Pharmacy Education (2024) 24(2) 9 - 16
https://doi.org/10.46542/pe.2024.242.916

IAI SPECIAL EDITION

RESEARCH ARTICLE

In silico approach through molecular docking and study ADME on imine derivative compounds as a potential antibacterial agent against *Staphylococcus aureus*

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Keywords
ADME
Antibacterial
Docking
Imine
Staphylococcus aureus

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Abstract

Background: Antibiotics are drugs or compounds that inhibit or kill bacteria. Most natural and synthetic pharmacophores have been reported to be antimicrobial agents. One of these is an imine compound. Compounds containing imine groups exhibit various pharmacological activities, including antibacterial, antifungal, anti-HIV, anti-cancer, anti-inflammatory, antimalarial, and antituberculosis activities. **Objective:** This study aimed to determine the potential of four imine-derived compounds as antibacterial agents using molecular docking. **Method:** The molecular docking environment (MOE) 2022.0901 software package was used to perform molecular docking. Determination of the physicochemical and pharmacokinetic properties of the four imine-derivative compounds was performed online via the website www.swissadme. **Results:** Based on the docking results, compound 1 has great potential as an antibacterial agent because its binding orientation was similar to that of the positive control. In addition, based on the Absorption, Distribution, Metabolism, and excretion (ADME) study, compound 1 was shown to be suitable for Lipinski's rule of five (RO5). **Conclusion:** It can be concluded that compound 1 is easily absorbed, has good permeability, and can be used as a promising agent against *Staphylococcus aureus*.

Introduction

Multidrug-resistant bacteria are responsible for a significant number of infections, resulting in approximately 700,000 infections per year. This number is expected to increase annually until 2050. Overuse and abuse of antibiotics have led to the development of bacteria that are resistant to known antibiotics, for which there is little or no effective treatment (Tanvir *et al*., 2021).

Microorganisms, such as bacteria, viruses, fungi, and protozoa can cause infectious diseases. Some of the bacteria that cause infection are *Clostridium botulinum, Corynebacterium diphteriae, Escherichia coli, Salmonella typhii, Shigella flexneri, Shigella shigae, and Staphylococcus aureus* (Oliveira *et al*., 2018). *S. aureus* is the primary cause of a wide variety of opportunistic infections. *S. aureus* is also a human pathogen that causes a number of toxin-mediated illnesses, including gastroenteritis, staphylococcal scalded skin syndrome, toxic shock syndrome, endocarditis, and soft tissue infections (Oliveira *et al*., 2018). Chloramphenicol is the most commonly used antibiotic for treating infections. Chloramphenicol is a broad-spectrum bacteriostatic antibiotic active against both aerobic and anaerobic microorganisms. In addition, chloramphenicol is active against *Neisseria meningitidis* (Batty *et al*., 2020), *Escherichia coli, Staphylococcus aureus, and Salmonella typhi* (Udo *et al*., 2021).

Most natural and synthetic pharmacophores have been reported as antimicrobial agents, including imine

compounds. Imine compounds are important pharmacophores because they are easy to synthesise from carbonyl and amino derivatives and have the ability to interact with different biological targets. Compounds containing imine groups exhibit some biological activities, such as antibacterial, antifungal, anti-HIV, anticancer, anti-inflammatory, antimalarial, and antituberculosis compounds (Kumar *et al*., 2022).

In silico studies have played a significant role in the design of medications (Frimayanti *et al*., 2021). To determine the optimal conformation with the lowest binding free energy, in silico research (molecular docking and pharmacokinetic properties) was employed in the drug design process. In addition, they can offer helpful data for forecasting how a proposed medicine will bind to its target (i.e. protein). However, the potential antibacterial activity of imine derivatives has not yet received much attention. Moreover, there are few reports on the discovery of antibacterial drugs using in silico tools, such as molecular docking and ADME calculations.

