Cumhuriyet Sci. J., 45(1) (2024) 29-34
DOI: https://doi.org/10.17776/csj.1390256

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Evaluation of Antibiofilm and Antimicrobial Activities of N-heterocyclic Carbene Complexes

Uğur Tutar [1,a,*], Cem Çelik [2,b], Mehmet Ataş [3,c]

[1] *Department of Botanica, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Türkiye.*
[2] *Department of Medical Microbiology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas,Türkiye.*
[3] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Sivas Cumhuriyet University, Sivas, Türkiye.*
**Corresponding author*

Research Article

History
Received: 13/11/2023
Accepted: 12/03/2024

ABSTRACT

In recent years, resistance to antimicrobials has become a global problem. Despite the need for new antibiotics with the increase of resistant bacteria, developing new antimicrobials is problematic. Biofilms formed by microorganisms play an essential role in the development of resistance. We aimed to investigate the antimicrobial and antibiofilm activities of N-heterocyclic carbene (NHC) complexes. In this study, previously synthesized and characterized NHC complexes on standard bacterial and fungal strains were investigated. The minimal inhibition concentration (MIC) test was used to determine the antimicrobial activities of the compounds, and the biofilm inhibition concentration test was used to determine the anti-biofilm activities. Compounds 2b and 2c showed potent antimicrobial activity on microorganisms between <=1.9 and 7.8µg/mL. Antimicrobial activity in salts of compounds (1a-1c) was weaker than silver compounds (2a-2c). The antibiofilm activity was between 27 and 79%, especially in silver-bound compounds (2a-2c). Benzimidazole derivative NHC compounds that we evaluated in our study were found to have significant antimicrobial and antibiofilm effects on pathogenic microorganisms. These compounds, which we assessed in our study, may be antimicrobial drug candidates that can be used in different areas. It will be essential to conduct further in vitro and in vivo studies on this subject.

Keywords: N heterocyclic carbene, Benzimidazole, Antimicrobial activity, Antibiofilm activity.

[a] *ututar5@gmail.com* *https://orcid.org/0000-0002-8058-0994*
[b] *cemcelik58@gmail.com* *https://orcid.org/0000-0002-7141-5874*
[c] *atasmehmet@gmail.com* *https://orcid.org/0000-0002-9425-0080*

Introduction

Although microorganisms are indispensable elements of our world, they are also the cause of many negative aspects in the fields of food and industry, especially infections [1-3]. While the common effort of those dealing with health sciences is to provide the best conditions for the environment and all living things, microorganisms are one of the most important obstacles [4].

Antimicrobial substances are used extensively for human and animal health, for the protection of nutrients in the food industry, and protection from the harmful effects of microorganisms in the sector [5]. However, in recent years, the resistance developed by microorganisms against these substances has emerged as a global problem. Efforts such as reducing the overuse of antimicrobials, improving sanitation, and infection control have not solved this problem completely, and new antimicrobials are needed has been understood [4,6]. Although resistance to antimicrobial agents has increased rapidly, new antimicrobial agents have not been developed at the same rate [7]. One of the reasons for the lack of discoveries is the increasing consumption of natural structures with antimicrobial activity [8]. Therefore, synthetic chemistry plays a crucial role in developing new strategies and antimicrobials [9].

Biofilms are one of the essential causes of resistance to antimicrobial agents. Biofilms, formed from a complex process, cause significant health and economic losses due to their important role in infectious diseases, increasing metal biodegradation processes in industrial sectors, decreasing food quality in agriculture and food sectors, and contaminating water systems. Many antibiotics are not able to destroy dense biofilms. For this reason, it is necessary to find effective substances that can destroy biofilms or prevent the formation of biofilms [10-12]. NHCs are heterocyclic species containing a carbene carbon and at least one nitrogen atom in the ring structure. An increasing number of publications today have focused on the medical applications of NHCs. Especially metal complexes of NHCs are evaluated as metallopharmaceuticals with great promise to be antibacterial and anticancer agents [13]. The successful isolation and characterization of an N heterocyclic carbene (NHC) at the end of the twentieth century have opened a new class of organic compounds for research. To date, NHCs have been among the most powerful tools in organic chemistry with numerous commercially important applications and have found applications in various fields, including medical fields [14]. This study aimed to determine the antibiofilm and antimicrobial properties of

Benzimidazole derivative NHC compounds previously designed and synthesized. We think that our study will contribute to the literature on studies on new compounds that have the potential to be antimicrobial agents.

