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參展科別 生物化學

作品名稱 利用虛擬篩選 LpxC 抑制劑的方式找出對抗多重抗藥性綠膿桿菌的新療法

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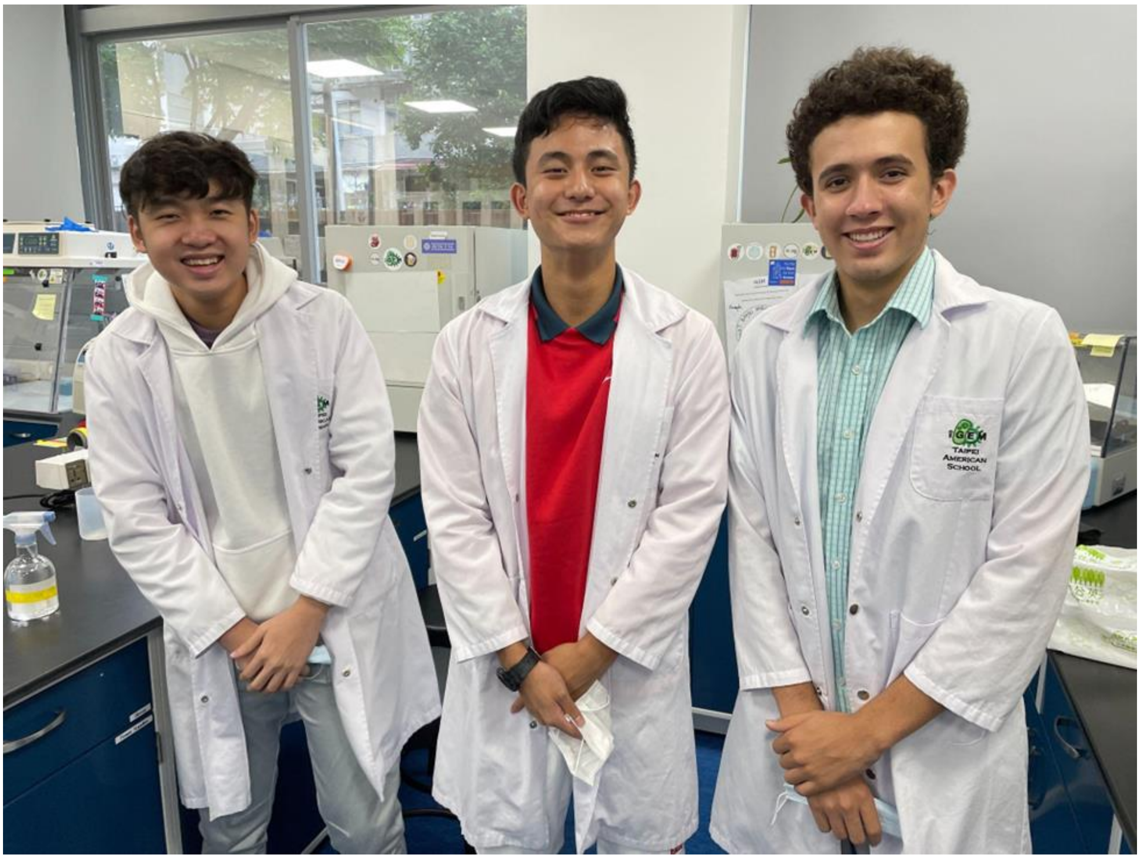
關鍵詞 多重抗藥性細菌(MDR Bacteria)、
官能團修飾(Functional Group Modification)、
LpxC

作者簡介



大家好，我們是台北美國學校的三人組：余昀翰 (左)、劉其桓 (中) 、 Ethan (右)。我們三位對環境科學以及生物化學都充滿熱忱，因此在打好基礎之後參加了各種奧林匹亞競賽以及在學校以社團的方式來推廣校園之中的科學文化。在這些經歷當中，我們發掘了我們對科學實驗和研究的興趣，因此這次組了團隊之後決定依照興趣以生物化學為研究領域。我們的夢想是希望有一天能藉由我們對科學具有的研究精神踏上國際舞臺，與其他在各自領域也頗有成就的學生共享，互相學習。我們每天進實驗室，每天跟指導老師學習，而在這些日子中學到的知識和得到的經驗，都成為了我們友情之間難能可貴的回憶和未來研究的基石。