Methods

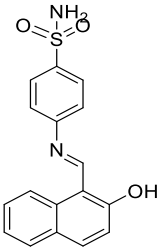
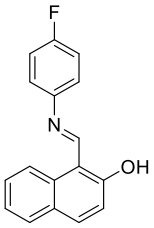
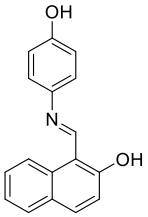
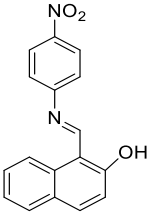
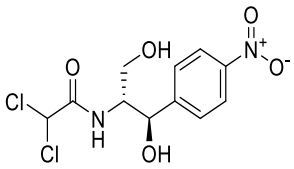
Ligand preparation

Chemdraw Professional 15.0 was used to sketch the structure of imine derivative compounds and chloramphenicol as a positive control and then saved in ”cdx" format. The molecular structures of the imine derivatives and chloramphenicol are listed in Table I. Files of molecular structures were imported and analysed using the Molecular Operating Environment (MOE) 2022.0901 (Chemical Computing Group) with a force field of MMFF94x and a gradient of 0.0001, producing the 3D structures of each ligand. It was then recorded as a database of ligands. A list of ligand and positive control are presented in Table 1.

Protein preparation

The protein structure used (PDB code: 1N67) was downloaded from the website www.rcsb.org in PDB format, this protein 1N67 consisted of 1 chain (A). Water molecules that bound with 1N67 protein were removed using the BIOVA Discovery Studio Visualiser (DSV) application. Then, the ligand attached to the protein is removed. The prepared structure is saved in the format “pdb" (1N67-prepI).

Table I: Molecular structure of ligands

No	Structure
1	 *(E)-4-(((2-hydroxynaphthalen-1-yl)methylene)amino)benzenesulfonamide*
2	 *(E)-1-(((4-fluorophenyl)imino)methyl)naphthalen-2-ol*
3	 *(E)-1-(((4-hydroxyphenyl)imino)methyl)naphthalen-2-ol*
4	 *(E)-1-(((4-nitrophenyl)imino)methyl)naphthalen-2-ol*
Positive control (Chloramphenicol)	 *2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide*

Additionally, a new preparation of the protein that had been created in the BIOVA Discovery Studio Visualiser (DSV) tool was made using the Molecular Operating Environment (MOE) 2022.0901 software package. The first step is to add hydrogen to the option for adding hydrogen. CHARMM27 was selected as the best force field, and the coordinates X, Y, and Z were set at 25.334, 46.535, and 66.305, respectively. RMS Gradient is set to 0.001 (kcal/mol/A). QuickPrep tools in MOE 2022.0901 were used for the protein preparation. Finally, this protein was saved in PDB format, and then it was ready to be used as a receptor.

Molecular docking

The database of ligands is in "MDB (Microsoft Access database)" format. It contained four structures of the prepared imine derivative compounds selected as ligands. Docking was performed with placement and refinement positions of 50 and 100, respectively. Furthermore, the best binding mode for each compound was selected and visualised in 2D and 3D.

Physicochemical and pharmacokinetic properties (ADME)

A pharmacokinetic profile was obtained, as described below. Molecular structure of imine derivative compounds with MarvinSketch and search for the SMILES formula using OpenBabel. After getting the SMILES formula, click "*run*". The pharmacokinetic profiles of the imine derivatives were analysed using SwissADME.

The pharmacokinetic profile of a drug can be influenced by its physicochemical properties. Analysis of the pharmacokinetic properties of drugs in the context of bioavailability includes molecular weight (MW) < 500 g/mol, Donor H-bond (HBD) <5, H-bond acceptor (HBA) < 10, and logarithmic calculation of the 1-octanol/water (cLogP) <5 partition coefficient. Research has been conducted on the physicochemical properties of compounds that have potential as drugs based on Lipinski's rule of five to describe the properties of the compound in terms of molecular weight, log P, hydrogen donor bonds, and acceptor hydrogen bonds.

