Research Article

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Antioxidant, Antimicrobial Properties and *In Silico* Study of a N,N'-(ethane-1,2-diyl)bis(1-(9H-fluoren-2-yl)methanimine)

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ABSTRACT

This study aimed to determine the antibacterial and antioxidant activities of the newly synthesized Schiff base and to support the laboratory results with molecular modeling studies. Antibacterial activity of schiff bases was demonstrated using Gram (-) *Pseudomonas aeruginosa* and *Acinetobacter baumanni* bacterial strains. Minimum Inhibition Concentration (MIC) values were determined to evaluate their antimicrobial activity against Gram (-) *Pseudomonas aeruginosa* and *Acinetobacter baumanni* bacterial strains. In antioxidant experiments, the responses to DPPH and ABTS radicals were calculated at certain concentration ranges and graphs were drawn. For the molecular modeling study, Autodock Vina and Discovery Studio 2020 package programs were used. The observed bacterial inhibition activity varied depending on the clinical isolate and the concentration of the samples tested. The highest inhibition activity was achieved at concentration of 75 µl -100 µl. *N,N'*-(ethane-1,2-diyl)bis(1-(9*H*-fluoren-2-yl)methanimine) samples. Molecular docking results show that *N,N'*-(ethane-1,2-diyl)bis(1(9*H*-fluoren-2-yl) methanimine) binds strongly to the 4ZIY and 4ZHU structures. It has been proven by molecular docking study that the synthesized Schiff base ligand has antibiotic resistance properties. N, N'-(ethane-1,2-diyl)bis(1-(9Hfluoren-2-yl) methanimine) synthesis compound showed moderate activity against A. baumannii and P. aeruginosa strains. It is known that Schiff bases have strong biological activities and antibacterial activity. In this study, the synthesis of Schiff base showed antibacterial and antioxidant activity. In addition, our results were supported by molecular modeling. Our findings can be taken to a higher level with in vivo and in vitro studies.

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Introduction

As in the whole world, *Pseudomonas aeruginosa (P.aeruginosa)* and *Acinetobacter baumannii (A.baumannii)* strains have started to be isolated with increasing frequency and multiple resistance in our country in recent years. This rapid increase in antimicrobial resistance reduces

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and prolongs the treatment options of clinicians, especially in intensive care units (ICU) infections caused by these microorganisms [1,2,3]. ICUs are the units most frequently encountered with nosocomial infections and resistant microorganisms due to the more frequent use of invasive procedures, long hospital stays, and the fact that hospitalized patients are often immunosuppressive, elderly, newborns or patients who have undergone an operation [4,5]. *A.baumannii* and *P.aeruginosa* are Gram (-) microorganisms that are the most common cause of nosocomial infections in ICU [6,7,8,9]. Since these bacteria are resistant to external environmental conditions, they can maintain their vitality for a long time in the hospital environment. In addition, the fact that they are naturally resistant to many antibiotics and can develop acquired resistance in a short time gradually limits the antimicrobials that can be used in the treatment of the infections they cause [11,12,13].

Researching a new drug and putting it on the market involves quite laborious processes. These processes, which require a long time, are also quite expensive. Today, the discovery and development processes of drugs can be realized in a shorter time and at less cost by using special design technologies [14]. Molecular docking studies can define that a drug can be an active ingredient in a structure [15]. By looking at the receptor and ligand structure, it is seen whether the compound carries a functional drug group [16]. Compounds containing the azomethine group (-HC=N-), which were first introduced by Hugo Schiff in 1864 and formed as a result of the reaction of a primary amine with an active carbonyl compound, and which are generally carried out by acid, base catalysis or heat, are called schiff bases [17,18]. Schiff bases are useful chelates because of their ease of preparation, structural variety, and steric and electronic control mechanisms [1]. These are privileged ligands and find a lot of use thanks to their advantages such as versatile synthesis and good solubility [1]. In azomethine derivatives, the C=N bond is required for biological activity. The nitrogen atom of azomethine is involved in the formation of components and interacts in normal cell processes [21]. It is known that heterocyclic structures containing an azole ring system and a phonol derivative have a wide range of biological applications for antifungal, antioxidant, antibacterial, antitumor, anti-inflammatory and antipyretic applications [16].

Reactive oxygen species (ROS) are free radicals produced during oxidative metabolism. ROS can attack nucleic acid, lipids, proteins, polyunsaturated fatty acids and carbohydrates and induce their oxidation, which can lead to oxidative damage such as protein alteration, membrane dysfunction, enzymatic inactivation, and rupture of DNA strains [17]. Therefore, ROS must be cleared by cellular voters. An antioxidant can inhibit or delay the oxidation of other molecules. Antioxidants can inhibit the formation of free radicals and also delay lipid

peroxidation, which leads to deterioration of food and pharmaceutical products during processing and storage. Antioxidants can protect the human body from ROS. Antioxidants are widely used in foods to prevent radical chain reactions that cause food spoilage [18].

In this study, which was carried out for the first time, it was determined that the synthesized *N*,*N*'-(ethane-1,2-diyl)bis(1-(9*H*-fluoren-2-yl)methanimine) (**1**) schiff base, which is frequently found in ICU (*A. baumannii*, *P. aeruginosa*) bacteria and to investigate the binding affinities and models in bacterial structures by in silico studies. New compounds need to be synthesized and investigated, since existing compounds show weak bacterial resistance.

Material and Methods

Chemistry

Compound **1** which bearing fluorene group was prepared in the air atmosphere. The solvents and all other reagents were commercially available from Sigma-Aldrich and ISOLAB chemical company and used without further purification. Melting point was identified in glass capillaries under air with an Electrothermal-9200 melting point apparatus. FT-IR spectrum was saved in the range 400-4000 cm-1 on Perkin Elmer Spectrum 100 FT-IR spectrometer. Proton (^{1}H) and Carbon (^{13}C) NMR spectra were recorded using either a Bruker AC300P FT spectrometer operating at 300.13 MHz (^{1}H) and 75.47 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane as an internal reference. Elemental analyses were performed by Inonu University Scientific and Technological Research Center (Malatya, TURKEY).