摘要

多重抗藥性（MDR）細菌已經在全世界的範圍內成為了一個重大威脅，而像是多重抗藥性的綠膿桿菌就是其中一種對大多數療法有抵抗力的病原體。在目前的治療方案無效之前，有必要開發出一種新型機制的抗生素能夠作為對抗的手段。我們通過電腦虛擬篩選的方式，並用一個脂多糖脂質 A (LipidA)生合成路徑的關鍵蛋白，LpxC，作為篩選的對象。在我們的第一次預測中，ZINC000001587011 (brequinar) 具有較低的結合能和較高的生物利用度。但由於其較高的 cLogP 值，使我們對其進行了官能團修飾，以期能有所改善。最後，我們在所有衍生物中找到了 N11，有最大的潛力能做為抗綠膿桿菌的藥物前驅物。

Abstract

Multidrug-resistant (MDR) bacteria are a significant threat to communities worldwide. MDR Pseudomonas aeruginosa, a major pathogen resistant to most therapies, can cause serious conditions such as endocarditis and pneumonia. The development of an antibiotic is needed before current treatments fail, and we are implementing the reverse pharmacology approach in conducting our research. With the use of PyRX AutoDOCK Vina, we targeted LpxC, a key protein for bio synthesizing lipid A of lipopolysaccharide, by in silico virtual screening of current approved therapies. In our first prediction, ZINC000001587011, also known as brequinar, had a low binding energy, high bioavailability, but an unfavorably high calculated octanol-water partition coefficient (cLogP), which signifies poor solubility in water. In hopes of lowering the high cLogP, we performed functional group modification on brequinar. Finally, after going through virtual screening of 20,000 candidates and 30 derivatives of ZINC000001587011, we propose that the N11 derivative of brequinar might have the most potential against P.aeruginosa lipid A synthesis due to its commendatory binding energy, favorable bioavailability, and low cLogP, making it a potential treatment for MDR P. aeruginosa.

壹、前言

Introduction

一、研究動機

Research Motive / Purpose

Multidrug-resistant (MDR) bacteria, bacterial strains resistant to drugs in three or more antimicrobial categories, pose a significant threat to communities worldwide with an estimated 2.8 million antibiotic-resistant infections occurring in the U.S. per year (1, 2). These infections are linked to increased morbidity and mortality, with around 35,000 deaths resulting from antibiotic-resistant infections in the U.S each year (2). MDR bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* have become resistant over time to most available antibacterial agents, including tetracycline and chloramphenicol (3, 4). Even newly developed antibiotics like vancomycin became ineffective against these MDR bacteria due to the antibiotic-resistant genes being generated in these strains after the abuse of antibiotics (4). Antibiotics eliminate the non-antibiotic-resistant bacteria, making resistant strains dominant within the microbial communities. As antibiotic-resistance genes form in bacteria, other antibiotics must be used as a treatment, and eventually, the bacteria will become resistant to the new antibiotic. The abuse of antibiotics, where antibiotics are over-prescribed, accelerates this process, and the bacteria will become more and more resistant to antibiotics (5). Eventually, MDR bacteria will evolve to overcome current antibiotic treatments, so alternative ways to remedy MDR bacteria must be investigated.