Results

Docking

The molecular docking results for all the ligands are shown in Table II. Figure 1 shows the spatial arrangement of chloramphenicol as a positive control. The spatial arrangement and superimposition of compound 1 is shown in Figure 2. Figure 3 shows the spatial arrangement of compounds 2, 3 and 4.

Table II: Docking results

No	Binding free energy (kcal/mol)	RMSD	Hydrogen bond	Hydrophobic interaction	Van der Waals interaction	Another interaction	Binding factor
Chloramphenicol (Positive control)	-6.7701	1.1876	Thr39, Arg395	Arg395	Asp385	Tyr448, Pro341, Tyr399, Ser447, Pro251, Tyr369, His252, Val288, Phe455, Ile488, Val490, Tyr436	15
1	-7.6154	0.9367	**Tyr43, Asp385**	**Arg395**		**His252**, Ile339, **Pro251, Val288,** Thr289, **Ile488, Phe455, Tyr448, Pro341,** Val450, **Tyr399, Tyr369**	12
2	-6.4478	0.9017	**Val288, Asp385**	**Arg395**	Asp340	Asn284, **Pro341,** Ile339, **Tyr436, Phe455, Ile488, Val490, Tyr399, Thr397,** Thr383, **His252**	11
3	-6.3965	0.7418	**Val288, Asp38, Phe455**	**Arg395**		Ile339, Asn284, **Pro341, Tyr436, Ile488, Val490, Tyr399,** Thr383, **Thr397, His252**	11
4	-6.7615	0.8355	**Val288, Asp385**	**Arg395**	Asp340	Asn284, **Pro341,** Ile339, **Ile488, Phe455, Tyr436, Tyr399, His252, Thr397,** Thr383	10

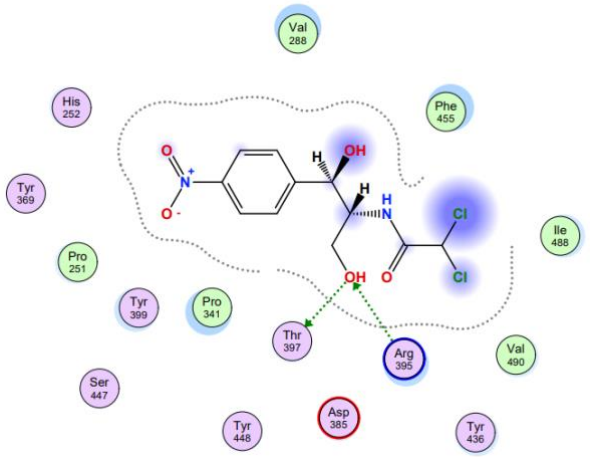


Figure 1: Spatial arrangement of the binding site for chloramphenicol as a positive control

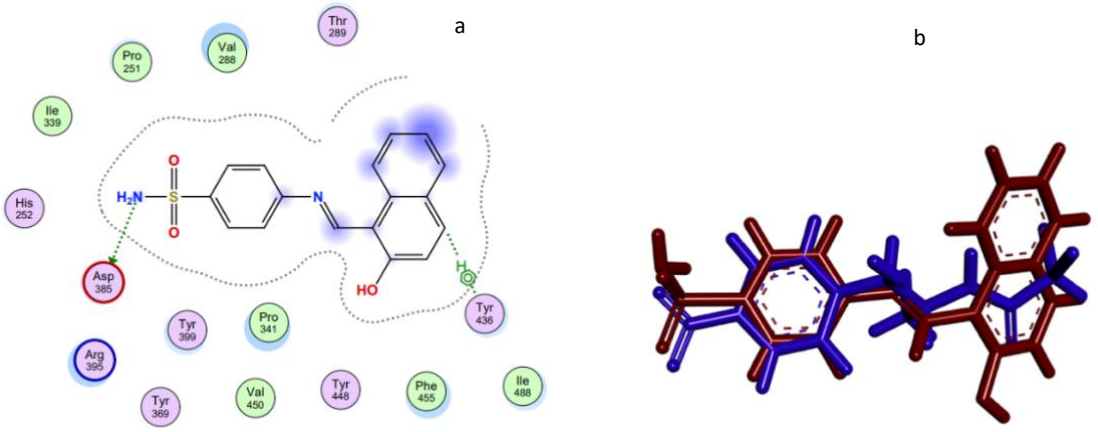


Figure 2: Visualisation of the spatial arrangement of compound 1(a); Superimposition of compound 1 (brown) and chloramphenicol (blue) (b)

