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RESEARCH ARTICLE

Investigation of Synthesis, X-Ray Characterization and Biological Activities of Copper(II) Imidazole Complexes

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SUMMARY

Copper, a transition metal, possesses antimicrobial and anticancer properties. This trace element plays a critical role in various biological functions in the human body. Copper complexes have been found to enhance the effects of pharmaceuticals, with significant interest in studying their biological activities, especially when coordinated with therapeutic ligands like thiosemicarbazones, phosphines, imidazole, and benzimidazole. In this research, copper complexes containing imidazole and benzimidazole derivatives were synthesized and thoroughly characterized. Their antimicrobial and cytotoxic properties were evaluated. The antimicrobial effects of these complexes against bacterial strains were assessed using the Microbroth Dilution Technique. Additionally, the cytotoxicity of the synthesized complexes was tested on the Panc-1 and Clone-9 cell lines through LDH and MTT assays. The antimicrobial tests revealed that the copper(II) complex with the imidazole ligand (Complex 1) exhibited superior antimicrobial activity compared to the benzimidazole-based complex (Complex 2). On the other hand, MTT and LDH assays indicated that Complex 2 had a stronger cytotoxic effect on Clone-9 cells than Complex 1. However, Complex 1 showed greater activity against Panc-1 cells overall. The findings suggest that the copper(II)-imidazole complex could serve as a potential candidate for developing alternative chemotherapeutic agents.

Keywords: *Antimicrobial Activity, Benzimidazole, Copper(II) Complexes, Cytotoxicity, Imidazole, Panc-1 Cell Line.*

Bakır(II) İmidazol Komplekslerinin Sentezi, X-Ray Karakterizasyonu ve Biyolojik Aktivitelerinin Araştırılması

ÖZ

Bir geçiş metali olan bakır, antimikrobiyal ve antikanser özelliklere sahiptir. Bu eser element, insan vücudundaki çeşitli biyolojik işlevlerde kritik bir rol oynamaktadır. Bakır komplekslerinin, özellikle tiyosemikarbazonlar, fosfinler, imidazol ve benzimidazol gibi terapötik ligandlarla koordine edildiğinde, ilaçların etkilerini artırdığı bulunmuş ve biyolojik aktivitelerinin incelenmesine önemli ilgi gösterilmiştir. Bu araştırmada, imidazol ve benzimidazol türevleri içeren bakır kompleksleri sentezlenmiş ve kapsamlı bir şekilde karakterize edilmiştir. Antimikrobiyal ve sitotoksik özellikleri değerlendirilmiştir. Bu komplekslerin bakteri suşlarına karşı antimikrobiyal etkileri Mikrobroth Dilüsyon Tekniği kullanılarak değerlendirilmiştir. Ayrıca, sentezlenen komplekslerin sitotoksisitesi Panc-1 ve Klon-9 hücre hatları üzerinde LDH ve MTT analizleri ile test edilmiştir. Antimikrobiyal aktivite testleri, imidazol ligandlı bakır(II) kompleksinin (Kompleks 1), benzimidazol bazlı komplekse (Kompleks 2) kıyasla daha etkili olduğunu ortaya koymuştur. Öte yandan, MTT ve LDH analizleri, Kompleks 2'nin Klon-9 hücreleri üzerinde Kompleks 1'den daha güçlü bir sitotoksik etkiye sahip olduğunu göstermiştir. Ancak Kompleks 1'in, genel olarak Panc-1 hücrelerine karşı daha yüksek aktivite gösterdiği sonucuna varılmıştır. Bulgular, bakır(II)-imidazol kompleksinin alternatif kemoterapötik ajanların geliştirilmesi için potansiyel bir aday olabileceğini düşündürmektedir.

Anahtar Kelimeler: *Antimikrobiyal Aktivite, Benzimidazol, Bakır(II) Kompleksleri, Sitotoksisite, İmidazol, Panc-1 Hücre Hattı.*

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INTRODUCTION

Copper plays an important role in regulating intracellular redox states by acting as a cofactor for several enzymes within the human body. It has been recognized for its antimicrobial and anticancer properties (Nakahata et al., 2017; Ünver et al., 2022).

The global rise of antibiotic resistance, coupled with the limited number of available antibiotics, poses significant risks to public health. The challenge of treating infections caused by resistant pathogens contributes to the increased prevalence of chronic diseases (Mahmood et al., 2019).

This underscores the urgent demand for new antimicrobial solutions. Among the promising candidates, transition metal complexes, especially those involving copper, have become a focal point in research due to their ability to serve as effective antimicrobial agents (Saddam Hossain et al., 2018). Copper complexes, in particular, have garnered significant attention for their demonstrated antimicrobial properties (Ren et al., 2011).

These copper complexes also exhibit synergistic effects when combined with other drugs (Ren et al., 2011). The ability of copper-imidazole and copper-benzimidazole complexes to demonstrate a wide array of biological activities continues to fuel interest in their synthesis (Mendoza et al., 2013). Previous studies have reported various biological activities of copper (II) complexes containing imidazole and benzimidazole ligands, such as antibacterial, antifungal, antiamoebic, and antiparasitic effects (Bandopadhyay et al., 2022; Beheshti et al., 2021; Chen et al., 2012; Mañozca-Dosman et al., 2025). For instance, Atria et al. (2011) demonstrated that copper complexes with imidazole derivatives exhibited antimicrobial properties against several bacterial strains. In another study, a copper complex containing an imidazole ring was shown to possess antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* (Chai et al., 2018). Furthermore, 2-benzimidazole derivatives have been shown to have both antibacterial and mycobacterial effects (Chen et al., 2012). In a study by Ajibola et al. (2020), a copper-benzimidazole complex demonstrated superior antibacterial activity against *S. aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* when compared to the benzimidazole ligand.