Synthesis of (N,N'-(ethane-1,2-diyl)bis(1-(9H-fluoren-2-yl)methanimine), 1

Compound **1** was synthesized by reacting 1 mL (0.9 g, 15 mmol) ethylenediamine with 2 equivalents of 9*H*-fluorene-2-carbaldehyde (5.83 g, 30 mmol) in ethanol (20 mL) for 2 hours. Then, the compound crystallized by cooling the mixture. The crystals formed were washed by filtration with diethyl ether. As a result white solid was obtained. Yield: 82% (5.07 g); m.p.: 97-98 °C; color: white. Anal. calc. for $C_{30}H_{24}N_2$: C: 87.35; H: 5.86; N: 6.79. Found: C: 87.07; H: 5.93; N: 6.85. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K), δ (ppm): 3.10 and 3.74 (t, 4H, *J*= 5.6 and 5.5 Hz, −NC***H***$_2$C***H***$_2$N-); 3.93 and 4.05 (s, 4H, Ar-C***H***$_2$-Ar); 7.32-8.01 (m, 14H, Ar-***H***); 8.40 and 8.43 (s, −N=C***H***-$_{imine}$). ^{13}C NMR (100 MHz, $CDCl_3$, 298 K), δ (ppm): 35.2 and 37.6 (Ar-***C***H_2-Ar); 60.4 and 61.2 (−N***C***H_2***C***H_2N-); 119.0, 125.2, 126.9, 127.4, 128.1, 128.6, 129.4, 130.3, 141.1, and 143.4 (Ar-***C***); 161.6 (−N=***C***H-$_{imine}$).

Antioxidant study Antimicrobial study

DPPH Radical Scavenging Activity

DPPH• free radical scavenging activity of synthesized compounds and standard antioxidants was performed by Blois method [19,44]. 1 lt of 0.1 mM DPPH• solution was prepared in ethanol and adjusted to 3 ml by adding solutions of different concentrations [9,15]. The prepared solutions were thoroughly vortexed and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm with a spectrophotometer. DPPH• radical scavenging activity was calculated using the following equation:

DPPH• scavenging effect (%) = (Absorbance of control vs. absorbance of sample) / Absorbance of control x 100

ABTS•$^+$ Scavenging Activity

It was made by the color change method, which is an indication that the dark blue/green colored ABTS•$^+$ cation radical has lost its radical property as a result of the treatment with antioxidants [22]. ABTS•$^+$ cation radical was obtained by mixing the ABTS solution prepared with 2 mmolL-1 H_2O and 2.45 mmolL-1 potassium persulfate ($K_2S_2O_8$) solution at a ratio of 1:2 and incubating for 14 hours in the dark and at room temperature. Before using the ABTS•$^+$ cation radical, the ABTS•$^+$ solution was diluted with sodium phosphate buffer (0.1 molL-1, pH 7.4) to obtain an absorbance of 0.750 ± 0.025 at 734 nm. Then, 10, 20, 30 µL of the stock solutions of the synthesized organic compounds were taken and phosphate buffer was added until the volume was 3 mL, and 1 mL of ABTS•$^+$ solution was added to them and vortexed. Inhibition was calculated at 734 nm for each concentration [21,22].

ABTS•$^+$ cation radical scavenging activity was calculated using the following equation:

ABTS•$^+$ radical scavenging activity (%) = ((Absorbance of control - Absorbance of sample) / Absorbance of control)) x 100

Antimicrobial study

Bacterial Strains

Pseudomonas aeruginosa and *Acinetobacter baumannii* bacterial strains were used to evaluate the antimicrobial activity. All multidrug-resistant clinical isolates were obtained from Bacteriophage Microlysis Therapeutic Bank (MTBB, Ankara, Turkey): *A. baumannii* (MTBB 120557), *P. aeruginosa* (MTBB 130203). Condalab branded Miller's LB Agar was used as the medium. In the macrodilution method, the effect of different concentrations of antimicrobial agent dilutions prepared using sterile tubes against a certain concentration of microorganism was investigated. With the effective concentrations of the substances synthesized against the used microorganism; MIC value is determined according to the presence or absence of growth

[23,24]. Optical density (OD) measurement and counting of viable microorganisms are the most commonly used methods for monitoring growth. The results obtained are more sensitive than the agar dilution method [23].

Antimicrobial activity

For bacterial inhibition assay, 1 ml of overnight cultures of the tested strains were diluted with 100 ml Luria Broth (LB) and grown until the turbidity equal to 0.1 to 0.3 McFarland at 37°C at 100 rpm. The dilutions of the Compound **1** in concentration range of 25-100 ul with cell suspensions were added to the test tubes and incubated at 37 °C with shaking at 150 rpm. The turbidity was measured for up to 5 h.

Preparation of medium

Miller's LB Broth (5.5 g) was weighed and diluted to 250 ml, then the density was adjusted according to McFarland standard (0.5).

Molecular modeling method

Ligand System

N,*N*'-(ethane-1,2-diyl)bis(1-(9*H*fluoren-2-yl) methanimine) material was imported in sdf format in ChemDraw 3d program. Converted from Open Babel GUI program to pdb format.

Protein system

4ZIY and 4ZHU crystal structures were obtained from the Protein Data Bank (www.rcsb.org). While choosing the crystal structures, care was taken to ensure that the resolution value of the proteins was a maximum of 3 Å.

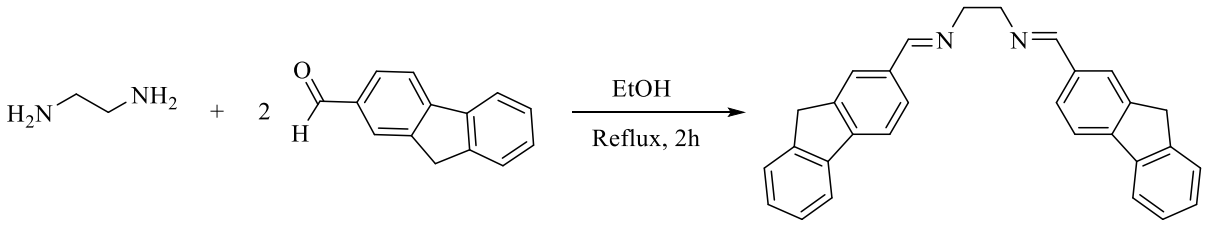
Molecular Modeling

Autodock 4.2.6 is used. AutoDockTools program was used to create modeling data entry files. In all models, a cube divided into squares with 40x40x40 point dimensions in x, y, z directions was created. A length of 0.375 Å (about one quarter the length of the carbon-carbon covalent bond) and a distance dependent function of the dielectric constant were used to calculate the energy of the couplings. 10 processes were carried out using Lamarckian genetic algorithm logic. Randomly placed fragments with an initial population of 50 were used with a maximum energy of 2.5 x 106 and a maximum of 2.7 x 104 formations. A mutation rate of 0.02 and a genetic change rate of 0.8 were chosen. Results that differed less than 0.5 Å in root mean square deviation (RMSD) were pooled and the results of the optimal free energy of coupling were chosen as the final complex structures. Ligand-protein interactions in the active site of **1** were investigated using Autodock Vna 1.1.2 [16] and Discovery Studio 2020 programs [23].