Materials and Methods

Compounds

In this study, previously synthesized and characterized benzimidazolium-based Ag(I)-NHC complexes; 1-allyl-3-(3-methylbenzyl) benzimidazolium chloride (1a), 1-allyl-3-(4-isopropylbenzyl) benzimidazolium chloride (1b),1-allyl-3-(4-ter-butylbenzyl) benzimidazolium bromide (1c), chloro [1-allyl-3-(3-methylbenzyl) benzimidazole-2-ylidene] silver(I) (2a), chloro[1-allyl-3-(4- isopropylbenzyl) benzimidazole-2-ylidene] silver (I) (2b), bromo[1-allyl-3-(4-ter-butylbenzyl) benzimidazole-2-ylidene] silver(I) (2c), [15,16]. were evaluated in terms of antimicrobial and antibiofilm properties.

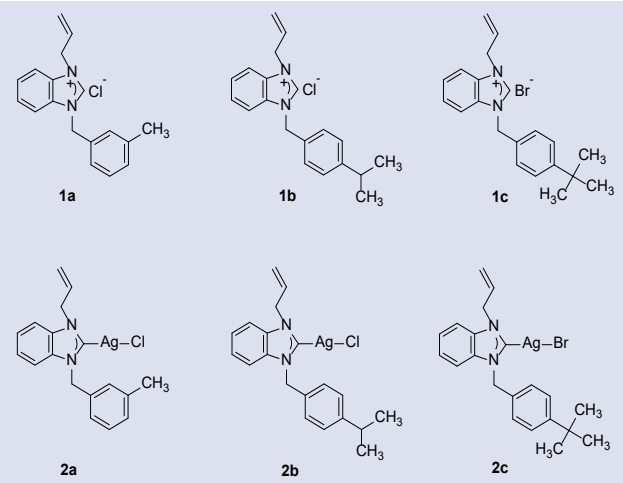

Figure 1. Structure of compounds (1a-c, 2a-c)

The minimal inhibition concentration (MIC) test was used to determine the compounds' antimicrobial activities, and the Biofilm inhibition concentration (BIC) test was used to determine their anti-biofilm activities.

Microorganism

In this study, ATCC 25922 *Escherichia coli*, ATCC 27853 *Pseudomonas aeruginosa*, ATCC 29213 *Staphylococcus aureus*, ATCC 29212 *Enterococcus faecalis*, and ATCC 10231 *Candida albicans* bacteria and yeast strains obtained from the standard strains of the American Type Culture Collection were used. Standard microorganisms were stored at -80 °C and revived at room temperature before the study.

Antimicrobial Activity, Determination of MICs

To determine the antimicrobial activities of the compounds, the broth microdilution method was used as applied by Şahin et al. [17]. Briefly, Overnight broth cultures were used to prepare inoculations of microorganisms and microorganism inoculations were adjusted to the standard turbidity of 0.5 McFarland (1 ×10^8 CFU/mL microorganism). Microorganism inoculations were adjusted to a final concentration of 5x10^5 CFU/ mL. Before starting the test, 2 mg of the compound was weighed and dissolved with 10% (v/v) DMSO in 4 mL of MHB to prepare a stock solution. Then, serial two-fold dilutions were prepared with MHB at concentrations ranging from 3.9 to 250 µg/ mL in 96-well plates. Ciprofloxacin and fluconazole were used as standard antibacterial and antifungal agents. Optical densities were measured after 24 hours of incubation at 37 °C. A microplate reader (SPECTROstar Nano, BMG LABTECH, Ortenberg/Germany) was used to measure the optical density at 620 nm. The lowest concentration that inhibits the growth of microorganisms was determined as MIC.