Cancer continues to be a major global health threat, with the World Health Organization forecasting that cancer-related deaths may rise to 13.1 million by 2030 (Paul et al., 2021). Since the 1970s, there has been a growing interest in developing more effective anticancer agents, particularly those with higher efficacy than platinum-based drugs (Alshehri et al., 2022). Copper, an essential endogenous metal, has gained attention for its low toxicity and high coordination ability, making it a promising candidate for anticancer therapy (Cai et al., 2021).

Increased concentrations of copper in the bloodstream have been associated with the advancement of several types of cancer, as malignant cells often accumulate more copper compared to normal cells. This heightened copper absorption could potentially inhibit angiogenesis, the process by which new blood vessels develop to nourish tumors with oxygen and nutrients (Qiao et al., 2014). Numerous studies have explored the anticancer potential of copper complexes in cancers such as liver, cervical, lung, and breast cancers (Manjuraj et al., 2018). Research indicates that copper-based therapeutics could offer a promising strategy for targeted cancer treatment (Qiao et al., 2014). In addition, copper complexes with therapeutic ligands such as thiosemicarbazones, imidazoles, and phosphines have attracted considerable attention in recent years (Alshehri et al., 2022).

In this research, we describe the synthesis of two copper (II) complexes, each incorporating imidazole and benzimidazole-derived ligands. Comprehensive structural analysis of these complexes was carried out using single-crystal X-ray diffraction techniques. Furthermore, the antimicrobial activities of the complexes were assessed against a variety of bacterial strains. Their cytotoxic effects were also evaluated on the Panc-1 human pancreatic cancer cell line and Clone-9 rat liver epithelial cells using LDH and MTT assays.

MATERIALS AND METHODS

General considerations

Copper(II) perchlorate hexahydrate ($Cu(ClO_4)_2 6H_2O$), 4-dimethylaminopyridine (DMAP), and solvents were purchased from commercial suppliers (TCI, Sigma, J&K Scientific). The imidazole-based ligand 1-(4-(trifluoromethyl)benzyl)-1*H*-imidazole (ImCF3) and the benzimidazole derivative 1-benzyl-1*H*-benzo[d]imidazole (Benzim) were synthesized following published procedures with slight modifications (RSC Adv., 2018, 8, 40000, doi: 10.1039/c8ra08897g). Elemental analysis was conducted with an Elementar Vario EL III microanalyzer. FT-IR spectra were obtained using a Perkin Elmer Spectrum 100 Spectrometer, covering the 4000–400 cm^{-1} range, with KBr pellets. The process for synthesizing the copper complexes is illustrated in Figure 1.

Synthesis of copper complexes 1 and 2

Complex 1 [$Cu(ImCF_3)_4(ClO_4)_2$]

A 10 mL methanol solution containing 4 equivalents of ImCF3 (0.4 mmol, 90.5 mg) was prepared, to which copper(II) perchlorate hexahydrate (0.1 mmol, 37 mg) was added. The mixture was stirred at 60°C for 24 hours. After filtration, the resulting deep blue solution was allowed to evaporate at room temperature, leading to the formation of deep blue crystals after five days. The copper complex was isolated with a yield of 49% (63 mg). The elemental analysis confirmed the chemical formula: $[Cu(C_{11}H_9N_2F_3)_4(ClO_4)_2]$. **Elemental Analysis:** Anal. Calcd (%) for $C_{44}H_{36}N_8O_8Cl_2F_{12}Cu$: C, 45.28; H, 3.11; N, 9.60. Found (%): C, 45.16; H, 3.18; N, 9.58. **FT-IR (KBr, cm^{-1}):** 3144, 1323, 1091.

Complex 2 [$Cu(Benzim)_2(DMAP)_2](ClO_4)_2$

In a 10 mL methanol solution, copper(II) perchlorate hexahydrate (0.1 mmol, 37 mg) was mixed with two equivalents of Benzim (0.2 mmol, 41.6 mg) and two equivalents of DMAP (0.2 mmol, 24.4 mg). The resulting mixture was stirred at 60°C for 12 hours. After 6 days, crystals were formed, and the final yield was 50% (52 mg). Elemental analysis confirmed the composition as $[Cu(C_{14}H_{12}N_2)_2(C_6H_{10}N_2)_2](ClO_4)_2$. **Elemental Analysis:** Anal. Calcd (%) for $C_{42}H_{44}N_8O_8Cl_2Cu$: C, 54.64; H, 4.80; N, 12.14. Found (%): C, 54.68; H, 4.75; N, 12.19. **FT-IR (KBr, cm^{-1}):** 3146, 1617, 1056.