二、研究背景

Research Background

A conventional way to kill pathogens is to block off crucial metabolic pathways of pathogens by targeting a key set of proteins (6). This method is simple, effective, and has been used extensively. One promising new way is to inhibit virulence factors of pathogens, including preventing the synthesis of endotoxins and exotoxins (7), blocking quorum sensing (8), and stopping biofilm formation (9). Our method in this study is to prevent the synthesis of an endotoxin, which is known as lipopolysaccharide (LPS), by inhibiting lipid A synthesis. The assembly of LPS starts with lipid A, then core polysaccharides, and finally the O-antigen. Lipid A is the membrane anchor domain of LPS, and it is vital to both protect gram-negative bacteria against external agents and is also essential for bacterial growth. Therefore, inhibiting the biopathway of lipid A should lead to the death of gram-negative bacteria (10).

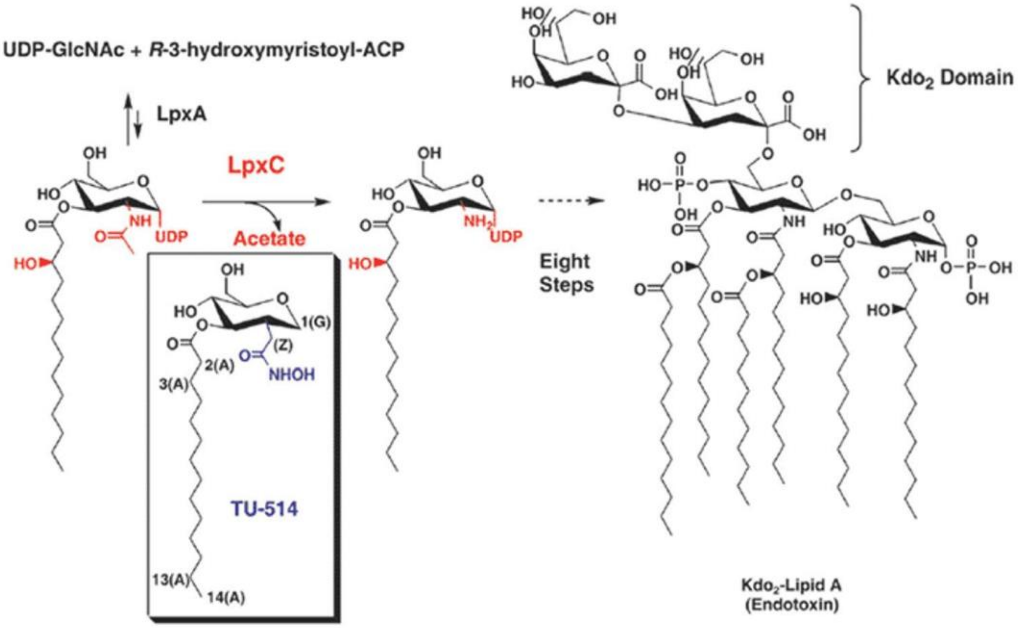


Figure 1: Biopathway of the synthesis of Lipid A. This figure was reprinted from figure 1 in Zhou et. al, 2003.

LpxC (EC: 3.5.1.108) is a hydrolase from the committed step in the lipid A biosynthesis pathway that hydrolyzes UDP-3-O-myristoyl-N-acetylglucosamine to UDP-3-O-myristoylglucosamine (**Figure 1**). LpxC can function as a monomer that binds with either cofactors Zn^{2+} or Fe^{2+} (11). LpxC is highly conserved among gram-negative bacteria, and potentially can be lethal if inhibited. Previous research has targeted LpxC to design potential inhibitors, such as CHIR-090 , BB78484 , LpxC-4 , and TU-514 (12-15)(**Figure 2**). However, no inhibitors are available commercially, with only one, MAK-181, in phase II clinical trials (CITATION). LpxC remains a relatively unexplored target for developing inhibitors. Our study will attempt to find a potential lead compound for LpxC inhibition in *P. aeruginosa* by using *in silico* docking.

Reverse pharmacology is a method contrary to classical pharmacology (16). Reverse pharmacology utilizes computational techniques to find potential drug candidates. A specific protein target is first selected based on the significance of the pathogenic mechanism, then a computer attempts to dock multiple molecules at a binding site on the protein. The candidates with the lowest binding energies have potential, as the molecule and enzyme are more likely to bind. For the process to be thermodynamically favorable, the binding energy must be negative.