Results and Discussion

Synthesis and characterization study

Compound **1** was synthesized from the ethylenediamine and 2 equivalents of the 9*H*-fluorene-2-carbaldehyde in ethanol (Scheme 1). This compound was obtained in moderate yields 82%. The compound's structure was determined using FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopic methods, and elemental analysis techniques. The proton peak was detected in the ^{1}H NMR spectra by a characteristic peak at the imine (N=CH), which appeared as a highly downfield shifted singlet at 8.40 and 8.43 ppm. The ethylene group (CH_2) proton peaks were observed as triplets at 3.10 and 3.74 ppm. Aliphatic methylene (CH_2) singlet peaks between two aromatic rings were observed at 3.93 and 4.05 ppm. Proton peaks were observed as multiplets in both aromatic rings, ranging from 7.32-8.01 ppm. In the ^{13}C NMR spectra of the compound, a carbon signal at 161.6 was attributed to imine carbon (N=CHN) at a lower area compared to other aromatic carbons. The ethylene group (CH_2) carbon peaks were observed at 60.4 and 61.2 ppm. Aliphatic methylene (CH_2) carbon peaks between two aromatic rings were observed at 35.2 and 37.6 ppm. All data are consistent with the proposed formula.



Scheme 1. *N,N'*-(ethane-1,2-diyl)bis(1-(9*H*-fluoren-2-yl)methanimine), **1**

Antioxidant activity results

DPPH Radical Scavenging Activity Results

The reducing capacity of DPPH• radicals was determined by the reduction in absorbance at 517 nm as a result of the induction of antioxidants. The maximum absorbance of a stable DPPH• radical in ethanol was recorded as 517 nm. They make the antioxidant molecules an inactive radical by donating a hydrogen proton to the DPPH$^{\bullet}$ radical. As a result of this reaction, low absorbance is obtained. A color change from purple to yellow is visually noticeable in this interaction. Therefore, DPPH$^{\bullet}$ is often used as a substrate to evaluate the antioxidant activity of antioxidant molecules [20,23]. DPPH$^{\bullet}$ is a stable free radical and takes an electron or a hydrogen radical to become a stable diamagnetic molecule [23]. Table 1 shows the decrease in the concentration of DPPH$^{\bullet}$radical due to the radical scavenging capacity of both the **1** extract and

the standards. At the same concentration (0.03 mg/ml), the scavenging effect of **1** extracts and standards on DPPH$^{\bullet}$ radical decreased in the order Trolox(90.5%) > BHA(75.8%) > BHT(73.8%) > **1** (49.8%). As a result of these results, **1** extract showed that it was effective in terms of free radical scavenging activity.

ABTS$^{\bullet+}$ Radical Scavenging Activity Results

The blue-green ABTS$^{\bullet+}$ radical cation scavenging activity of the synthesized compounds was measured according to the radical scavenging activity of the standard antioxidants BHA, BHT and Trolox. In this method, antioxidants oxidize the ABTS$^{\bullet+}$ dark cation radical, resulting in a reduction of dark color. The color change that occurs with this reaction is used as a parameter for the measurement of antioxidant potential [31]. The scavenging effect on ABTS$^{\bullet+}$ radical decreased in the order of Trolox (90.5%) > BHA(75.6%) > BHT(73.7%) > **1** (27%) is seen in Table 1. As a result of these results, **1** extract showed that it has an effect on free radical scavenging.

Table 1 The scavenging activity of compound **1** on ABTS$^{\bullet+}$ radical

Extract	**DPPH[b]** 0.03 mg/ml	**ABTS[b]** 0.009 mg/ml
1	49.8 ± 2.6	27 ± 0.5
BHA[a]	75.8 ± 5.9	75.6 ± 6.2
BHT[a]	73.8 ± 5.3	73.7 ± 5.7
TROLOX[a]	90.5 ± 6.4	90.5 ± 6.7

Data mean ± standard deviation,
[a]standard antioxidant
[b]The percent (%) of ABTS and DPPH radical scavenging activity

Antimicrobial activity results

The effects of *N*,*N*'-(ethane-1,2-diyl)bis(1-(9*H*-fluoren-2-yl)methanimine) (**1**) on *P.aeruginosa* and *A.boumannii* bacteria were evaluated by measuring their optical density. It has been shown that compound **1** has a high inhibitory effect on *A.boumannii* and a low level inhibitory effect on *P.aeruginasa* (Table 2). The effect of compound **1** on multidrug resistant strains is shown in

Table 3 as IC_{50} and % inhibition values. The effectiveness of compound **1** for *P.aeruginosa* and *A.boumannii* bacteria is shown in Figure 1 and Figure 2, where the MIC50 values for *P.aeruginosa* at 225 minutes and for *A.boumannii* bacteria at 120 minutes are between 75 µl and 100 µl.

Table 2 Inhibitory effect of compound **1** on *A.boumannii* and *P.aeruginosa*

Compound	***P. aeruginosa***	***A. boumannii***
1	-+	-+++

%25 - %50 inhibition -+ (less active), %50-%75 inhibition -++ (modarate active), %75 - %100 inhibition -+++ (highly active).

Table 3 % inhibition and IC_{50} values of compound **1** due to multidrug resistance against bacterial strains

Sample	**IC_{50} µg/ml**		**% Inhibition**		
Compoıund	*Pseudomonas aeruginosa* (MTBB 130203)	*Acinetobacter baumannii* (MTBB 120557)		*Pseudomonas aeruginosa* (MTBB 130203)	*Acinetobacter baumannii* (MTBB 120557)
1	75 µg/ml	80 µg/ml	25 µg/ml	10	20
			50 µg/ml	25	35
			75 µg/ml	30	45
			100 µg/ml	50	70