Biofilm Inhibition Concentration (BIC) Assay

The microtiter plate test was applied to evaluate the antibiofilm activities of the compounds against microorganisms, similar to the work of Şahin et al. with some modifications [18]. Overnight broth cultures were used to prepare inoculations of microorganisms. Briefly, 100 µL of Tryptic Soy Broth (TSB) containing 1% glucose (w/v) and microorganism at a density of 0.5 McFarland (1x10^8 CFU/ mL) was dispensed into 96-well U-bottom microplates. Then, 100 µL of different compound concentrations (1.9 - 250 µg/ mL) was dispensed into different wells. Only 200 µL of TSB solution was dispensed into the negative control well, and only 200 µL of the microorganism solution in the positive control well. The prepared microplates were incubated at 37 °C for 24 hours. At the end of the period, all wells were washed three times with 300 µL phosphate buffer solution (PBS), and planktonic cells were removed. Then, the biofilm in all wells was fixed with 95% methanol for 15 minutes. After fixation, 0.1% crystal violet (w/v) was added to the wells, dried at room temperature, and stained for 30 minutes. At the end of the period, the excess dye was removed with PBS 3 times and left to dry. A 33% acetic acid solution was added to dissolve the dye in the wells and evaluated at 570 nm in a microplate reader (SPECTROstar Nano, BMG LABTECH, Ortenberg/Germany). All experiments were repeated three times.

The percent biofilm inhibition was calculated using the formula given below.

Biofilm inhibition (%) = { (Ac −As) / Ac } 100

(Ac and As are the absorbance of the control and sample, respectively)

Results and Discussion

Compounds 2b and 2c showed strong antimicrobial activity against bacteria and yeast strains. These compounds were found to have inhibition concentrations close to the control antimicrobial agents (ciprofloxacin and fluconazole). Compound 2a showed moderate antimicrobial activity compared to other silver compounds in our study. Although antimicrobial activity was observed in the salts of the compounds (1a-1c), this effect was found to be weaker than in the silver compounds (2a-2c) (Table 1).

Table 1. Minimum-inhibitory concentrations (MIC, in µg/ mL) of compounds

Compounds	Microorganisms				
	ATCC 25922 *E.coli*	ATCC 27853 *Pseudomonas aeruginosa*	ATCC 29213 *Staphylococcus aureus*	ATCC 29212 *Enterococcus faecalis*	ATCC 10231 *Candida albicans*
1a	62.5	62.5	125	125	62.5
1b	125	125	125	125	125
1c	62.5	125	62.5	125	62.5
2a	15.6	31.25	62.5	31.25	31.25
2b	<=1.9	3.9	3.9	3.9	3.9
2c	3.9	3.9	7.8	<=1.9	3.9
Ciprofloxacin	<= 1.9	<=1.9	<= 1.9	<= 1.9	
Fluconazole					<= 1.9

When we evaluated the antibiofilm activities of the compounds we studied at concentrations sub-MIC, it was found that all compounds had this effect at different concentrations. The antibiofilm activity was found between 27 and 79% against microorganisms, especially silver-bound compounds (2a-2c). Other salt compounds (1a-1c) also inhibited biofilms of microorganisms at different rates at values sub-MIC. (Table 2).

Nowadays, the processes of discovering and producing new antibiotics are problematic. Therefore, there is an antimicrobial resistance crisis affecting the whole world. The process of developing a new antibiotic involves excellent difficulties. Traditional methods for finding antimicrobial agents have not produced a new antibiotic for a long time [19].

As a result, there has been a severe decrease in new antibacterial agents in recent years. However, new antibiotics are urgently needed due to the threat posed by increased highly resistant pathogenic bacteria [20].

Table 2. Reduction in biofilm formation on ½ MIC value of compounds (%)

Compounds	Microorganisms				
	ATCC 25922 *E.coli*	ATCC 27853 *Pseudomonas aeruginosa*	ATCC 29213 *Staphylococcus aureus*	ATCC 29212 *Enterococcus faecalis*	ATCC 10231 *Candida albicans*
1a	31.25/24±0.5	31.25/8±0.5	62.5/27±0.5	62.5/22±1.0	31.25/14±1.0
1b	62.5/45±0.5	62.5/14±1.0	62.5/29±0.5	62.5/39±0.5	62.5/42±2.0
1c	31.25/41±1.0	62.5/16±1.0	31.25/37±0.5	62.5/11±0.5	31.25/31±0.5
2a	7.8/68±0.5	15.6/27±0.5	31.25/44±0.5	15.6/49±0.5	15.6/47±0.5
2b	NT	1.9/38±1.0	1.9/68±1.0	1.9/62±0.5	1.9/70±1.0
2c	1.9/79±1.0	1.9/34±0.5	3.9/61±0.5	NT	1.9/66±1.0

NT: Non-tested (The biofilm inhibition test could not be studied because the MIC value was at the lowest concentration studied.