三、研究目的

Research Purpose

Here, we performed an *in silico* virtual screening on LpxC in *P. aeruginosa* to find a new drug against this MDR bacteria. We then made several functional group modifications to the lead compound, and the modified molecules were re-docked. We hypothesized that using computational techniques of molecular docking, we could find a potential lead compound that can prevent the biosynthesis of LPS leading to the death of the pathogen. In this study, we found a promising lead compound that can inhibit LpxC in *P. aeruginosa* and modified it to increase its bioavailability, which can be further studied for human use.

貳、研究過程與方法

Materials and Methods

1、 研究設備

Materials Used for Research

Two online databases were used; RCSB PDB (https://www.rcsb.org/) and ZINC15 (https://zinc15.docking.org/) used to download protein structure files and ligands file, respectively. Docking platforms used were AutoDockTools 1.5.6 and PyRX 0.9.x. Molecule remodeling programs used were ChemDraw 20.0 and Chem3D. Protein-ligand interaction figures in this study were made with Pymol 2.4. SwissADME was used for lead compound elimination and modified compound elimination.

二、研究過程

Experimental Methods

(1) 電腦虛擬篩選 Molecular Docking

The ligand files from the Alfa-Chemistry catalog were downloaded and converted to PDBQT format with the Open Babel function on PyRX. The docking center was set to -14.39, 14.97, -28.08 (x,y,z) with the grid box dimensions (Å) of 41.25, 20.25, 20.25 (x,y,z). The AutoDock VINA panel in PyRX was used for docking, with exhaustiveness set to 10. The two candidates with the lowest binding energies were selected for further remodeling.

(2) 先導化合物消除 Lead Compound Elimination

The compounds with the lowest binding energies from docking were successively analyzed with SwissADME, focusing on the molecular weights, ClogP, and the Abbot bioavailability scores. To make the final decision about the candidate to modify, these values were considered along with the binding energy.

(3) 官能團修飾 Functional Group Modification

The candidates' original ligand structures were input into ChemDraw using the SMILES code. Chloroalkane, nitro, and aldehyde groups were added on to the candidates' backbone carbons one at a time. The modified molecules were copied to Chem3D and saved as SDF files. The molecular docking process was then repeated with these files.

參、研究結果與討論

Results

Table 1: Top three molecules with the lowest binding energies after docking with LpxC

Molecule	MW (Da)	R	A	D	cLogP	Binding Energy (kcal/mol)	Abbot
ZINC000001042092	546.57	6	6	0	4.89	-11.7	0.55
ZINC000001710746	516.59	6	4	0	7.75	-11.5	0.17
ZINC000001587011	375.37	3	5	1	5.09	-10.9	0.85

MW: molecular weight; R: number of rotatable bonds; A: number of hydrogen acceptors; D: number of hydrogen donors.

To identify a lead compound, we docked twenty thousand substances from the catalogs of Alfa-Chemistry in the ZINC15 database. Around 0.865% of the compounds had a binding energy of -9 kcal/mol and under, with only two compounds having a binding energy of less than -11 kcal/mol. ZINC000001042092 had an exceptionally low binding energy of -11.7 kcal/mol. ZINC000001710746 also had a low binding energy of -11.5 kcal/mol (**Table 1**).

Table 2: Docking results of ZINC000001587011 derivatives.