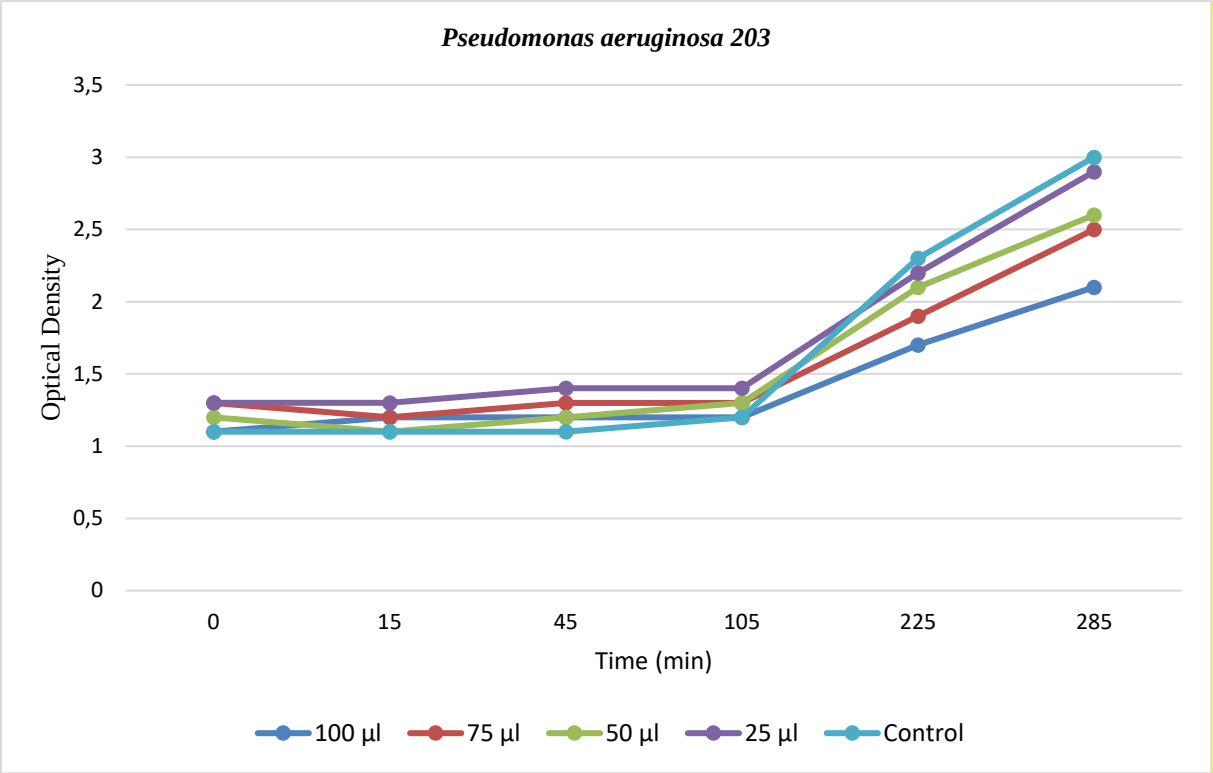


Fig *1* *Pseudomonas aeruginosa* 203 Optical Density of multidrug-resistant bacterial strains.

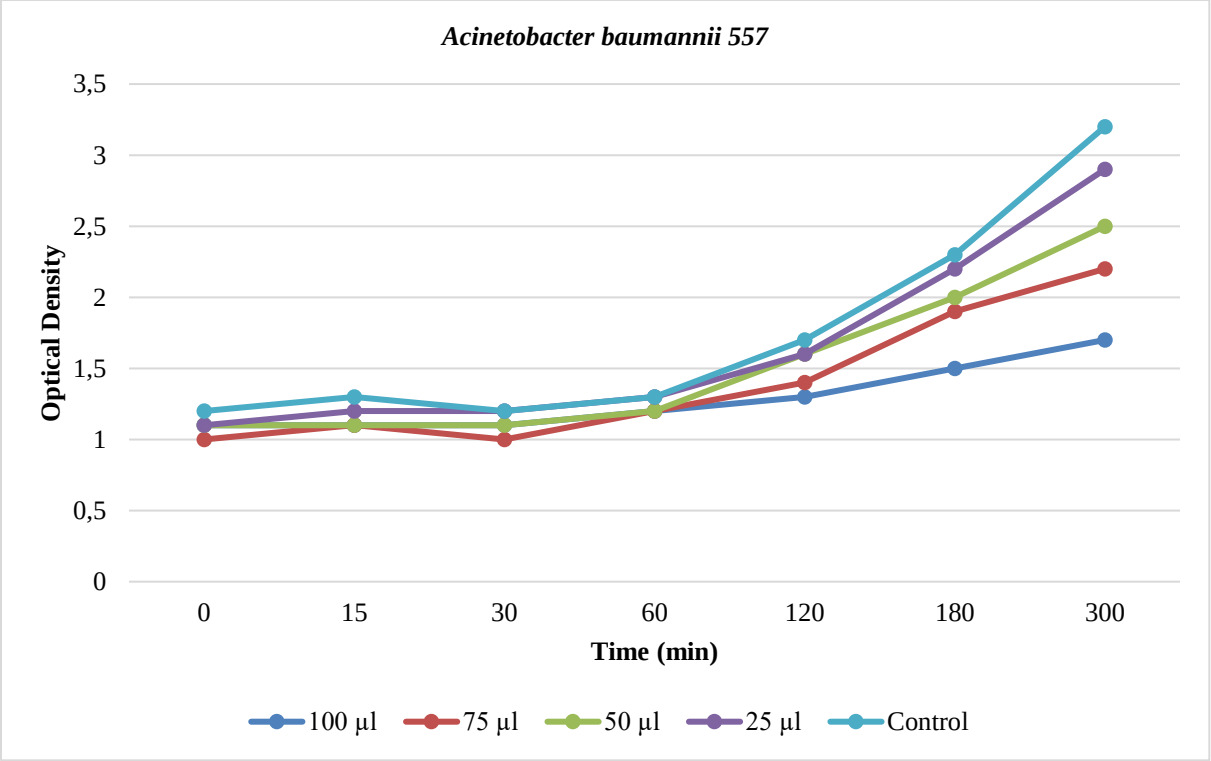


Fig 2 *Acinetobacter baumannii* 557 Optical Density of multidrug-resistant bacterial strains.

In silico Study Results

According to the molecular docking results, the docking score of Compound **1** molecule to the protein structure of 4ZHU (*Pseudomonas aeruginosa*) was found -8.8 kcal/mol (Table 4).

Table 4 In Silico study results of ligand **1** in 4ZHU (*Pseudomonas aeruginosa*)

Results Analysis Software	Visualization Sofware	Protein	Ligand	Docking Score	Amino Acid Residue
Autodock Vina	3 D BIOVIA Discovery Stduio Visualizer	4ZHU	**1**	-8.8	LEU53, ILE169, VAL176, PRO178
Autodock 4.2	3 D BIOVIA Discovery Stduio Visualizer	4ZHU	1	-8.8	PHE127, LEU130, ALA131, VAL135, GLY148, ARG171

Molecular docking model; The protein structure of 4ZHU (*Pseudomonas aeruginosa*) is shown in Figure 3 of the Compound **1** molecule.