The main goal of medicinal chemistry is to design and synthesize bioactive compounds that can be safe and effective drugs [21]. Synthetic chemistry is critical for the discovery and development of new drugs. Chemical syntheses play an important role in pharmaceutical research and development. In the last century, many new synthesized compounds have led to the discovery and development of important life-changing drugs. Today, there is a critical need for new syntheses to develop new drugs that will guide future treatments [22].

Our study evaluated benzimidazole-derived Ag-NHC compounds' antimicrobial and antibiofilm activities and their salts, which were previously synthesized but whose antimicrobial and antibiofilm properties were unknown. The results of our study showed antimicrobial and antibiofilm activities similar to those of the control antimicrobials (ciprofloxacin and fluconazole), especially in the silver-bound 2b and 2c compounds (Table 1, 2).

There are publications in the literature on the antimicrobial activities of NHC molecules. Sakamoto et al. [23]. demonstrated the antimicrobial activity of the Ag(I)-NHC compounds they synthesized in their study and reported that most of this effect was due to the Ag metal. Sarı et al. [24]. Showed the antimicrobial activities of

benzimidazolium-based Ag(I)-NHC compounds they synthesized on Gram-positive and Gram-negative bacteria and yeasts. It is understood that Ag(I)-NHC compounds, whose activities we evaluated in our study, are more effective on microorganisms than those reported in this study. Gök et al. [25]. Demonstrated the antimicrobial activities of the benzimidazolium-based Ag(I)-NHC compounds they synthesized on Gram-positive and Gram-negative bacteria and yeasts. Butorac et al. [26]. reported that silver and gold-bound NHC compounds showed antimicrobial activity on *S. aureus*, *Bacillus subtilis*, *E. coli,* and *P. aeruginosa*. Siegmund et al. [27]. Their study with NHC complexes reported that their synthesised compounds exhibited strong antimicrobial activity on Gram-positive bacterial strains at low concentrations. Researchers reported that these compounds are important for developing new metal-based antibiotic agents. The silver-bound NHC complexes that we evaluated in our study also showed strong antimicrobial activity at low concentrations on all microorganisms we studied. Therefore, we think these compounds, which we evaluated in our study, may be important for developing new metal-based antibiotics.

About 80% of human bacterial infections are related to biofilms [28]. Therefore, it is critically important to design and develop anti-biofilm molecules that can be used in the treatment of biofilm-related diseases [29]. Benzimidazole derivatives are widely used as antimicrobial, antihistamine, antiviral, antidiabetic, anticancer, antifungal, anti-inflammatory, analgesic, etc., due to their pharmacological activities; it has become very important in recent years in the field of medicinal chemistry. There are currently few benzimidazole derivatives on the market as drug candidates against various diseases. However, intensive studies show that benzimidazole-based drug candidates can increase rapidly [30]. The previous study reported benzimidazolium salts with methicillin-resistant *S. aureus* bacteria are potent antibiotic and anti-biofilm agents. In this study, the researchers reported that new drugs should be developed to combat resistant bacteria and that it is important that these drugs can prevent or destroy biofilms [31]. We think that benzimidazole-derived compounds, which we evaluated in our study, may be among the future antimicrobials against resistant microorganisms with their strong antibiofilm and antimicrobial effects.

Üstün et al. [32]. They studied the antimicrobial activity and antibiofilm activity in the synthesised benzimidazole derivative NHC compounds. Researchers found antimicrobial activity against microorganisms in salts between 31.25 -250 µg/ mL concentrations, while they found much higher effects in their silver-bound complexes at concentrations <= 1.9-15.6 µg/ mL. Again, researchers reported that salts at sub-MIC concentrations inhibited microorganism biofilms by 8-51.6%, while silver-bound NHC compounds inhibited microorganism biofilms up to 77% at sub-MIC concentrations. While the salts of the compounds we evaluated in our study had antimicrobial and antibiofilm activity similar to the studies of Üstün et al., much stronger activities were found in Ag(I)-NHC complexes, especially in compounds 2b and 2c. We think that these compounds should be included in further in vitro and in vivo studies to be future antimicrobials.