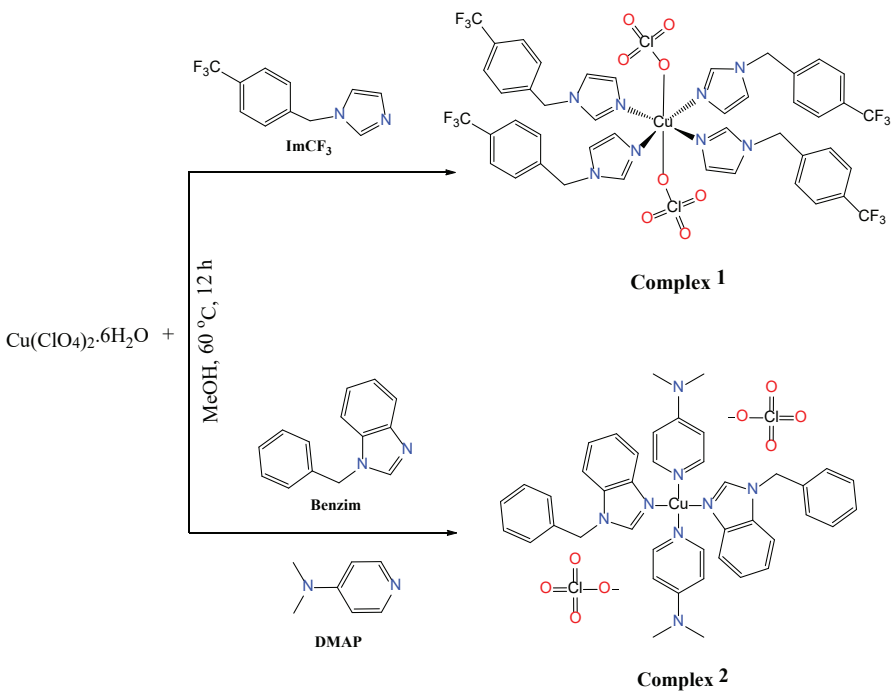

Figure 1. Synthesis procedure of two copper(II) complexes

X-ray crystallography

Single crystals of Complexes 1 and 2 were obtained by evaporating the solvents used in the reactions. X-ray diffraction analysis was conducted at 296.15 K using a Bruker APEX-II D8 Venture diffractometer with Mo Kα radiation (λ = 0.71073 Å). The data were processed with Bruker SMART software, and the structure was solved using the SHELXS program. Refinement was carried out using full-matrix least-squares (Shedrick, 2008), with hydrogen atoms placed at calculated positions. Molecular structures were visualized using the MERCURY software (Macrae et al., 2006).

Antimicrobial activity with MTT assay

Test bacteria

Acinetobacter baumannii ATCC 19606, *Enterococcus faecalis* ATCC 29210, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 70063, *Klebsiella pneumoniae* MCTC 13438, MRSA ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* PA01, *Staphylococcus aureus* ATCC 25923, and *Staphylococcus epidermidis* CECT 4183 bacteria were used in the experiment. These strains were stored in a -86°C (EQUITEC) incubated in brain-heart infusion broth (Merck) at 37°C for 24 hours before use.

Minimum inhibitory concentration (MIC)

The MIC values were determined using the Microbroth Dilution Method (CLSI, 2006). Copper complexes containing imidazole and benzimidazole derivatives (Complex 1, Cu, Imi¸ Complex 2, DMAP, and Benzim) were dissolved in¸ dimethyl sulfoxide (DMSO) and then diluted in Mueller Hinton Broth. Following bacterial inoculation, the plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration at which no purple color appeared upon adding MTT reagent. Chloramphenicol was used as the positive control, while DMSO served as the negative control.

Minimum bactericidal concentration (MBC)

To ascertain the MBC, the wells that showed the MIC were streaked onto Mueller-Hinton agar plates and incubated at 37°C for 24 hours. The MBC was noted as having the lowest concentration where no bacterial growth was visible (Er et al., 2023).

Cytotoxicity assessment in Panc-1 and Clone-9 cell lines

Cell lines and culture conditions

Panc-1 (human pancreatic carcinoma, ATCC CRL-1469) and Clone-9 (rat liver epithelium, ATCC CRL-1439) cell lines were cultured under standard 37°C, 5% CO_2 conditions using DMEM or F-12K media (Capricorn Scientific GmbH) supplemented with 10% FBS (Sigma-Aldrich) and 1% penicillin–streptomycin. Complexes 1 and 2 were dissolved in DMSO (Sigma-Aldrich), with the final solvent concentration maintained below 0.01%. Cells were exposed to 0.0078–0.062 mg/mL of each compound, and viability was assessed after 24 and 48 hours.

MTT reduction assay

The cytotoxic effects of Complexes 1 and 2 were assessed via an MTT viability assay, following established protocols (Liu et al., 1997; Mosmann, 1983). Cells were seeded at 1×10^4 per well in 96-well plates and allowed to adhere for 24 hours before treatment. Serial concentrations of each compound (0.16–33.3 μM) were added for either 24 or 48 hours. After exposure, the medium was replaced with 100 μL of MTT solution (0.5 mg/mL) (Sigma-Aldrich Co.) and incubated for 3 hours at 37 °C to allow for formazan formation. The supernatant was then removed, and 200 μL of DMSO was added to dissolve the crystals. Absorbance at 570 nm was recorded using a BioTek 800 TS reader. All conditions were tested in triplicate, each with eight technical replicates.