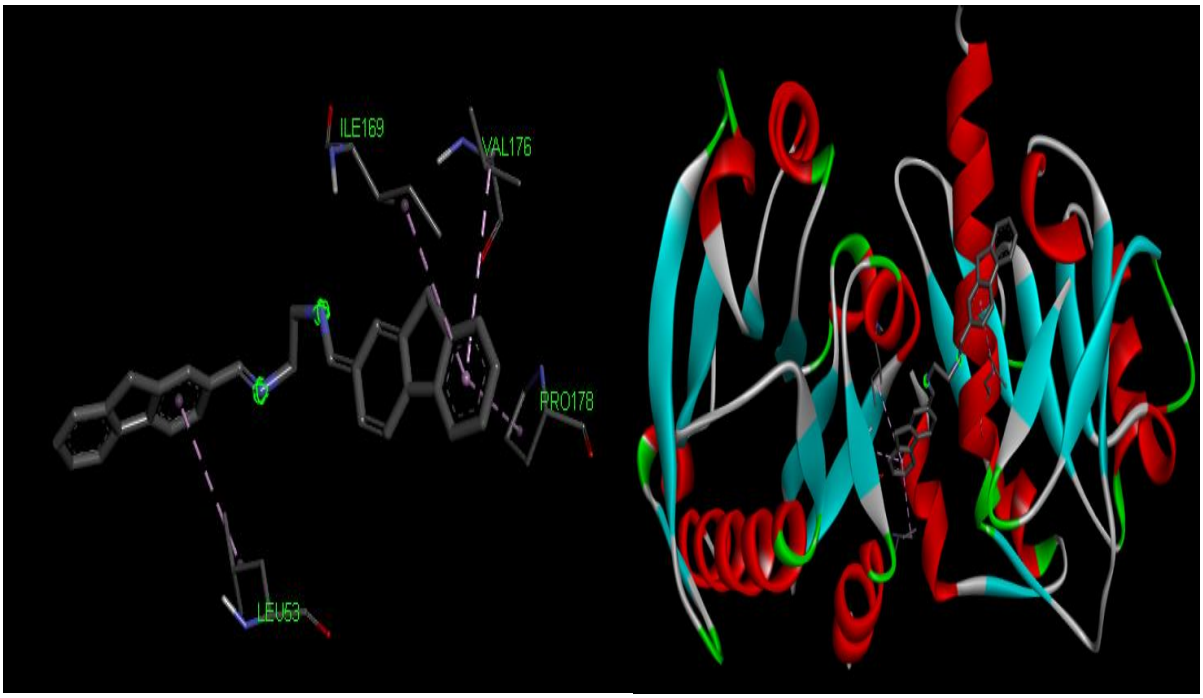


Fig 3 Docking patterns of Compound **1** ligand 4ZHU (amino acid residues of 4ZHU whose binding points were determined by Discovery Studio according to Autodock Vina results)

4ZHU appears to form pi-alkyl bond with LEU53, ILE169, VAL176, PRO178 with Compound ligand 1 (Figure 4).

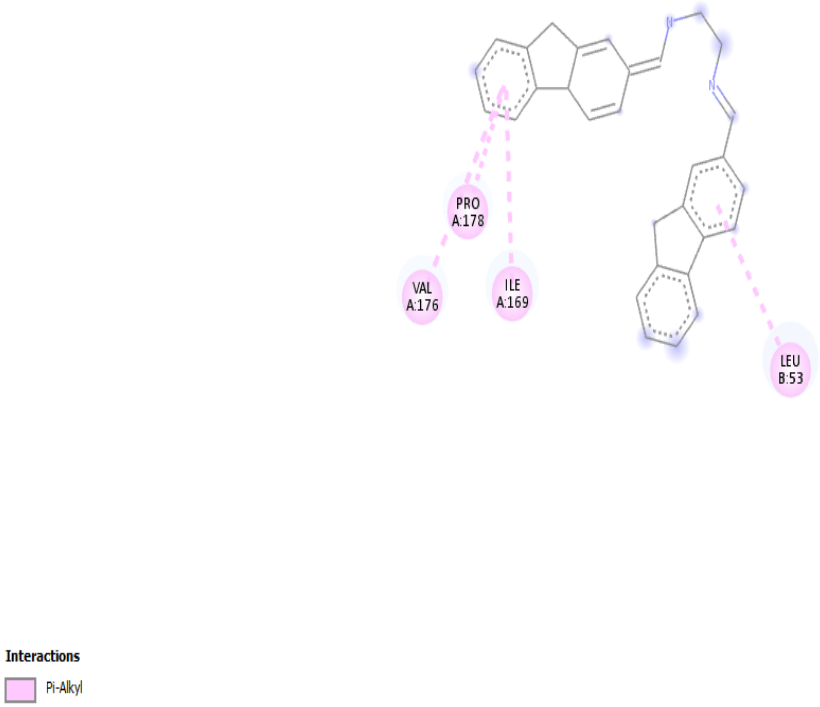


Fig 4 Bond structures of Compound ligand **1** in 4ZHU. (4ZHU appears to form pi-alkyl bond with LEU53, ILE169, VAL176, PRO178 with ligand **1**).

The sites where ligand Compound **1** is thought to be responsible for its biological effect in 4ZHU and where it interacts best with the target site appear (Figure 5).