Molecule	MW (Da)	cLogP	Binding Energy (kcal/mol)	Tunnel	**Molecule**	MW (Da)	cLogP	Binding Energy (kcal/mol)	Tunnel
N11	420.37	4.29	-10.2	Y	**N12**	420.37	4.3	-8.7	N
N20	420.37	4.28	-9.9	Y	**CHO10**	403.38	4.78	-8.7	N
CHO20	403.38	4.77	-9.8	Y	**CL23**	409.81	5.57	-8.7	N
CL20	409.81	5.55	-9.8	Y	**CL12**	409.81	5.6	-8.7	N
CL19	409.81	5.59	-9.8	Y	**N9**	420.37	4.32	-8.6	N
CL11	409.81	5.61	-9.8	Y	**CHO1**	403.38	4.55	-8.6	N
N19	420.37	4.26	-9.7	Y	**CHO11**	403.38	4.78	-8.6	N
CHO19	403.38	4.79	-9.5	Y	**CHO5**	403.38	4.78	-8.6	N
CL6	409.81	5.62	-9.5	Y	**CHO12**	403.38	4.8	-8.6	N
N5	420.37	4.32	-9.3	Y	**CL1**	409.81	5.3	-8.6	N
CL10	409.81	5.61	-9.1	N	**N10**	420.37	4.32	-8.5	N
N1	420.37	3.97	-8.9	N	**CHO23**	403.38	4.73	-8.5	N
N23	420.37	4.27	-8.9	N	**CHO6**	403.38	4.81	-8.4	N
N6	420.37	4.34	-8.9	N	**CHO9**	403.38	4.77	-8.3	N
CL5	409.81	5.59	-8.8	N	**CL9**	409.81	5.61	-8.3	N

MW: molecular weight; Y and N for the Tunnel column dictates whether the molecule reaches into the small tunnel in the active site.

The "Lipinski rule of 5" is most commonly used to eliminate non-ideal candidates. This rule states that a lead compound which violates more than one of these rules will have poor absorption or permeation: 1 -More than 5 hydrogen bond donors; 2 - More than 10 hydrogen bond acceptors; 3 - Molecular weight of more than 500 Dalton; 4 - Calculated octanol-water partition coefficient (cLogP) is calculated to be greater than 5 (17). Although both

ZINC000001042092 and ZINC000001710746 only violate one of these rules, their high molecular weight and the number of benzene rings lead to concerns about permeability and solubility. Taking these into consideration, we chose ZINC000001587011, as it had a much lower molecular weight and much higher Abbot bioavailability score while maintaining an acceptable binding energy of -10.9 kcal/mol. Moreover, this compound, known as brequinar, is already clinically used to treat autoimmune diseases, such as rheumatoid arthritis, by inhibiting human dihydroorotate dehydrogenase (18).

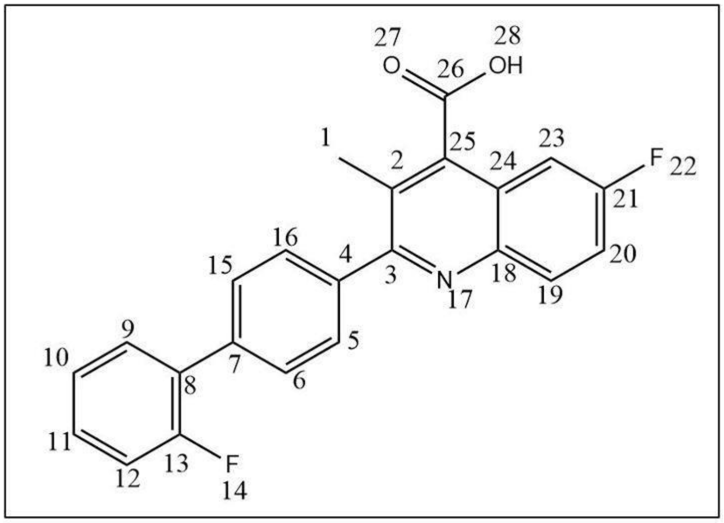


Figure 2: Structure of ZINC000001587011. The numbers are used to identify all individual atoms except for hydrogen.