Membrane integrity: LDH release assay

The integrity of the cell membrane was assessed by measuring LDH release using the CytoTox-ONE kit (Promega). Clone-9 and Panc-1 cells (10×10^3 per well) were treated with Complexes 1 and 2, or DMSO (solvent control), after 24 and 48 hours. Cytotoxicity was assessed by comparing the LDH release in treated cells with control cells (Garcia et al., 2012).

Statistical analysis

All experiments were performed in triplicate, and data were analyzed with one-way ANOVA using SPSS 22.0. Results are presented as mean ± SD, with statistical significance defined as $p \leq 0.05$. IC_{50} values were determined by nonlinear regression from at least three independent assays using GraphPad Prism 6.01 (GraphPad Software, La Jolla, CA, USA). Selectivity Index (SI) values were determined by calculating the ratio of IC_{50} values in normal cells to those in cancer cells (SI = IC50 normal / IC50 cancer). A SI value greater than 1 indicates preferential cytotoxicity toward cancer cells.

RESULTS AND DISCUSSION

Structural characterization of complexes 1 and 2

The formation of two copper(II) complexes, referred to as Complex 1 and Complex 2, was confirmed using X-ray diffraction analysis. Figure 2 presents the ORTEP representations of their molecular structures, and Tables 1 and 2 summarize the crystallographic data, including bond lengths and angles.

Table 1. Crystal data and structure refinement parameters for copper(II) complexes.

	Complex 1	Complex 2
Molecular Formula	$[Cu(ImCF_3)_4(ClO_4)_2]$	$[Cu(Benzim)_2(DMAP)_2](ClO_4)_2$
CCDC number	1989602	1956477
Empirical formula	$C_{22}H_{18.16}ClCu_{0.5}F_{5.84}N_4O_4$	$C_{21}H_{22}ClCu_{0.5}N_4O_4$
Formula weight	580.79	461.64
Temperature	273.15 K	296.15
Wavelength	0.71073 Å	0.71073 Å
Crystal system, space group	Triclinic, P-1	Monoclinic, $P2_1/c$
Unit cell dimensions	a = 9.0363(17) Å alpha = 65.120(4)° b = 16.804(3) Å beta = 90° c = 18.146(3) Å gamma = 90°	a = 11.366(2) **Å alpha = 90°** b = 9.716(2) **Å beta** = 101.193(13)° c = 19.329(4) **Å gamma = 90°**
Volume	2499.7(8) A^3	2093.9(8) A^3
Z, Calculated density	4, 1.543 mg/m^3	4, 1.464 mg/m^3
Absorption coefficient	0.647 mm^{-1}	0.713 mm^{-1}
F(000)	1177.0	958.0
Crystal size	0.3 × 0.2 × 0.2 mm	0.1 × 0.08 × 0.07 mm
Theta range	6.258 to 56.982 deg	3.652 to 56.862 deg
Limiting indices	-12 ≤ h ≤ 12, -22 ≤ k ≤ 22, -24 ≤ l ≤ 24	-15 ≤ h ≤ 15, -12 ≤ k ≤ 12, -23 ≤ l ≤ 25
Reflections coll. / unique	63706/ 12564 [R_{int} **= 0.1899**]	20884/ 5138 [R_{int} = 0.0461]
Completeness to theta	99.3% 28.491	97.9% 28.431
Absorption correction	Integration	Integration
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/rest/parameters	12564/21/698	5138/0/279
Goodness-of-fit on F^2	0.946	1.051
Final R indices [I>2sigma(I)]	R_1 **= 0.0821, wR_2 = 0.1817**	R_1 = 0.0505, wR_2 = 0.1239
R indices (all data)	R_1 **= 0.2816, wR_2 = 0.2606**	R_1 = 0.1105, wR_2 = 0.1492
Largest diff. peak and hole	0.45 and -0.37 $e.A^{-3}$	0.58 and -0.47$e.A^{-3}$

Complex 1 crystallizes in the triclinic system with the P-1 space group, whereas Complex 2 adopts a monoclinic structure with the P21/c space group. Both complexes exhibit mononuclear coordination. In Complex 1, the copper center is coordinated by four nitrogen atoms from monodentate $ImCF_3$ ligands and two oxygen atoms from perchlorate anions. The nitrogen and oxygen bond lengths are 1.987–1.999

Å and 2.597 Å, respectively. The overall geometry of Complex 1 is a distorted octahedron, attributed to the elongated copper-oxygen bonds. The observed bond angles include N(1)-Cu(1)-O(1'), N(1)-Cu(1)-N(3), and N(3)-Cu(1)-O(1'), measuring 80.40°, 87.86°, and 90.51°, respectively.

In Complex 2, the copper ion coordinates with two benzimidazole (Benzim) and two 4-DMAP ligands. The bond distances range between 1.994 and 2.001 Å. The coordination geometry is classified as distorted square planar, with notable bond angles such as N(3)-Cu(1)-N(1), N(3)-Cu(1)-N(1'), and O(1)-Cl(1)-O(3), recorded at 89.67°, 90.33°, and 109.10°, respectively. Additionally, both complexes exhibit π–π stacking and hydrogen bonding interactions, which enhance their crystallographic stability.