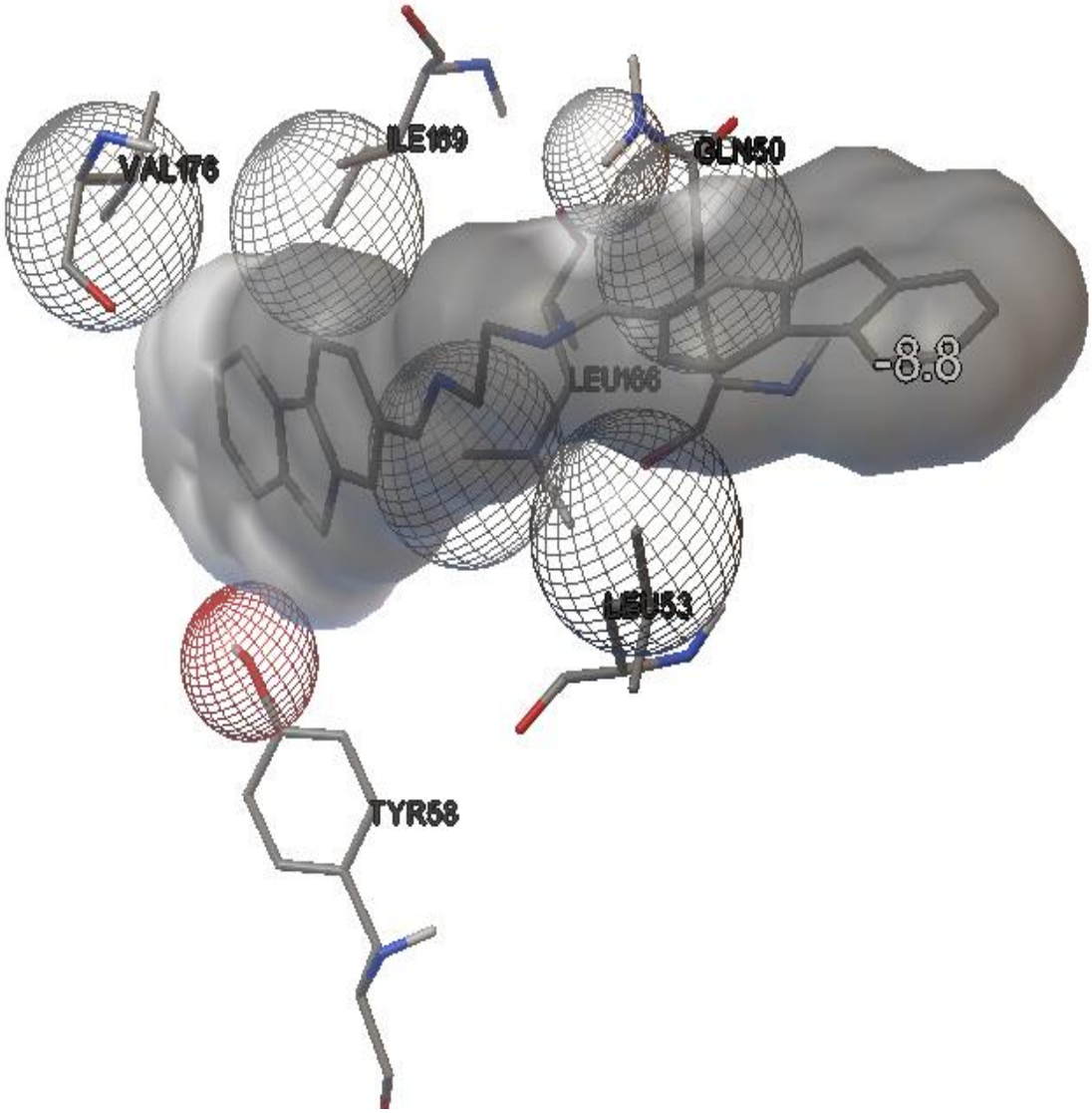


Fig 5 Pharmacophore pattern of ligand Compound **1** in 4ZHU. (The sites where ligand **1** is thought to be responsible for its biological effect in 4ZHU and where it interacts best with the target site appear.)

According to the molecular docking results, the docking score of Compound **1** molecule to the protein structure of 4ZIY (*A.baumannii*) was found -10.2 kcal/mol (Table 5).

Table 5 In silico study results of ligand Compound **1** in 4ZIY (*Acinetobacter baumannii*)

Results Analaysis Software	Visualization Sofware	Protein	Ligand	Docking Score	Amino Acid Residue
Autodock Vina	3 D BIOVIA Discovery Studio Visualizer	4ZIY	**1**	-10.2	GLY124, LYS125, THR127, HIS292, ARG327
Autodock 4.2	3 D BIOVIA Discovery Studio Visualizer	4ZIY	1	-10.2	GLY124, LYS125, THR126, PHE288, ALA289, HIS292, ASN293, GLY323, ARG327,LEU328, PHE330, ASN344

Molecular docking model; The protein structure of 4ZIY (*Acinetobacter baumannii*) is shown in Figure 6 of the **1** molecule.

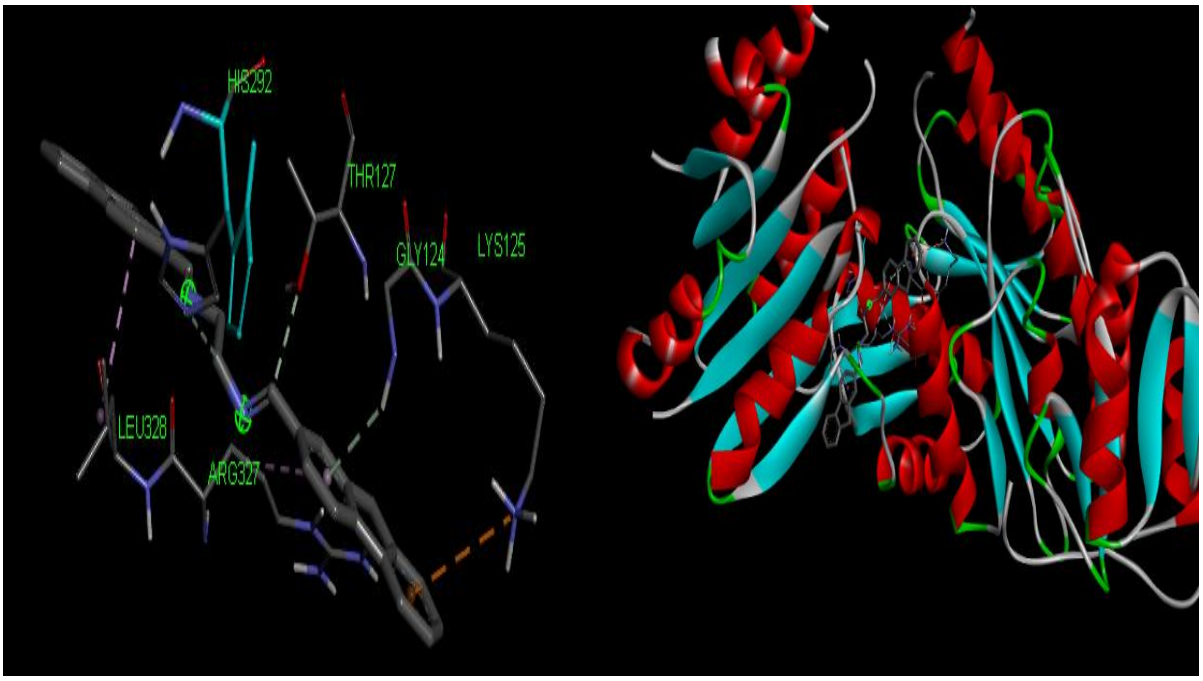


Fig 6 Docking patterns of Compound **1** ligand 4ZIY (amino acid residues of 4ZIY, whose binding points were determined by Discovery Studio according to Autodock Vina results)

To ligand Compound **1** of 4ZIY; It is observed that it forms pi-cation bonds with LYS125, pi-donor hydrogen bonds with GLY124, THR127, HIS292, carbon hydrogen bonds with HIS292, pi-alkyl bonds with LEU328 (Figure 7).