In order to improve our lead compound, we modified it with chloroalkane, nitro, and aldehyde groups. We then re-docked these derivatives of brequinar. We specifically chose these groups because they have the potential to increase the electron acceptor groups, making it easier to interact with the binding site. Carbon atoms, which can be modified with functional groups, are C_1, C_{10}, C_{11}, C_{12}, C_{19}, C_{20}, C_{23} (**Figure 3**). No improvement in binding energy was observed out of the 30 derivatives, but cLogP values varied widely. For molecules modified with chloroalkane groups, the cLogP unexpectedly increased, while others modified with nitro groups and aldehyde groups usually decreased in cLogP (**Table 2**). The lowest achieved cLogP, N1, had a difference in cLogP of 1.12 compared to the original lead compound. The reason for analyzing the cLogP is because lower values usually have better performances in drug-likeness tests due to increased solubility in water.

Next, we analyzed the 3D structure of the modified compounds docked with LpxC. We found that a Zn^{2+} ion was inside the active site which has two openings (**Figure 4**). One opening is connected to a small tunnel, which only allows certain structures to enter it. However, all compounds that could enter this tunnel had tighter interactions with LpxC (**Figure 5; Table 2**). In fact, these compounds had the 10 lowest binding energies of all the modified molecules. We found that only 2 benzyl rings could reach inside the tunnel, so modifications made on those rings would have a large effect on the binding energies. Only C_5 and C_6, which is located at the neck of the small tunnel, had space for small molecular modifications without drastic changes to the binding energy **(Figure 5**). We were able to perform larger molecular modification without affecting the binding energy in the phenyl ring near the center of the active site , at C_{19} and C_{20} C_{23}, on the same phenyl ring, is too close to the Zn^{2+} binding site of the candidates, leaving no space for modifications.

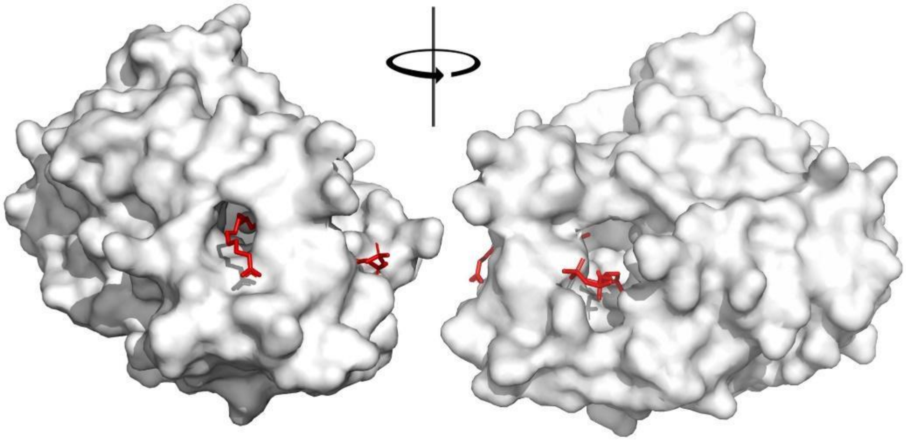


Figure 3: Active pocket on LpxC with two openings. The one on the left is a small tunnel, the one on the right is much bigger opening. The substrate is colored in red.

