

## Investigation of the Antimicrobial and Cytotoxic Activities of Two Different Schiff Bases with Thiazole Ring

Tuğçe Deniz Karaca <sup>1,a</sup>, Mustafa Çiçek <sup>2,b</sup>, İbrahim Yılmaz <sup>3,c</sup>, Alev Doğan <sup>4,d,\*</sup>

<sup>1</sup> Department of Medical Services and Techniques, Health Service Vocational Schools, Gazi University, Ankara, Türkiye.

<sup>2</sup> Department of Biology, Kamil Özdağ Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Türkiye.

<sup>3</sup> Department of Chemistry Education, Faculty of Education, Bolu Abant İzzet Baysal University, Bolu, Türkiye.

<sup>4</sup> Department of Science Education, Faculty of Education, Gazi University, Ankara, Türkiye.

\*Corresponding author e-mail address: dogan.alev@gmail.com

### Research Article

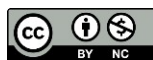
#### History

Received: 25.11.2025

Accepted: 02.03.2026

### ABSTRACT


Multidrug resistance to antimicrobial and anticancer drugs has been identified as a global threat by the World Health Organization. Therefore, the search for new biological agents to be used in treatment has accelerated. In this sense, Schiff bases are pharmaceutically important compounds due to the functional group found in their structures, and the investigation of their antimicrobial and anticancer properties has become extremely important. Therefore, in this study, the antimicrobial activities of two different thiazole-ringed Schiff bases against *Escherichia coli* (ATCC 700728), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus cereus* (NRRL B-3711), *Staphylococcus aureus* (ATCC 9144), *Staphylococcus epidermidis* (ATCC 12228), and *Candida albicans* (NCPF 3179) were investigated. Antimicrobial activities were determined using Disk Diffusion and Minimum Inhibitory Concentration (MIC) methods. Additionally, the in vitro cytotoxic activities of Schiff bases were determined on breast cancer (MCF7) and liver cancer (Mahlavu) cell lines using the MTT method. According to disk diffusion and MIC analysis results, compound 2 was found to be more effective against all microorganisms, exhibited antibacterial activity against *Bacillus cereus* (MIC = 260.75 µg/mL) comparable to that of the standard antibiotic ampicillin (MIC = 250 µg/mL). Also noteworthy are the cytotoxic activities of both Schiff Bases on cancer cell lines.




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**Keywords:** Antimicrobial effects, Anti-proliferative properties, Schiff bases, Thiazole ring.

<sup>a</sup>  0000-0002-7471-1759

<sup>c</sup>  0000-0002-9447-3065

<sup>b</sup>  0000-0002-7109-6500

<sup>d</sup>  0000-0002-8907-1344

## 1. Introduction

Antimicrobial resistance is a significant global public health problem. This negatively impacts clinical and therapeutic outcomes, leading to treatment failures and high morbidity and mortality rates. Therefore, this problem has necessitated the search for new generations of antimicrobial agents from novel sources that can combat or prevent multidrug resistance and, consequently, their use in therapeutic treatments. However, cancer remains a major cause of death globally, and similarly, existing drugs are often inadequate for treatment. Therefore, both problems have made the search for new biological agents crucial [1].

In this context, Schiff bases are organic compounds that exhibit a wide range of biological activities due to the presence of carbon-nitrogen double bonds. The ability of these bases to form functional groups is of great importance in biological experiments. These compounds are synthesized via a condensation reaction between a primary amine and an aldehyde or ketone in a suitable solvent and are extremely important due to their strong properties such as adaptability, simplicity and

functionality [2-4]. There are various biological studies in the literature regarding these bases, including antimicrobial activities [5-9].

The Thiazole group has also become an important heterocycle in chemical research. Due to its aromatic structure, the ring possesses numerous reactive positions, including donor-acceptor, nucleophilic, oxidation reactions, and other functions. Molecules containing thiazole rings exhibit unpredictable behavior in physiological systems. The thiazole ring is a five-membered heterocyclic structure containing S and N atoms and is found in a number of drugs including Sulfathiazole, Nitazoxanide, Abafungin, Ravuconazole, Fentiazac, Nizatidine, Fanetizole and Cinalukast [10]. These molecules can activate or inhibit biochemical pathways and enzymes, or stimulate or block receptors in biological systems. There are also various studies on these compounds such as antimicrobial, anticancer, anti-inflammatory, activities [11-19].

Therefore, in this study, the antimicrobial activities of two different Schiff Bases with Thiazole ring were investigated and their effects on cell viability were also

evaluated. These Schiff bases [(E)-Trans-2-(2-(biphenyl-4-ylmethylene)hydrazinyl)-4-(3-methyl-3-phenylcyclobutyl)thiazole, (Compound 1) and 5-Diethylamino-2-[[4-(3-methyl-3-phenylcyclobutyl)thiazol-2-yl]-hydrazonomethyl]phenol, (Compound 2)] were tested against two Gram-negative bacteria [*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*)], three Gram-positive bacteria [(*Bacillus cereus* (*B. cereus*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*)), and the fungal strain *Candida albicans* (*C. albicans*). Furthermore, the cytotoxic potential of these Schiff bases was determined using two human cancer cell lines, MCF7 and MDA-MB-231.