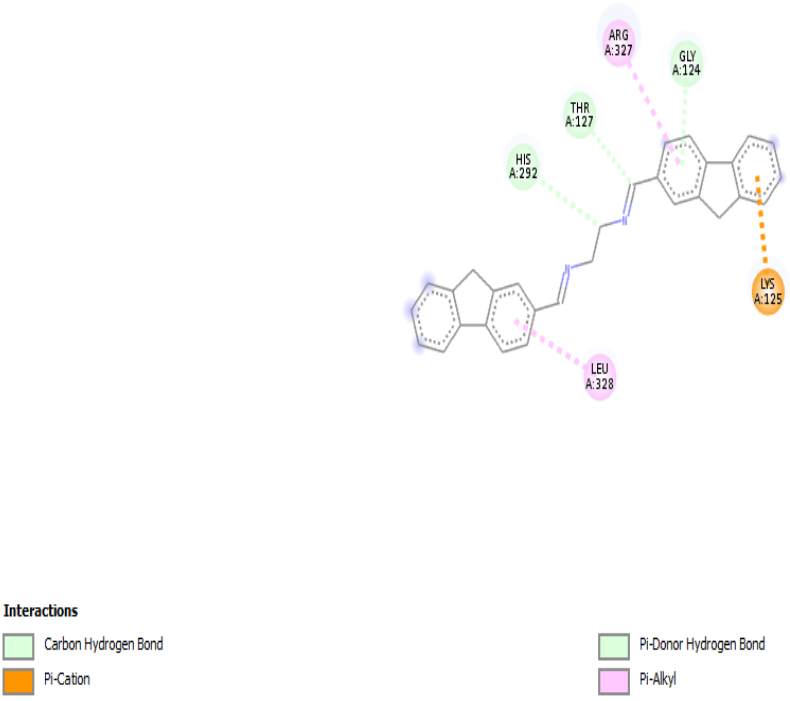


Fig 7 Bond structures of ligand **1** at 4ZIY (to ligand **1** of 4ZIY; It is observed that it forms pi-cation bonds with LYS125, pi-donor hydrogen bonds with GLY124, THR127, HIS292, carbon hydrogen bonds with HIS292, pi-alkyl bonds with LEU328).

Where Compound **1** ligand is thought to be responsible for its biological effect in 4ZIY and where it interacts best with the target site appears (Figure 8).

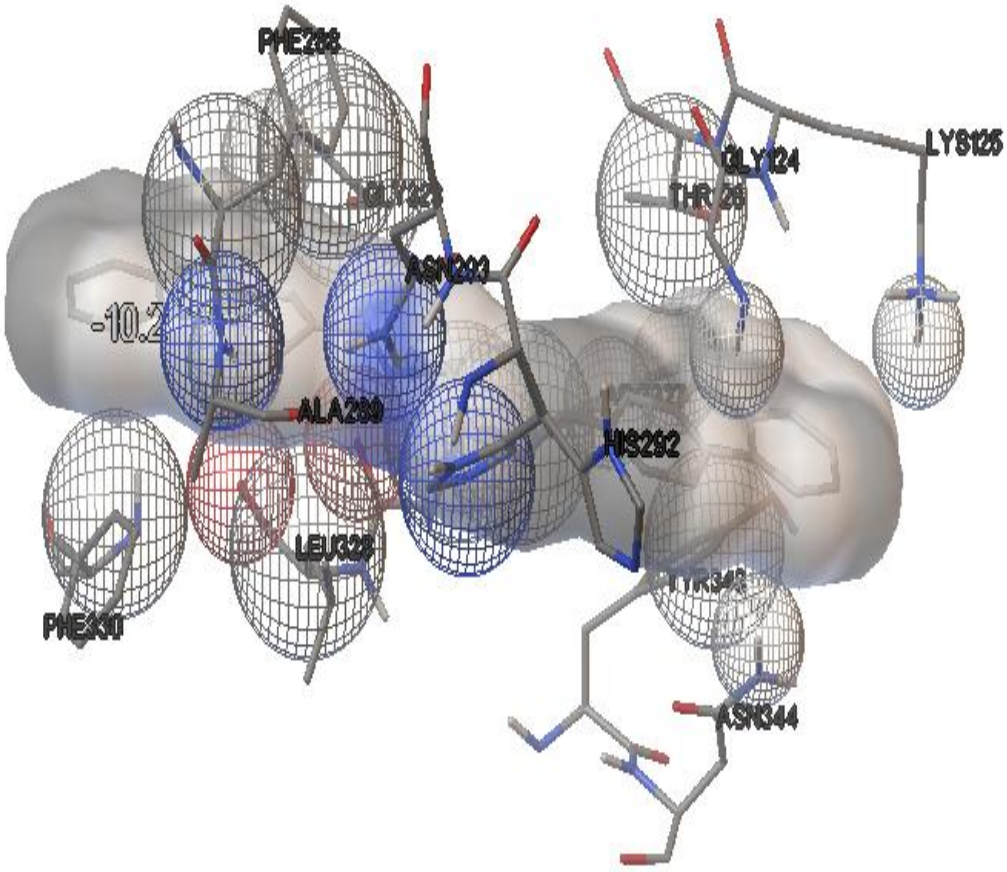


Fig 8 The pharmacophore pattern of ligand **1** in 4ZIY (where **1** ligand is thought to be responsible for its biological effect in 4ZIY and where it interacts best with the target site appears.)

Schiff bases are represented by the general formula RCH=NR'. In this notation, R and R' are aryl or alkyl substituents. It was observed that Schiff bases synthesized from the reaction of primary amines and carbonyl compounds were synthesized in two main stages. In the first step, a carbonyl amine intermediate is formed from the condensation of the primary amine and the carbonyl group. In the second step, Schiff base is formed as a result of the dehydration of the carbonyl amine intermediate [26].