Table 2. Selected bond distances (Å) and bond angles of two copper(II) complexes

Complex 1	
Bond Distances (Å)	**Bond Angles (°)**
Cu(1)-N(1) 1.987	N(1)-Cu(1)-N(3) 87.86
Cu(1)-N(3) 1.999	N(1)- Cu(1)-O(1') 83.40
Cu(1)-O(1') 2.597	N(3)- Cu(1)-O(1') 90.51
Complex 2	
Bond Distances (Å)	**Bond Angles (°)**
Cu(1)-N(1) 2.001	N(3)-Cu(1)-N(1) 89.67
Cu(1)-N(3) 1.994	N(3)-Cu(1)-N(1') 90.33
Cl(1)-O(1) 1.370	O(1)-Cl(1)-O(3) 109.10

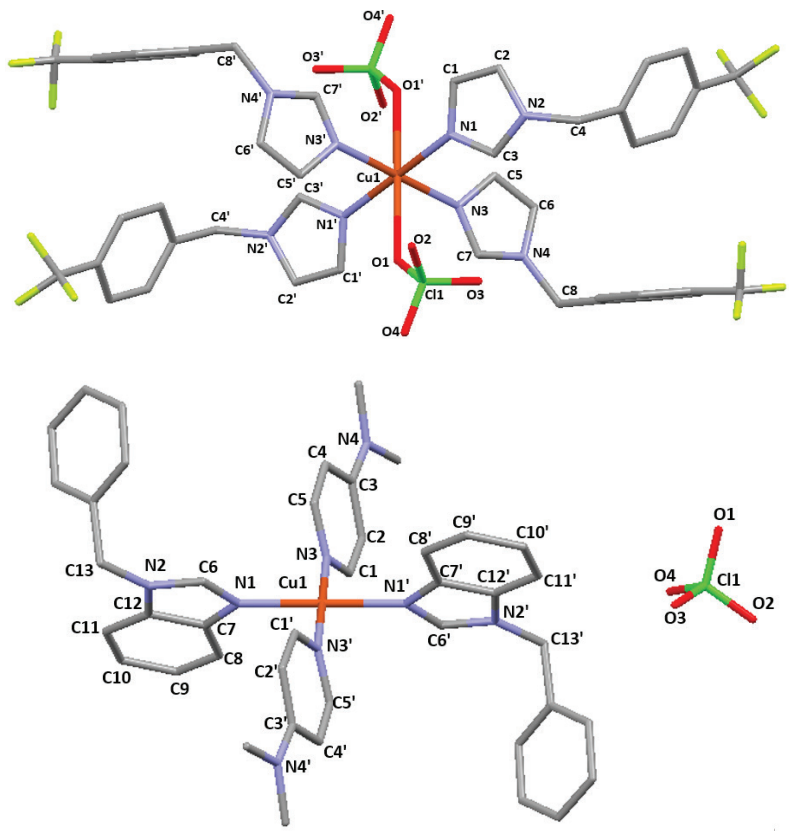

Figure 2. Molecular structures of copper complexes with thermal ellipsoid plots at the 20% probability level. Hydrogen atoms of both complexes were omitted for clarity.

Antimicrobial activity

Table 3 presents the MIC and MBC determined for the synthesized compounds. The results of the study showed that Complex 1 of the MIC values of *E. coli* ATCC 25922, *E. faecalis* ATCC 29210, *S. aureus* ATCC 25923 and *S. epidermidis* CECT 4183 bacteria were found to be 0.5, 0.5, 1, and 0.5 mg / mL, respectively. MIC values of other microorganisms (*A. baumannii* ATCC 19606, *K. pneumoniae* ATCC 70063, *K. pneumoniae* MCTC 13438, *P. aeruginosa* ATCC 27853, *P. aeruginosa* PA01, and MRSA ATCC 43300) were found above 2 mg / mL. The bactericidal activity of Complex 1 was observed above 2 mg/mL, except for *S. aureus* ATCC 25923 (1 mg / mL). When we look at the antimicrobial activity results of the Complex 2, the MIC and MBC values of all tested microorganisms were found to be above 2 mg / mL, except for the MIC value of *E. faecalis* ATCC 29210 (0.5 mg / mL) (Table 3.).

The Benzim ligand has MIC and MBC values similar to those of Complex 2. However, the MIC values of Imi ligand against Escherichia coli ATCC 25922, MRSA ATCC 43300, and Staphylococcus aureus ATCC 25923 bacteria were recorded as 0.25, 0.5, and 0.25 mg/mL, respectively (Table 3.). Imi ligand was observed to have a higher antibacterial effect against these bacteria compared to Complex 1. In Complex 1, imidazole is bound to copper, while in the Imi ligand it is free. Therefore, it is thought that the antimicrobial effect may be higher. No studies on this subject were found in the literature.