## 2. Materials and Methods

### 2.1 Chemicals

All chemicals and reagents used in this study were original and purchased from Sigma-Aldrich, Serva, and

Merck. Cell culture media were purchased from Serana and Lonza, and 96-well microtiter plates were purchased from Nest Scientific.

### 2.2 Synthesis of (E)-Trans-2-(2-(biphenyl-4-ylmethylene)hydrazinyl)-4-(3-methyl-3-phenylcyclobutyl)thiazole, (Compound 1)

The compound 1 was synthesized and characterized according to our previous manuscript (Figure 1.) [20]. Briefly, thiosemicarbazide (0.9113 g, 10 mmol) was added to a solution of 4-biphenylcarboxaldehyde (1.8222 g, 10 mmol) in 50 mL ethanol. Then a solution of 1-methyl-1-phenyl-3-(2-chloro-1-oxoethyl)cyclobutane (2.2271 g, 10 mmol) in 20 mL absolute ethanol was added. After adding  $\alpha$ -chloroketone, the temperature was raised to approximately 55 °C and held at this temperature for about 2 hours. The solution was then cooled to room temperature and the resulting precipitate, after neutralization, was crystallized and dried.

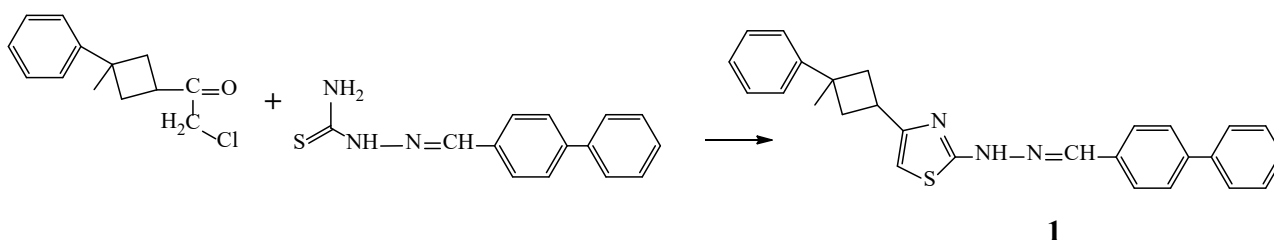


Figure 1. (E)-Trans-2-(2-(biphenyl-4-ylmethylene)hydrazinyl)-4-(3-methyl-3-phenylcyclobutyl) thiazole

### 2.3 Synthesis of 5-Diethylamino-2-[[4-(3-methyl-3-phenylcyclobutyl)thiazol-2-yl]-hydrazonomethyl]phenol, (Compound 2)

Compound 2 was also synthesized and characterized in the same way as in our previous article (Figure 2.) [21]. Briefly, 4-Diethylamino-2-hydroxy-benzaldehyde (10 mmol, 1.9324 g) was added to thiosemicarbazide (10 mmol, 0.9114 g) in 50 ml of ethanol and stirred at room

temperature. Subsequently, a solution of 1-methyl-1-phenyl-3-(2-chloro-1-oxoethyl) cyclobutane (2.2271 g, 10 mmol) in 20 ml of pure ethanol was added. After the addition of  $\alpha$ -chloroketone, the temperature was raised to 50-55 °C and kept at this temperature for approximately 2 hours. The solution was crystallized in ethyl alcohol at room temperature, as in the case of the first compound.

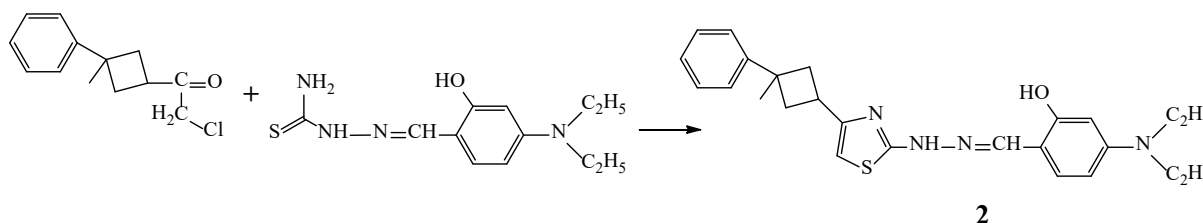


Figure 2. 5-Diethylamino-2-[[4-(3-methyl-3-phenylcyclobutyl)thiazol-2-yl]-hydrazonomethyl]phenol