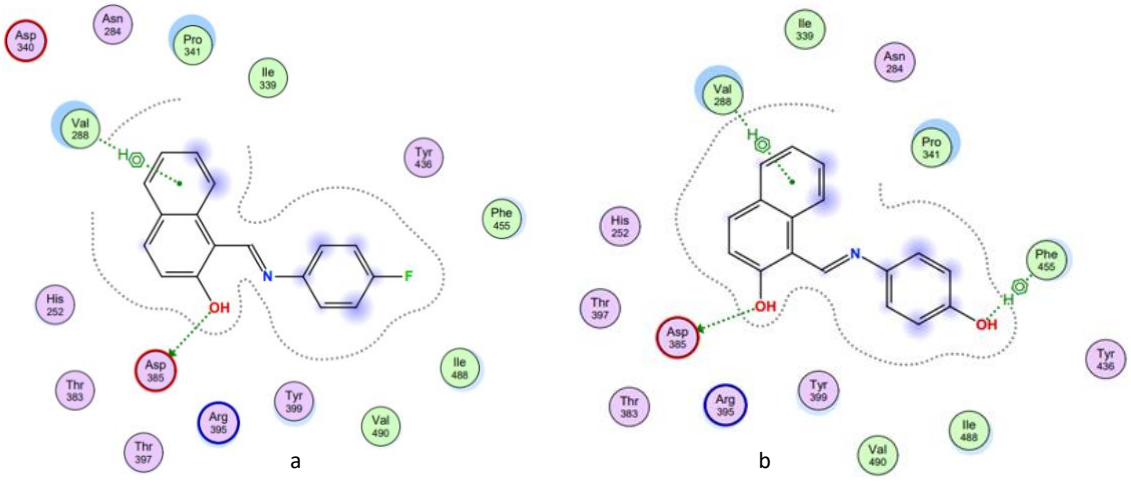


Figure 3: Spatial arrangement of compounds 2 (a) and 3 (b)

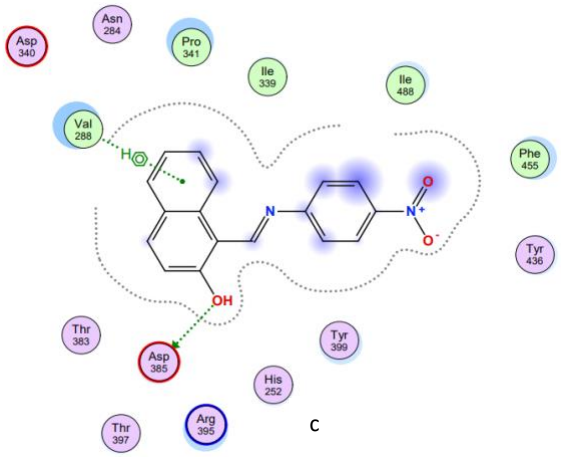


Figure 3: Spatial arrangement of compound 4 (c) (continued)

ADME profiling and toxicity prediction

Prediction of physicochemical properties was carried out using SwissADME, which is based on Lipinski's rule of five and requires several physicochemical parameters. Drug-likeness or drug similarity is used to estimate the probability of a molecule becoming an oral drug by considering the qualitative bioavailability of the compound (Lipinski *et al*., 2001; Sarfaraz *et al*., 2020).