Table 3. Antibacterial activity results of Complex 1 and Complex 2 (mg / mL). MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

Microorganism name	Imidazole						Benzimidazole						Cloramphenicol	
	Complex 1		Cu		Imi		Complex 2		DMAP		Benzim			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
***Acinetobacter baumannii* ATCC 19606**	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 1	〉 1
***Escherichia coli* ATCC 25922**	0.5	〉 2	〉 2	〉 2	0.25	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	0.0078	0.0078
***Enterococcus faecalis* ATCC 29210**	0.5	〉 2	〉 2	〉 2	〉 2	〉 2	0.5	〉 2	〉 2	〉 2	〉 2	〉 2	0.0156	0.0156
***Klebsiella pneumoniae* ATCC 70063**	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	0.125	〉 1
***Klebsiella pneumoniae* MCTC 13438**	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	0.125	〉 1
***Pseudomonas aeruginosa* ATCC 27853**	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 1	〉 1
***Pseudomonas aeruginosa* PA01**	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	〉 1	〉 1
MRSA ATCC 43300	〉 2	〉 2	〉 2	〉 2	0.5	0.5	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	0.0156	0.0156
***Staphylococcus aureus* ATCC 25923**	1	〉 2	〉 2	〉 2	0.25	0.5	〉 2	〉 2	〉 2	〉 2	〉 2	〉 2	0.0156	0.0312
***Staphylococcus epidermidis* CECT 4183**	0.5	1	〉 2	〉 2	0.5	0.5	〉 2	〉 2	〉 2	〉 2	1	1	0.0156	0.0156

In a study conducted by Rodríguez-Argüelles et al. (2005), MIC of imidazole-containing copper complexes on *E. coli* and *S. aureus* bacteria was reported above 0.1 mg / mL. In another study, MIC and MBC values of different copper complexes containing imidazole on *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were generally found to be 0.3 mg / mL and above 0.3 mg / mL (Gulya et al., 2013). In a study by Maru and Shah (2015), the MIC values of 2 different benzimidazole-containing copper complexes on *E. coli* MTCC 442, *P. aeruginosa* MTCC 44, and *S. aureus* MTCC 96 were found to be 0.5 mg / mL, 0.5 mg / mL, and 1 mg / mL, respectively.

Lobana et al. (2018) investigated the antimicrobial activity of copper complexes containing 11 different azole groups on different bacteria. As a result of the study, it was determined that each compound has different effects on the microorganism; it has been reported that the MIC values of 8 compounds on *S. aureus* bacteria vary between 10 and 1000 μg / mL, while the other 3 compounds have no effect. In addition, the MIC values of 10 compounds on *S. epidermidis* bacteria varied between 5-500 μg / mL, the other 1 compound had no effect on this bacteria; MIC values of 11 compounds on *E. faecalis* bacteria varied between 7-500 μg / mL; It has been reported that the MIC value of 1 compound on *E.coli* bacteria is 500 μg / mL, while the other 10 compounds have no effect on this bacteria.

Cytotoxic activity

The cytotoxic effects of Complexes 1 and 2 were assessed in Clone-9 normal hepatocytes and Panc-1 pancreatic cancer cells using MTT and LDH assays after 24 and 48 h of exposure to concentrations ranging from 0.0078 to 0.0625 mg/mL. These assays evaluate mitochondrial metabolic activity and membrane integrity, respectively. The results are shown in Figures 3A–D and 4A–B.

Both MTT and LDH assays indicated that Complex 1 did not induce appreciable cytotoxicity at lower concentrations (0.0078–0.015 mg/mL) in either cell line at either time point. In contrast, higher concentrations (0.031 and 0.0625 mg/mL) caused a statistically significant reduction in cell viability. After 24 h, this effect was more pronounced in Panc-1 cells, with viability decreases of approximately 25% and 45%, compared with reductions of approximately 14% and 54% in Clone-9 cells.

After 48 h of exposure, Clone-9 cell viability decreased by approximately 20% and 83% at 0.031 and 0.0625 mg/mL, respectively. In Panc-1 cells, viability remained comparable to controls at lower concentrations but declined markedly at higher concentrations, with reductions of approximately 68% and 97% at 0.031 and 0.0625 mg/mL, respectively.

The MTT assay showed that Complex 2 reduced Clone-9 cell viability in a time- and dose-dependent manner (Figure 3C.), with viability decreasing to approximately 50% and 25% at 0.062 mg/mL after 24 and 48 h, respectively. In Panc-1 cells, no cytotoxic effect was observed at lower concentrations (0.0078–0.031 mg/mL) after 24 h; however, viability declined by approximately 75% at the highest concentration. After 48 h, Panc-1 cell viability decreased in a concentration-dependent manner, reaching an approximate 80% reduction at 0.062 mg/mL (Figure 3D.).