### 2.4 Inoculum Preparation

Compounds 1 and 2 were evaluated for their antimicrobial potential against *Escherichia coli* ATCC 700728, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 9144, *Staphylococcus epidermidis* ATCC 12228, and *Candida*

*albicans* NCPF 3179. For this purpose, single colonies of bacteria were pre-cultured in Mueller Hinton Broth (MHB) through overnight incubation at 37°C. Then, using the 0.5 McFarland standard, each bacterial culture was adjusted to a concentration of  $1.5 \times 10^8$  CFU/mL [22]. To prepare the fungal inoculum, *C. albicans* isolate was incubated in

Sabouraud Dextrose Broth (SDB) for overnight at 37°C and turbidity of the fungal culture was matched to that of 0.5 McFarland standard [ $1.4 \times 10^6$  CFU/mL] [23].

### 2.5 Disk Diffusion Assay

The disk diffusion technique was utilized to test for antimicrobial susceptibility. 100  $\mu$ L of standardized inoculum of microorganisms were evenly dispersed throughout agar plates' surface. Sterile disks with a diameter of 6 mm, impregnated with 20  $\mu$ L of Compounds 1 and 2 at a concentration of 2400  $\mu$ M were placed on the agar surface. The plates were then incubated at 37°C for 24 hours, and the diameters of the inhibition zones surrounding the disks were measured. Gentamicin (10  $\mu$ g/disc) was used as the standard antibiotic against bacterial strains, whereas Terbinafine-HCl (10  $\mu$ g/disc) was employed as the standard antifungal agent against *C. albicans*.

### 2.6 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MICs) of Compounds 1 and 2 were determined using the two-fold serial dilution method adapted for 96-well microtiter plates [24]. For this purpose, the standardized microorganism suspensions were diluted 1:100 (for bacterial suspensions) or 1:10 (for *C. albicans*) with liquid medium. Then 100  $\mu$ L of cell suspension was mixed with equal volumes of 1X, 0.5X, 0.25X, 0.125X, 0.0625X and 0.03125X concentrations of the compounds at a ratio of 1:1 (v/v) in a microplate well. Control wells were prepared with untreated inoculum, test compound dilutions without microorganisms, and uninoculated growth media. Following an incubation period of 24 hours

at 37 °C, the MIC values were determined as the lowest concentrations that did not exhibit any visible growth of the test microorganisms. In the experiments, ampicillin (500  $\mu$ g/mL) was used as the standard antibiotic and terbinafine hydrochloride (Terbinafine-HCl) (500  $\mu$ g/mL) was used as the standard antifungal.

### 2.7 Cell culture and MTT assay

Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Pen/Strep) was used to culture the human hepatocellular carcinoma cell line Mahlavu and the estrogen receptor positive (ER+) breast cancer cell line MCF7. In a humidified chamber with 5% CO<sub>2</sub>, the cells were grown as monolayers at 37°C. To assess the effects of Compounds 1 and 2 on cell proliferation and viability, an MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay was performed. Mahlavu and MCF7 cells were seeded in 96-well microtiter plates with a final volume of 100  $\mu$ L of growth media at densities of  $2 \times 10^3$  and  $5 \times 10^3$  cells/well, respectively. The growth media were changed the next day, and the cells were cultured in new media with varied concentrations of the compounds (0-400  $\mu$ M) for a duration of 72 hours. Following incubation, the cells were treated for 4 hours with 10  $\mu$ L of a 5 mg/ml MTT solution, and then for another 16 hours, they were incubated with 10% sodium dodecyl sulfate (SDS) in 0.01 M hydrochloric acid (HCl) [25]. To ascertain the viability of the cells, absorbance at 570 nm was measured using a microplate spectrophotometer. Cell viability was determined using the given formula, and each experiment was run in triplicate (Equation 1.)

$$\% \text{ Cell viability} = \frac{A(\text{Treated Cells}) - A(\text{Blank})}{A(\text{Control Cells}) - A(\text{Blank})} \times 100 \quad (1)$$

### 2.8 Statistical Analysis

Each experiment was performed at least in triplicates. Results are presented as mean  $\pm$  standard errors of the mean (SEM). GraphPad Prism software was used to determine IC<sub>50</sub> values from cell viability assay results. ANOVA followed by Tukey's test was used to make multiple statistical comparisons using the indicated software. Differences between groups were considered significant when p values were less than 0.05.

## 3. Results and Discussion

In this study, the antimicrobial and cytotoxic activities of two different Schiff bases, which we named compound 1 and compound 2 containing a thiazole ring, were evaluated against different microorganisms. Data according to the disk diffusion assay results are given in Table 1 and the MIC values against microorganisms are

given in Table 2. Table 3 contains the IC<sub>50</sub> values of the studied compounds against Mahlavu and MCF7 cells.