Shanty et al. In 2017, Schiff bases were stated to have antioxidant activity. [26]. Antibacterial, fungicidal, anti-carcinogenic, catalytic and biological activity is attributed to the presence of the imine group (N=CH-) [27,28]. Schiff bases and their complexes are versatile compounds synthesized from the condensation of an amino compound with carbonyl compounds and widely used for industrial purposes, and are known to have antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties [29].

The synthesis of Schiff bases and the measurement of their biological activities have long been of interest to researchers. It is seen that many studies have been conducted in the literature in this area [29,30]. Sharma et al. 2019 determined the antibacterial effects of Schiff base ligands and metal complexes synthesized by the reaction of salicyaldehyde with o-phenylenediamine and p-phenylenediamine, 2-furaldehyde with o-phenylenediamine and p-phenylenediamine against the *E. coli, B. subtilis, P. Aereuguinosa, S. aureus* bacteria. They determined the MIC values of some complexes and the minimum inhibitory concentration (MIC) of complex number one against *B. subtilis* and *S. aureus* as 75 μmol mL[31]. It is seen to be compatible with the results obtained in our research. It is recorded in literature studies that Schiff bases show antibacterial and antifungal properties, but this activity is greater with the addition of metal complexes [30, 31]. The antibacterial activity of Schiff bases can be explained by the presence of keto-amine structure. In addition, the fact that they have an enol-imine structure rather than a keto form suggests that they may be effective in showing antibacterial activity. Using the turbidity reduction assay, we showed clinical isolates of *A. baumannii* and *P. aeruginosa* to be considerably affected by **1** regarding the growth inhibition activity. The concentration of **1** had notable impact on bacterial inhibition activity. This study reveals that **1** have potential agent to deal with multidrug-resistant bacteria either alone or in combination with conventional antimicrobials.

In general, it is thought that schiff bases will exhibit good antioxidant properties due to their chelating properties. Antioxidant determination of synthetic compound **1** was made by the method of reducing capacity of DPPH• radicals and ABTS•$^{+}$ radical cation scavenging activity. In this study, in which ethanol was used as a solvent, values of 49.8 and 27 were found for DPPH• and ABTS•$^{+}$, respectively. DPPH• method; It is widely used to measure the ability of antioxidants to scavenge free radicals. In this spectrophotometric method, a stable free radical, DPPH• (2,2-diphenyl-1- picrylhydrazil) reagent is used. It is based on the measurement of antioxidants' ability to reduce the DPPH• radical, which is reduced to hydrazine when this radical interacts with hydrogen donors. According to this method, strong hydrogen donor groups are required for compounds to show good antioxidant properties. The fact that the synthesis compound is close to the BHA compound indicates that its antioxidant property is low. It was found that synthetic compound **1** showed moderate and low inhibitory properties in terms of antioxidant activity compared to DPPH• and ABTS•$^{+}$ methods, respectively. Studies that similar to our findings are available in the literature [32,33]. This situation is thought to be related to the low hydroxyl group in the synthesized compound **1**. It is stated that the ethanolic extract of the synthetic schiff base compound, which has strong hydrogen donors, shows very

strong antioxidant activity [34]. In general, there are many factors that affect antioxidant activity in synthetic compounds. The high number of electron donating groups in the synthesis compound is one of the mechanisms that increase antioxidant activity. The type of solvent used stands out as another factor affecting the antioxidant result. It can be said that this solvent was used in the literature studies as the reason why ethanol was preferred in the research.

A. baumannii and *P. aeruginosa* have an important place among the infectious agents because they are resistant to adverse conditions. These bacteria, in addition to being naturally resistant to many antibiotics, can also develop resistance during antimicrobial use. Therefore, the infections they cause especially in ICU patients progress with high mortality and morbidity. It has been reported that there has been a remarkable increase in antibiotic resistance and multiple resistance in *A. baumannii* and *P. aeruginosa* over the years [35,36]. *A. baumannii* and *P. aeruginosa* are in the priority 1 (critical) group in the list of priority pathogens that urgently need new antibiotics, published by the World Health Organization (WHO) in February 2017 [37]. Centers for Disease Control states that multi-resistant *Acinetobacter* isolates cause 7300 infections and 500 deaths annually, while multi-resistant *Pseudomonas* isolates cause 6700 infections and 440 deaths annually [37].

These factors, which cause hospital-acquired pneumonia in particular, are most commonly isolated from respiratory materials. In studies, it is seen that the most frequently isolated material type is tracheal aspirate [38, 39]. 46% of A. baumannii strains and 52% of P. aeruginosa strains were isolated from tracheal aspirate samples. It is seen that blood is the sample type from which A. baumannii isolates in the second most frequency, while urine is the sample type from which P. aeruginosa strains are isolated in the second frequency. While many studies conducted in our country reported that these agents were detected most frequently in the anesthesia department among ICUs, 37% of A. baumannii strains and 39% of P. aeruginosa strains used in our study were isolated from the general ICU [40, 41, 42].

In vitro resistance rates detected especially against A. baumannii isolates isolated from the ICU are quite high [43]. The agents that can be used in the treatment of infections caused by bacteria are very limited, as they are naturally resistant to most antibiotics and develop resistance to almost all other antibiotics.

Conclusion

In studies on Schiff bases and metal complexes, it was concluded that complex Schiff bases are more effective on bacteria than free Schiff bases. However, the degree of action of the metal complexes of Schiff bases varies among themselves. But, in this study, it was concluded that

although Schiff bases showed antibacterial effects at different rates, free Schiff bases could be effective on bacteria as well as metal complexes. It was observed that the synthesized 1 schiff base ligand showed different levels of activity in terms of reducing capacity of DPPH• radicals and removing ABTS•+ radical cation, especially DPPH• activity was at a normal level. It has been found that the antioxidant activity of synthesis schiff base 1 is low in general. Molecular modeling insertion studies were conducted to reveal the binding mechanism and effect of ligand 1 to 4ZIY (A. baumannii) and 4ZHU (P. aeruginosa). The docking results show that ligand 1 binds strongly to the 4ZIY and 4ZHU constructs. Due to the binding strength of the 1 ligand, it reveals a unique structure for A. baumannii and P. aeruginosa, and it is thought that it can be a reference for designing new molecules with antibiotic resistance with the same structure and applying these molecules in in vivo and in vitro studies.

Abbreviations
ICU: Intensive care unit ; ROS: Reactive oxygen species; $K_2S_2O_8$: Potassium persulfate; MTBB: Bacteriophage Microlysis Therapeutic Bank; MIC: minimum inhibitory concentration; WHO: World Health Organization

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