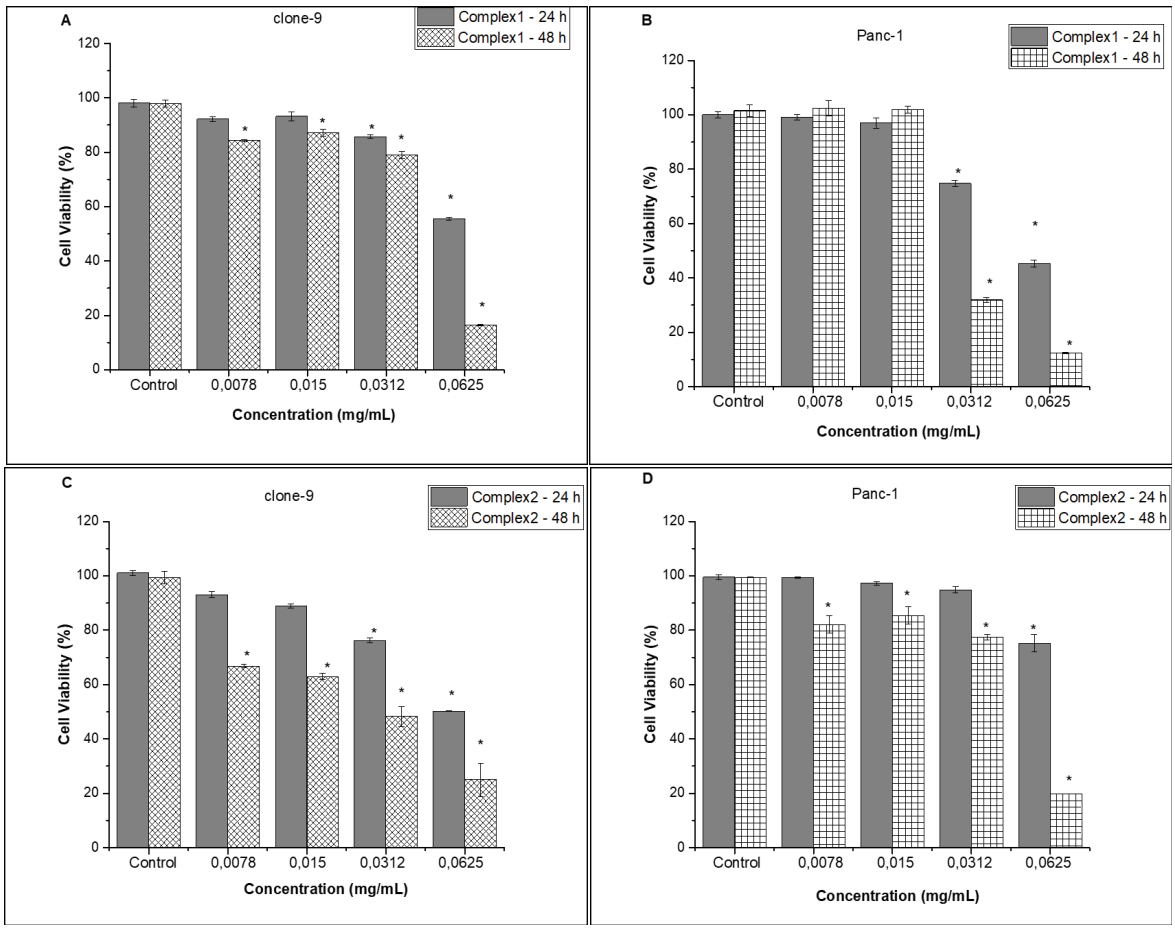

Figure 3. *In vitro* cytotoxic activities of complex 1 and complex 2. **A**) Cytotoxic activity of complex 1 on clone - 9 cell line. **B)** Cytotoxic activity of complex 1 on panc-1 cell line. **C)** Cytotoxic activity of complex 2 on clone - 9 cell line and **D)** Cytotoxic activity of complex 2 on panc - 1 cell line. Data are expressed as the mean ± SD. *Indicates significant difference from the control group (untreated cells) by the Tukey test ($p < 0.05$). The cytotoxic effect of cisplatin on the clone-9 and panc-1 cell lines should be described. Cisplatin $IC5_0$ value was used as a positive control. No data is provided.

Similar to the MTT results, Complex 1 showed no obvious cytotoxicity at lower concentrations (0.0078–0.015 mg/mL) in both cell lines in the LDH assay (Fig. 4A–B.). At the highest concentration, cytotoxicity cells were determined 40% and 80%, respectively, after 24 h and 48 h in clon-9 cells. However, in panc-1 cells, cytotoxicity reached 50% and 90% at 24 h and 48 h, respectively, at the highest concentration of Complex 1. Furthermore, in Clone-9 cells, cytotoxicity was increased in a concentration-dependent manner following 24 h and 48 h treatments. The significant increase in cell viability observed at the highest concentration (0.0625 mg/mL) of complex 2 was approximately % 80 (Figure 4A.). Complex 2 treatment of panc-1cells resulted in minimal changes in cytotoxicity after 24 h with lower concentrations (<0.0625 mg/mL), but the highest concentration (0.0625 mg/mL) cytotoxicity was increased to 30% and 83 % respectively after 24h and 48h (Figure 4B.).

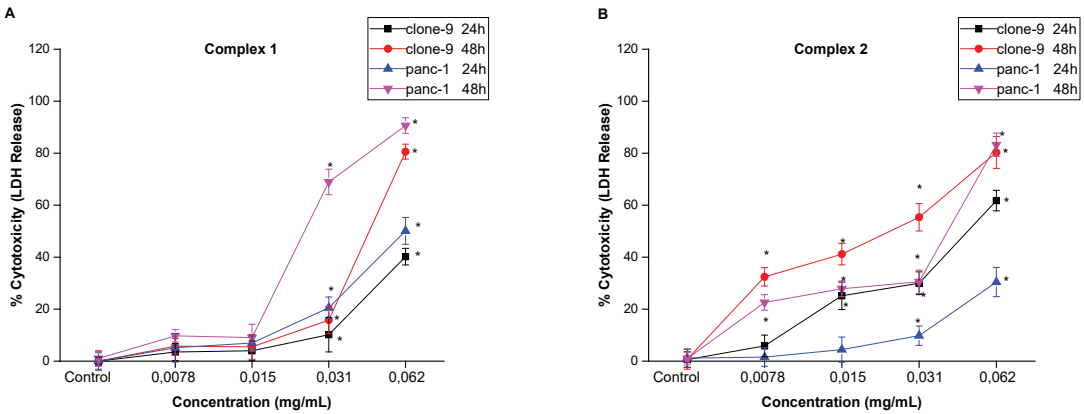

Figure 4. In vitro cytotoxic activities of complex 1 and complex 2. **A)** Complex 1 cytotoxic activity on clone-9 and panc-1 cell lines. **B)** Complex 2 cytotoxic activity on clone-9 and panc-1 cell lines. Data are expressed as the mean ± SD. *Indicates significant difference from the control group (untreated cells) by the Tukey test ($p < 0.05$).