Drug-likeness based on analysis using SwissADME online application can provide access to five types of existing rules with a wide range of drug-like categories for each rule. In this study, drug-likeness analysis was used according to Lipinski's rule, also known as the rule of five (RO5). The parameters of Lipinski's law are Log P, molecular weight, hydrogen bond acceptors (HBA), and hydrogen bond donors (HBD). The results of physicochemical predictions are presented in Table III.

Table III: SwissADME results

No	Molecular weight (g/mol)	Log P	Hidrogen bond donor (HBD)	Hidrogen Bond akseptor (HBA)	Rotatable bond	Druglikeness
1	326.37 g/mol	2.83	2	5	3	yes
2	265.28 g/mol	4.43	1	3	2	Yes
3	263.29 g/mol	3.44	2	3	2	yes
4	292.29 g/mol	3.06	1	4	3	yes
Chloramphenicol	323.13 g/mol	0.53	3	5	7	yes

Discussion

Molecular docking

The orientation between the ligand and receptor can be determined using molecular docking simulation (Prieto-Martinez *et al*., 2018; Chiemela *et al*., 2022). Generally, there are two types of docking: blind and directed. Oriented docking is a docking procedure carried out by knowing the precise location of the receptor's active site, whereas blind docking is a docking process carried out without this knowledge (Sampath & Padmanabhan, 2009; Anuradha *et al*., 2022). In this study, the docking process was performed using the blind docking method because the downloading process of the protein contained a native ligand attached to the protein. The molecular structure of this native ligand is different from that of chloramphenicol; thus, the active site of the protein with chloramphenicol is unknown.

Parameters that can be used for molecular docking are Binding free energy (S), which is the energy required for a ligand to bind to proteins (receptors). Smaller binding free energy indicated that interaction between ligand-protein is more stable. The RMSD value indicated that deviations or errors occurred during docking. Smaller RMSD values indicated more minor deviations or errors that occurred during docking. The optimal complex ligand-protein poses were chosen based on the lowest

binding free energy value as well as the lowest RootMean Squared Deviation (RMSD) value.

Based on the docking results as presented in Table 1, the positive control (chloramphenicol) had a binding free energy value of -6.7701 kcal/mol with an RMSD value of 1.1876. Chloramphenicol was able to bind with 15 amino acid residues on the active site of the receptor, namely the amino acids Thr397, Arg395, Asp385, Tyr448, Pro341, Tyr399, Ser447, Pro251, Tyr369, His252, Val288, Phe455, Ile488, Val490, and Tyr436. The visualisation results showed that chloramphenicol could form hydrogen bonds with amino acid residues Thr397 and Arg395. Both amino acids are bonded through hydrogen bonds with the hydroxy groups. The hydroxy groups that act as hydrogen bond donors are marked with green dotted lines with arrows pointing to the amino acid Thr397. The hydroxyl group that acts as a hydrogen bond acceptor is marked with a green dotted line with arrows pointing towards the hydroxyl group. The van der Waals bonds formed by the Asp385 amino acid residue are marked with a red ring. In addition, chloramphenicol also has one active site of the acid residue from the protein Arg395 with a hydrophobic bond marked with a blue ring.

