macromolecular targets. *Informatics in Medicine Unlocked*, *5*, 1–14.

Hanwell, M. D., Curtis, D. E., Lonie, D. C., Vandermeerschd, T., Zurek, E., & Hutchison, G. R. (2012).

Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics*, *4*(8), 1–17.

Hongmao, S. (2016). Structure-Based Ligand Design I: With Structures of Protein/Lead Compound Complex Available. *A Practical Guide to Rational Drug Design*, 15–60. https://doi.org/10.1016/B978-0-08-100098-4.00002-8

Ji, C., Svensson, F., Zoufir, A., & Bender, A. (2018). eMolTox: prediction of molecular toxicity with confidence. *Bioinformatics*, *34*(14), 2508–2509.

Khanna, N. R., & Gerriets, V. (2021). Beta Lactamase Inhibitors. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK557592/

Kulanthaivel, L., Jeyaraman, J., Biswas, A., Subbaraj, G. K., & Santhoshkumar, S. (2018). Identification of potential inhibitors for Penicillin binding protein (PBP) from *Staphylococcus aureus*. *Bioinformation*, *14*(9), 471.

Kwaśniewska, D., Chen, Y. L., & Wieczorek, D. (2020). Biological activity of quaternary ammonium salts and their derivatives. *Pathogens*, *9*(6) , 1–12. https://doi.org/10.3390/pathogens9060459

Lautié, E., Russo, O., Ducrot, P., & Boutin, J. A. (2020). Unraveling Plant Natural Chemical Diversity for Drug Discovery Purposes. *Frontiers in Pharmacology*, *11*, 397.

Maharao, N., Antontsev, V., Hou, H., Walsh, J., & Varshney, J. (2020). Scalable in silico Simulation of Transdermal Drug Permeability: Application of BIOiSIM Platform. *Drug Design, Development and Therapy*, *14*, 2307.

Malmir, M., Serrano, R., & Silva, O. (2017). Anthraquinones as potential antimicrobial agents-A review. In A. Mendez-Vilas (Ed.), *Antimicrobial research: Novel bioknowledge and educational programs*, 55–61. Formatex Research Center S.L.

Mohammed, M. M. (2016). Structure Antimutagenicity Relationship of Anthraquinones. *Natural Products Chemistry & Research*, *4*(5).

Morris, G. M., Ruth, H., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., & Hutchison, G. R. (2011). Open Babel: An Open chemical toolbox. *Journal of Cheminformatics*, *3*(10), 1–14.

Öztürk, H., Ozkirimli, E., & Özgür, A. (2015). Classification of Beta-Lactamases and Penicillin Binding Proteins Using Ligand-Centric Network Models. *PLoS ONE*, *10*(2).

Park, S. H., Kim, J. J., Park, B. J., Go, E. A., Sim, M. H., Lee, E. J., Sin, Y. G., Noh, J. G., Kim, M. K., Park,K. H., Jeong, C. S., & Lee, W. J. (2010). *Cosmetic Components Comprised of the Rubiadin Having Anti-Allergy Activity* (Patent No. KR101337564B1). Kolmar Co., Ltd. https://patents.google.com/patent/KR101337564B1/en

Pollo, L. A. E., Martin, E. F., Machado, V. R., Cantillon, D., Wildner, L. M., Bazzo, M. L., Waddell, S. J., Biavatti, M. W., & Sandjo, L. P. (2021). Search for Antimicrobial Activity Among Fifty-Two Natural and Synthetic Compounds Identifies Anthraquinone and Polyacetylene Classes That Inhibit *Mycobacterium tuberculosis*. *Frontiers in Microbiology*, *0*, 3619.

Roy, K., Kar, S., & Das, R. N. (2015). Computational Chemistry. *Understanding the Basics of QSAR for Applications in Pharmaceutical Sciences and Risk Assessment*, 151–189.

Schrödinger, L., & DeLano, W. (2020). *PyMOL*. http://www.pymol.org/pymol

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. (2018). Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *International Journal of Molecular Sciences,19*(6), 1578.

Turk, S., Verlaine, O., Gerards, T., Živec, M., Humljan, J., Sosič, I., Amoroso, A., Zervosen, A., Luxen, A., Joris, B., & Gobec, S. (2011). New Noncovalent Inhibitors of Penicillin-Binding Proteins from Penicillin- Resistant Bacteria. *PLoS ONE*, *6*(5)

Watroly, M. N., Sekar, M., Fuloria, S., Gan, S. H., Jeyabalan, S., Wu, Y. S., Subramaniyan, V., Sathasivam, K. V., Ravi, S., Rani, N. N. I. M., Lum, P. T., Vaijanathappa, J., Meenakshi, D. U., Mani, S., & Fuloria,N. K. (2021). Chemistry, Biosynthesis, Physicochemical and Biological Properties of Rubiadin: A Promising Natural Anthraquinone for New Drug Discovery and Development. *Drug Design, Development and Therapy*, *15*, 4527–4549

Wax, R. G., Lewis, K., Salyers, A. A., & Taber, H. (Eds.). (2007). *Bacterial Resistance to Antimicrobials* (2nd ed.). CRC Press.

Xi, J., Wu, Q., Xu, Z., Wang, Y., Zhu, B., Fan, L., & Gao, L. (2018). Aloe-Emodin/Carbon Nanoparticle Hybrid Gels with Light-Induced and Long-Term Antibacterial Activity. *ACS Biomaterials Science and Engineering*, *4*(12), 4391–4400.

Zapun, A., Contreras-Martel, C., & Vernet, T. (2008). Penicillin-binding proteins and β-lactam resistance. *FEMS Microbiology Reviews*, *32*(2), 361–385.