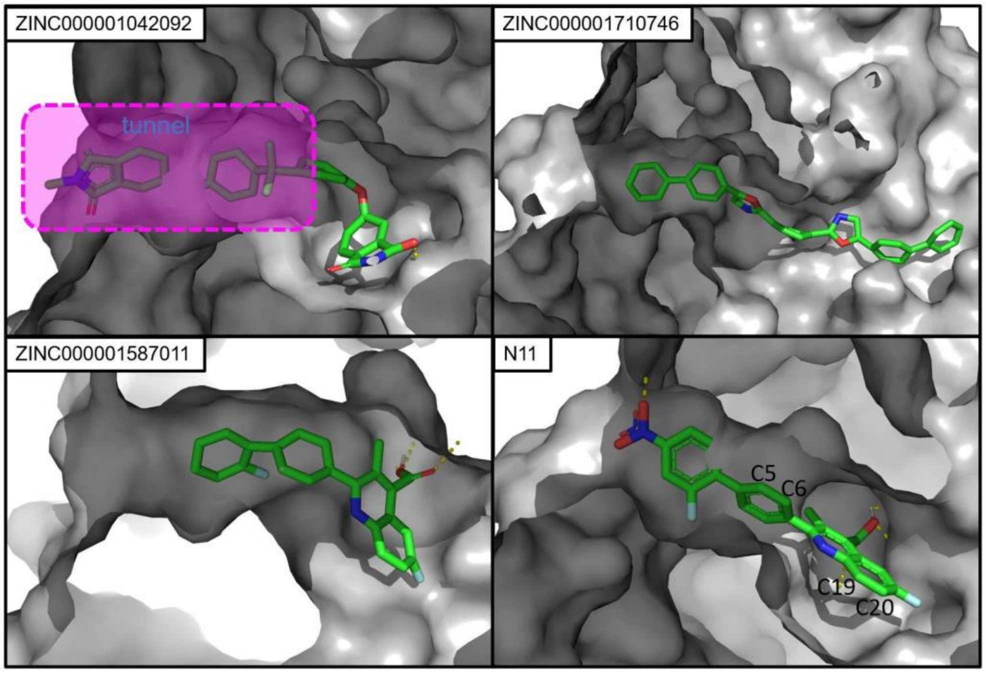


Figure 4: Ligand-protein 3D images. In the upper-left quadrant, the pink region refers to the small tunnel in the active site. Green: Carbon, Blue: Nitrogen, Red: Oxygen, Light Blue: Fluorine.

To compare our candidates to inhibitors from previous research, CHIR-090, BB78484, LpxC-4 and TU-514, Simplified Molecular Input Line Entry System (SMILEs) files were pasted into Chem3D and exported as SDF files for molecular docking. We see that our candidates are similar in structure; however, their inhibitors have much higher binding energies, between -9.2 and -7.0 kcal/mol (**Table 3**). As mentioned earlier, we found that molecules that could reach inside the smaller tunnel in the binding site had better interactions. Most of these candidates reach into the tunnel, the purple-shaded region in Figure 5. CHIR-090 and LpxC-4 both use their benzyl ring structures to reach into the tunnel. TU-514 uses a long carbon chain to reach into the tunnel. Only BB78484 does not reach into the tunnel due to its structure.