Şahin et al. [17]. They evaluated the newly synthesized NHC precursors and Ag(I)-NHC complexes regarding antibacterial, antifungal, and antibiofilm activities. Researchers compared the biological activities of the synthesised products in their studies with standard drugs, as in our study. Researchers who reported that the compounds have moderate antibacterial and antibiofilm activities reported that especially compound 2a inhibited *E. coli* biofilms, and compound 2d inhibited *C. albicans* biofilms at the highest rate. Bernardi et al. [33]. Investigated the antibiofilm properties of NHC compounds to which they bind different metals on Gram (+) and Gr (-) bacteria. This study reported that very strong antibiofilm activity was observed in Ag(I)-NHC compounds. The researchers stated that the results obtained in this study would open new perspectives in developing metal-based drugs that can be used to treat infections associated with biofilms. We also think that the strong antimicrobial and antibiofilm activity we obtained in our study may contribute to developing new antimicrobials with antibiofilm properties. O'Beirne et al. [34]. Their study with NHC compounds reported that the compounds showed excellent inhibition on *E. coli*, *P. aeruginosa,* and *K. pneumoniae*. The researchers reported that the compounds did not have an antibiofilm effect on *P.aeruginosa* but showed antibiofilm activity on Methicillin-resistant *S.aureus* (MRSA) and *C.parapsilosis.* The compounds we evaluated in our study also showed antibiofilm activity in *P.aeruginosa* biofilms. There are many studies on the antimicrobial properties of Ag(I)-NHCs in the literature. Kaloğlu et al [13] evaluated the antimicrobial activity of synthesized Ag-(I) NHC compounds on different microorganisms. They reported that these compounds showed strong activity.

The studies show that Ag(I)-NHC compounds have the potential to be applied in medicine due to their antimicrobial activity and low toxicity. The slow transport of silver ions across the cell membrane disrupts the cell's electron transport system and the structure of enzymes. The disadvantage of using silver compounds in pharmaceutical studies is that they quickly lose their effectiveness due to the rapid release of Ag+ ions. Therefore, the synthesis of silver complexes with strong coordinating ligands is crucial to prevent the rapid release of silver ions.

Conclusion

There is a great need for new antibiotics with the increase of highly resistant pathogenic bacteria. However, new antimicrobial agents have not been developed as needed in recent years. Benzimidazole derivative NHC compounds, whose antimicrobial and antibiofilm activities were evaluated in our study, were found to have

significant antimicrobial and antibiofilm effects on pathogenic microorganisms. Especially compounds 2b and 2c showed strong antimicrobial activity on microorganisms between <=1.9 and 7.8µg/ mL l. The antibiofilm activity was found between 27 and 79%, especially in silver-bound compounds (2a-2c). In the literature, there are few studies on the antibiofilm activities of benzimidazole-derived NHC compounds. These compounds, which we evaluated in our study, can be drug candidates that can be used in different fields. It will be essential to conduct further in vitro and in vivo studies on this subject.

Acknowledgements

The authors would like to thank Neslihan Şahin for providing us with the compounds.

Conflict of interest

The authors declare no conflicts of interest.

References

[1] Kowalchuk GA., Jones SE., Blackall LL., Microbes Orchestrate Life on Earth, *ISME J*., 2(8) (2008) 795-796.

[2] Lorenzo JM., Munekata PE., Dominguez R., Pateiro M., Saraiva JA., Franco D., Main Groups of Microorganisms of Relevance for Food Safety and Stability, *Innovative Technologies for Food Preservation.,* (2018) 53–107.

[3] Lindahl JF., Grace D., The Consequences of Human Actions on Risks for Infectious Diseases: a Review, *Infect Ecol Epidemiol.,* (5) (2015) 30048.

[4] McEwen SA., Collignon PJ., Antimicrobial Resistance: A One Health Perspective, *Microbiol Spectr.,* 6 (2) (2018) 10.1128.