Compound 1 had a binding free energy of -7.6154 kcal/mol, with an RMSD of 0.9367. There are 12 amino acids bonded with compound 1, and these amino acid residues are in common with the positive control, namely amino acid residues Tyr436, Asp385, Arg395, His252, Pro251, Val288, Ile488, Phe455, Tyr488, Pro341, Tyr399, and Tyr369. The visualisation results showed that compound 1 was unable to perform van der Waals interactions, but this compound was constructed by hydrogen bonding between amino acid residues Tyr436 and Asp385 with naphthalene and amine groups, respectively. In this case, the amine groups. Based on the visualisation of docking results and the superimposition results, compound 1 was shown to be an active agent against *S. aureus* (Frimayanti *et al*., 2020a; Frimayanti *et al*., 2020b). Compounds 2, 3, and 4 had binding free energies of -6.4478, -6.3965, and 6.7615 kcal/mol, respectively. All three compounds had another spatial arrangement with the positive control; thus, these compounds could not bind and superimpose well with the positive control. Although compounds 2, 3, and 4 formed a hydrogen bond with the val2888 residue, they could not be regarded as potentially active ligands because their binding free energy values were more significant than those of the positive control (Cournia *et al*., 2017). Additionally, compounds 2 and 4 showed van der Waals contact with Asp340; however, the binding was distinct from that of the positive control because of its different amino acid sequence. Therefore, these substances cannot be considered possible inhibitors of *S. aureus*. Validation of the docking method by superimposing the ligand with a positive control. It was used to determine the number of crucial residues that could bind well with the ligands during the binding process. Superimposition is also used to confirm the possibility and identify the characteristics of the active substances that can stabilise the connection between the ligand and protein target (Cournia *et al*., 2017; Tripathi & Bankaitis, 2017; Abrusan & Marsh, 2019). Superimposition was also used to simultaneously evaluate the orientation of the ligands.

Physicochemical and pharmacokinetic properties (ADME)

Based on Lipinsky, a compound is difficult to absorb and has low permeability if it has a molecular weight higher than 500, log value of octanol/water partition coefficient (log P) higher than 5, Hydrogen Bond Donors (HBD), which is expressed by the number of O-H and N-H groups, higher than 5, and Hydrogen Bond Acceptors (HBA) expressed by the number of O and N atoms, higher than 10 (Lipinski *et al*., 2001). Molecular weight affects the ability of a compound to penetrate cell membranes by passive diffusion; if a compound has a molecular weight (BM) greater than 500 g/mol, its ability to diffuse across the membrane becomes more difficult. The log P parameter describes the ability of a compound to dissolve in octanol/water (biological membrane). If log P is high, the compound becomes more hydrophobic. Hydrophobic compounds tend to have a higher level of toxicity because they last longer in the lipid bilayer and are widely distributed throughout the body, thereby reducing the selectivity of target binding. However, if the log p of a compound becomes increasingly negative, it becomes difficult for the compound to cross the lipid bilayer. Donor hydrogen bonds and acceptor hydrogen bonds are parameters used to describe the hydrogen bonding capacity of a compound required in the absorption process; therefore, if the number of hydrogen bonds in the donor is greater than five and the acceptor is greater than 10, the energy required in the absorption process increases. Hydrogen bonding can affect the chemical and physical properties of a compound, such as its boiling and melting points, solubility in water, ability to form chelates, and acidity. In general, Lipinski's five-law is used to describe solubility (Benet *et al*., 2016).

Compounds 1, 2, 3, and 4 fulfilled Lipinski's rule of five without any deviation in their parameters. From the prediction results, it can be predicted that compounds 1, 2, 3, and 4 are easily absorbed and have good permeability. According to Lipinski, if a compound fails to fulfil Lipinski's rule of five, there is a high probability

of problems related to oral absorption. However, a compound that fulfills Lipinski's five laws does not guarantee good activity because Lipinski's law is not related to the specific chemical structure contained in a compound.

Based on the results of the molecular docking and ADME studies, it was found that compound 1 has similarities between the ligand and the positive control (chloramphenicol), suggesting that compound 1 has potential as an antibacterial agent. In addition, because compound 1 also complies with Lipinski's law or the rule of five (RO5), this compound is easily absorbed and has good permeability.

Conclusion

Based on the molecular docking of four imine-derivative compounds, only compound 1 (E-(((2-hydroxynaphthalen-1-yl)methylene)amino) benzenesulfonamide) had a lower binding free energy value than the positive control. The binding free energy and RMSD of compound 1 were − -7.6154 kcal/ mole and 0.9367, respectively. This compound also contained 12 amino acids, similar to the positive control. Thus, compound 1 has the potential for use as an antibiotic. Based on SwissADME, it is also predicted that Compound 1 is easy to absorb, has good permeability and is used as a promising agent against *S. aureus.*

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