Table 4. IC_{50} and selectivity index values of complex 1 and 2 on clone-9 and panc - 1 cell lines.

Complex	IC_{50} values mg/mL				Selectivity Index (SI)	
	Clone-9		**Panc-1**			
	Incubation Time					
	24 h	**48 h**	**24 h**	**48 h**	**24 h**	**48 h**
Complex 1	0.070 ± 1.56	0.04 ± 2.04	0.056 ± 0.78	0.034 ± 1.46	1.25	1.18
Complex 2	0.062 ± 1.63	0.029 ± 1.88	ND*	0.046 ± 2.25	<0.99	0.63

*ND: Not determined IC_{50} value >0.0625 mg/mL

In our study, for Complex 1, the IC_{50} values at 24 hours were 0.070 mg/mL and 0.056 mg/mL for Clone-9 and Panc-1 cells, respectively. After 48 hours, these values decreased to 0.04 mg/ and 0.034 mg/mL. For Complex 2, the IC_{50} for Clone-9 cells was 0.062 mg/mL at 24 hours, dropping to 0.029 mg/mL at 48 hours. In Panc-1 cells, the 24-h IC50 value could not be determined, as Complex 2 did not induce a sufficient reduction in cell viability. In contrast, the IC_{50} value at 48 h was calculated to be 0.041mg/mL (Table 4.). The selectivity of the tested complexes toward pancreatic cancer cells was evaluated by calculating the Selectivity Index (SI) based on IC50 values obtained in normal Clone-9 hepatocytes and panc-1 pancreatic cancer cells. For Complex 1, SI values were 1.25 at 24 h and 1.18 at 48 h, indicating a modest but consistent preferential cytotoxicity toward Panc-1 cells compared with normal cells. In contrast, Complex 2 did not demonstrate cancer cell selectivity. At 24 h, the IC_{50} value in Panc-1 cells exceeded the highest tested concentration, resulting in an estimated SI value below 1, while at 48 h, the SI decreased further to 0.63, suggesting greater sensitivity of normal cells relative to cancer cells. Overall, these findings indicate that Complex 1 exhibits limited yet favorable selectivity toward pancreatic cancer cells, whereas Complex 2 lacks selective anticancer activity.

The increased cytotoxicity observed for Complex 2 in normal Clone-9 cells compared with PANC-1 pancreatic cancer cells may be attributed to cell line–specific biological differences and intrinsic drug-resistance mechanisms. PANC-1 cells are well known for their chemoresistant phenotype, characterized by enhanced drug efflux, elevated anti-apoptotic protein expression,

and efficient DNA damage repair, which collectively limit intracellular drug accumulation and reduce cytotoxic efficacy (Amrutkar & Gladhaug, 2017).

In contrast, Clone-9 cells exhibited greater sensitivity to Complex 2, indicating that this compound has not yet achieved cancer cell selectivity despite its pronounced cytotoxic potential. This interpretation is supported by Selectivity Index values below 1. Overall, these findings suggest that although Complex 2 displays substantial cytotoxic activity, further optimization is required to improve its selective anticancer efficacy, potentially through targeted delivery strategies and mechanistic investigations of cellular uptake.

Although no studies have examined the cytotoxic effects of copper complexes with imidazole and benzimidazole on Panc-1 and Clone-9 cell lines, their effects on other cell lines, including M14, MCF-7, HeLa, A-549, HT-29, SGC7901, and EC109, have been reported (Alshehri et al., 2022; Paul et al., 2021; Zhao et al., 2013). Raducka et al. (2021) determined IC50 values for benzimidazole-based copper complexes on A-549 cells as 264.32–608.7 μM, while Alshehri et al. (2022) reported IC_{50} values of 6.65 and 8.83 mg/mL for imidazole-based copper complexes on M14 cells.

CONCLUSION

This research reports the preparation, structural analysis, and biological evaluation of copper(II) complexes containing imidazole and benzimidazole ligands. The complexes exhibited notable antibacterial activity, especially Complex 1, which showed better performance compared to Complex 2. In terms of cytotoxicity, Complex 1 demonstrated moderate effects, particularly against Panc-1 cells. In summary, the results suggest that Complex 1 may possess limited but favorable selectivity toward pancreatic cancer cells, while Complex 2 demonstrates reduced selectivity, warranting cautious interpretation and further investigation. The IC_{50} values suggest that both complexes could be promising candidates for further investigation in antimicrobial and anticancer drug development.

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AUTHOR CONTRIBITION STATEMENT

S.E. and H.Ü. designed and directed the project; S.E. wrote the manuscript with support from H.Ü. and B.B.; H.Ü. synthesized the test substances, S.E. performed the antimicrobial activity experiments, and B.B. performed the cell culture experiments.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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