[5] Topal M., Uslu Şenel G., Arslan Topal EI., Öbek E., Antibiotics and Usage Areas, *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi.,* 31(3) (2015) 121-127.

[6] Ventola CL., The Antibiotic Resistance Crisis: Part 1: Causes and Threats, *P T*., 40 (4) (2015) 277-283.

[7] Conly J., Johnston B., Where Are All the New Antibiotics? The New Antibiotic Paradox, *Can J Infect Dis Med Microbiol*., 16 (3) (2005) 159-160.

[8] Chen CH., Lu TK., Development and Challenges of Antimicrobial Peptides for Therapeutic Applications, *Antibiotics (Basel).,* 9 (1) (2020) 24.

[9] Konaklieva MI., Addressing Antimicrobial Resistance Through New Medicinal and Synthetic Chemistry Strategies, *SLAS Discov*., 24 (4) (2019) 419-439.

[10] Jain A., Gupta Y., Agrawal R., Khare P., Jain SK., Biofilms--A Microbial Life Perspective: A Critical Review, *Crit Rev Ther Drug Carrier Syst*., 24 (5) (2007) 393-443.

[11] Dumitru C., Bacterial Biofilms and Their Biological Significance, *Rev Rom Med Vet*., 28 (2) (2018) 35-40.

[12] Dincer S., Masume Uslu F., Delik A., Antibiotic Resistance in Biofilm. Bacterial Biofilms, *London, UK: IntechOpen Limited,* (2020).

[13] Hopkinson MN., Richter C., Schedler M., Glorius F., An Overview of N-heterocyclic Carbenes, *Nature*, 510 (7506) (2014) 485-496.

[14] Smith CA., Narouz MR., Lummis PA., Singh I., Nazemi A., Hung Li C., Crudden CM., N-heterocyclic Carbenes in Materials Chemistry, *Chem Rev*., 119 (8) (2019) 4986-5056.

[15] Şahin-Bölükbaşı S., Şahin N., Tahir MN., Arıcı C., Cevik E., Gürbüz N., Özdemir İ., Cummings BS., Novel N-heterocyclic Carbene Silver (I) Complexes: Synthesis, Structural Characterization, and Anticancer Activity, *Inorganica Chim Acta*., 486 (2019) 711-718.

[16] Şahin N., Şahin-Bölükbaşı S., Tahir MN., Arıcı C., Çevik E., Gürbüz N., Özdemir İ., Cummings BS., Synthesis, Characterization, and Anticancer Activity of Allyl Substituted N-heterocyclic Carbene Silver (I) Complexes, *J Mol Struct*., 1179 (2019) 92-99.

[17] Şahin N., Üstün E., Tutar U., Çelik C., Gürbüz N., Özdemir İ., Antimicrobial Activity, Inhibition of Biofilm Formation, and Molecular Docking Study of Novel Ag-NHC Complexes, *J Organomet Chem*., 954–955 (2021) 122082.

[18] Tutar U., Çelik C., Antibiofilm and Antimicrobial Properties of 1-allyl-3-(2-diisopropylaminoethyl) Benzimidazolium Chloride and Its Silver(I)-NHC Complex, *Cumhuriyet Sci J*., 43 (3) (2022) 432-436.

[19] Lewis K., The Science of Antibiotic Discovery, *Cell*, 181 (1) (2020) 29-45.

[20] Wohlleben W., Mast Y., Stegmann E., Ziemert N., Antibiotic Drug Discovery, *Microb Biotechnol*., 9 (5) (2016) 541-548.

[21] Boström J., Brown DG., Young RJ., Keserü GM., Expanding the Medicinal Chemistry Synthetic Toolbox, *Nat Rev Drug Discov*., 17 (10) (2018) 709-727.

[22] Campos KR., Coleman PJ., Alvarez JC., Dreher SD., Garbaccio RM., Terrett NK., Tillyer RD., Truppo MD., Parmee ER., The Importance of Synthetic Chemistry in the Pharmaceutical Industry, *Science*, 363 (6424) (2019) eaat0805.