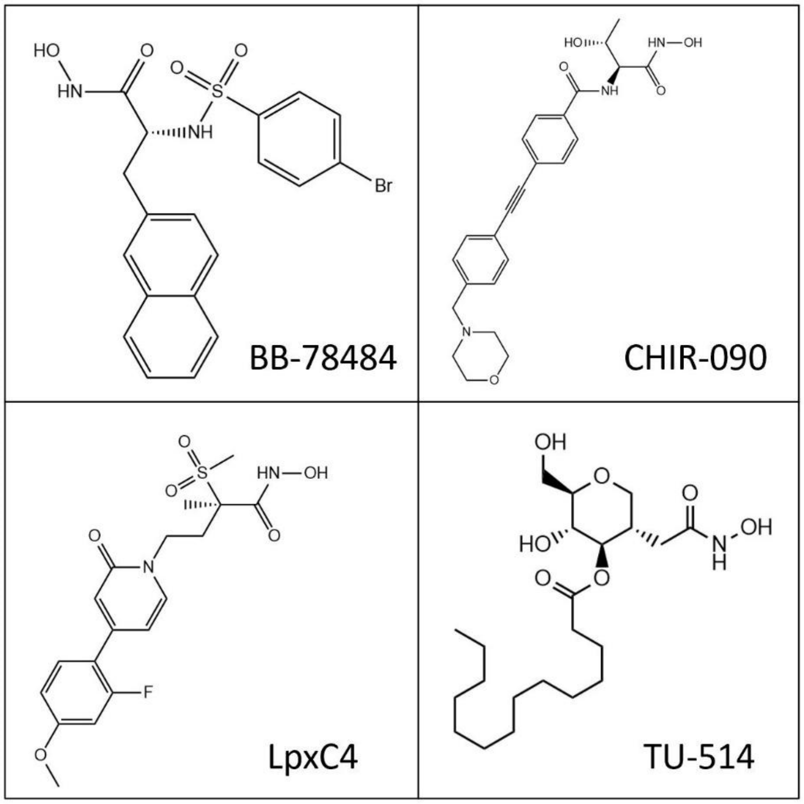


Figure 5: Structure of LpxC inhibitors from previous research. These structures were obtained from PubChem and redrawn in ChemDraw.

Table 3. Docking results of inhibitors from previous research

Molecule	MW (Da)	cLogP	Binding Energy (kcal/mol)	Tunnel
CHIR-090	437.49	1.53	-9.2	Y
LpxC4	412.43	1.53	-8.8	Y
BB-78484	449.32	2.95	-8.8	N
TU-514	431.56	3.3	-7	Y

MW: molecular weight; Y and N for the Tunnel column dictates whether the molecule reaches into the small tunnel in the active site.

肆、結論與應用

Discussion

In our research, we eliminated ZINC000001042092 and ZINC000001710746, our top two lead compounds with the lowest binding energies, due to concerns of permeability and solubility despite their extremely low binding energies. We used ZINC000001587011 as our lead compound and modified it with functional groups, as it had good permeability and solubility while maintaining a low binding energy. We then docked functional-group-modified versions of our lead compound and analyzed the results. We propose that N11 is our best candidate due to its low cLogP, which makes the compound soluble in the water and thus more easily absorbed by the human body. Although its binding energy is slightly worse than the original ZINC000001587011, the cLogP value improved while maintaining the same bioavailability as the original, making it our best candidate.

Bioavailability is very complicated in calculation, and even small modifications to the original compound can lead to large differences in bioavailability score. Although some nitro-modified molecules had a decrease in bioavailability scores, the scores are still slightly higher than the two original eliminated candidates. The chloroalkane and aldehyde modified molecules had the exact same bioavailability score as brequinar, so these are more ideal for human consumption.

When compared to candidates from other research, we saw that previous candidates have a much lower cLogP, between 1.53 to 3.3. However, their binding energies are not as favorable as ours, ranging from -7 to -9.2 kcal/mol. Future studies should be done on the modification of ZINC000001587011 to lower the cLogP to enhance drug performance. After analyzing the 3D structure of our results, we believe that future work should start with modification on C_{19} or C_{20} to better occupy the entire binding site. We predict that proper modifications on these carbons will lead to an improvement in binding energy.

In this study, we found that the N11 modification of ZINC000001587011 is an ideal candidate for the inhibition of LpxC in *P. aeruginosa*, due to its favorable binding energy, low cLogP, and high bioavailability. With further *in vitro* testing, this candidate is a promising treatment of the ongoing problem of MDR bacteria and can benefit communities worldwide.

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【評語】080010

本研究通過電腦虛擬篩選的方式，並用一個脂多糖脂質 A (LipidA)生合成路徑的關鍵蛋白 LpxC 作為篩選的對象。利用 molecular ducking 方法，找出對抗多重抗藥性綠膿桿菌的潛力抗菌藥物，並在篩選過程中經由官能團修飾找出可能 cLogP 值低的衍生物，最終篩選出最有潛力能做為抗綠膿桿菌的藥物前驅物分子 N11。

建議：

1. 這研究應該合成此 N11 分子和其衍生物，測定這些分子對抑制 LpxC 活性的 IC50 劑量與毒殺綠膿桿菌的最低劑量，有了這些結果才使本研究更完整。
2. 要接官能基需考慮合成途徑，看是否有可能合成 ZINC1587011 的衍生物。
3. 也須測試預測出來的 LpxC 抑制劑是否真的能抑制表達出來酵素的活性。