[23] Sakamoto R., Morozumi S., Yanagawa Y., Toyama M., Takayama A., Kasuga NC., Nomiya K., Synthesis, Characterization, and Structure-Activity Relationship of the Antimicrobial Activities of Dinuclear N-heterocyclic Carbene (NHC)-Silver(I) Complexes, *J Inorg Biochem*., 163 (2016) 110-117.

[24] Sarı Y., Akkoç S., Gök Y., Sifniotis V., Özdemir İ., Günal S., Kayser V., Benzimidazolium-Based Novel Silver N-heterocyclic Carbene Complexes: Synthesis, Characterization and In Vitro Antimicrobial Activity, *J Enzyme Inhib Med Chem*., 31 (6) (2016) 1527-1530.

[25] Gök Y., Akkoç S., Erdoğan H., Albayrak S., In Vitro Antimicrobial Studies of New Benzimidazolium Salts and Silver N-heterocyclic Carbene Complexes, *J Enzyme Inhib Med Chem*., 31 (6) (2016) 1322-1327.

[26] Butorac RR., Al-Deyab SS., Cowley AH., Antimicrobial Properties of Some Bis(iminoacenaphthene (BIAN)-Supported N-heterocyclic Carbene Complexes of Silver and Gold, *Molecules*, 16 (3) (2011) 2285-2292.

[27] Siegmund D., Lorenz N., Gothe Y., Spies C., Geissler B., Prochnow P., Nuernberger P., Bandow JE., Metzler-Nolte N., Benzannulated Re(i)-NHC Complexes: Synthesis, Photophysical Properties and Antimicrobial Activity, *Dalton Trans*., 46 (44) (2017) 15269-15279.

[28] Jiang Y., Geng M., Bai L., Targeting Biofilms Therapy: Current Research Strategies and Development Hurdles, *Microorganisms*, 8 (8) (2020) 1222.

[29] Roy R., Tiwari M., Donelli G., Tiwari V., Strategies for Combating Bacterial Biofilms: A Focus on Anti-biofilm Agents and Their Mechanisms of Action, *Virulence*, 9 (1) (2018) 522-554.

[30] Vasava MS., Bhoi MN., Rathwa SK., Jethava DJ., Acharya PT., Patel DB., Patel HD., Benzimidazole: A Milestone in the Field of Medicinal Chemistry, *Mini Rev Med Chem*., 20 (7) (2020) 532-565.

[31] Tessier J., Schmitzer AR., Benzimidazolium Salts Prevent and Disrupt Methicillin-Resistant *Staphylococcus aureus* Biofilms, *RSC Adv*., 10 (2020) 9420-9430.

[32] Üstün E., Şahin N., Çelik C., Tutar U., Özdemir N., Gürbüz N., Özdemir İ., Synthesis, Characterization, Antimicrobial and Antibiofilm Activity, and Molecular Docking Analysis of NHC Precursors and Their Ag-NHC Complexes, *Dalton Trans*., 50 (42) (2021) 15400-15412.

[33] Bernardi T., Badel S., Mayer P., Groelly J., de Frémont P., Jacques B., Braunstein P., Teyssot ML., Gaulier C., Cisnetti F., Gautier A., Roland S., High-Throughput Screening of Metal-N-heterocyclic Carbene Complexes Against Biofilm Formation by Pathogenic Bacteria, *ChemMedChem*., 9(6) (2014) 1140-1144.

[34] O'Beirne C., Piatek ME., Fossen J., Müller-Bunz H., Andes DR., Kavanagh K., Patil SA., Baumann M., Tacke M., Continuous Flow Synthesis and Antimicrobial Evaluation of NHC* Silver Carboxylate Derivatives of SBC3 In Vitro and In Vivo, *Metallomics*., 13 (2) (2021) mfaa011.

[35] Kaloğlu N., Kaloğlu M., Özdemir İ., Günal S., Özdemir İ., Silver–N-heterocyclic carbene complexes: Synthesis,characterization, and antimicrobial properties, *J Chin Chem Soc*., 64(4) (2017) 420–426.

[36] Nayak S., Gaonkar,S L., Coinage Metal N-Heterocyclic Carbene Complexes: Recent Synthetic Strategies and Medicinal Applications, *ChemMedChem*, 16(9) (2021) 1360–1390.