Based on our disk diffusion assay results (Table 1), it is evident that both Compound 1 and Compound 2 exhibit antimicrobial activity, showing inhibition zones of 6 mm against *S. aureus* and *E. coli* only. *S. aureus* is a significant microorganism known for its drug resistance, particularly methicillin resistance. Also, the recent rise in multidrug resistance in *E. coli* strains is making the treatment of many serious infections increasingly challenging. Therefore, these results from Schiff bases are valuable. The lack of detectable activity for both compounds against *B. cereus*, *S. epidermidis*, *P. aeruginosa*, and *C. albicans* suggests that the compounds may not have diffused effectively from the disk, thereby limiting their antimicrobial activity, or that the organisms may simply be insensitive to the concentrations tested.

Table 1. Inhibition zones of compound 1 and 2

| Compounds       | Zone of inhibition in mm |                       |                  |                      |                 |                    |
|-----------------|--------------------------|-----------------------|------------------|----------------------|-----------------|--------------------|
|                 | <i>B. cereus</i>         | <i>S. epidermidis</i> | <i>S. aureus</i> | <i>P. aeruginosa</i> | <i>E. coli</i>  | <i>C. albicans</i> |
| Compound1       | ND <sup>†</sup>          | ND <sup>†</sup>       | 6 ± 0.1          | ND <sup>†</sup>      | 6 ± 0.1         | ND <sup>†</sup>    |
| Compound 2      | ND <sup>†</sup>          | ND <sup>†</sup>       | 6 ± 0.1          | ND <sup>†</sup>      | 6 ± 0.1         | ND <sup>†</sup>    |
| Gentamicin      | 24 ± 0.1                 | 24 ± 0.1              | 16 ± 1           | 20 ± 1               | 11 ± 1          | NA <sup>‡</sup>    |
| Terbinafine-HCl | NA <sup>†</sup>          | NA <sup>†</sup>       | NA <sup>†</sup>  | NA <sup>†</sup>      | NA <sup>†</sup> | 13 ± 1             |

The antimicrobial effects of the compounds were also tested against the same microorganisms using the minimum inhibitory concentration method. The results of

the MIC experiment, given in Table 2, reveal significant insights into the antimicrobial efficacy of the tested compounds.

Table 2. MIC values of compounds 1 and 2 against test microorganisms

| Compounds       | MIC (µg/mL)    |                  |                  |                       |                      |                    |
|-----------------|----------------|------------------|------------------|-----------------------|----------------------|--------------------|
|                 | <i>E. coli</i> | <i>B. cereus</i> | <i>S. aureus</i> | <i>S. epidermidis</i> | <i>P. aeruginosa</i> | <i>C. albicans</i> |
| Compound 1      | >1000          | >1000            | >1000            | >1000                 | >1000                | >1000              |
| Compound 2      | 260.75         | 260.75           | 260.75           | 521.51                | >1043                | 521.51             |
| Ampicillin      | 62.5           | 250              | 7.81             | 7.81                  | ‡ND                  | ‡NA                |
| Terbinafine-HCl | ‡NA            | ‡NA              | ‡NA              | ‡NA                   | ‡NA                  | 15.63              |

‡ND; not detected ‡NA;not applicable

According to the results, Compound 1 did not demonstrate effective antimicrobial activity (>1000µg/mL) against the tested microorganisms under the tested conditions. In contrast, Compound 2 exhibited inhibitory activity against all test microorganisms. While Compound 2 had the lowest MIC value (260.75 µg/mL) on *B. cereus*, the MIC value on *P. aeruginosa* was determined to be >1043 µg/mL. In addition, the MIC value against *C. albicans* was determined as 521.51 µg/mL. Compound 2 has a specific MIC value in the microorganisms studied. However, compared to the antibiotic, Compound 2 can be said to have a more reasonable MIC value against *B. cereus*. In this case, it is thought that the potential of Compound 2 can be increased with structural modifications. When the literature is examined, some of the Schiff bases have demonstrated significant antibacterial and antifungal activities against a range of pathogenic microorganisms [26]. Studies have shown that Schiff bases exhibit potent antibacterial effects against Gram-negative bacteria such as *E. coli* and Gram-positive bacteria like *S. aureus* and *B. cereus*. For instance, recently thiazole-based Schiff base compounds have been reported to exhibit promising antibacterial activities against a group of clinical isolates with inhibition zones comparable to that of standard antibiotics amoxicillin [27].

Also, significant results have been obtained regarding the antibacterial and antifungal activity of 5-chlorosalicylaldehyde derivative Schiff bases. Among the compounds tested, (E)-4-chloro-2-((4-fluorobenzylimino)

methyl)phenol was found to show the best antimicrobial activity against both *E. coli* and *S. aureus* [28]. In another study investigating the antimicrobial effects of two novel Schiff bases, it was reported that metal compounds of the Schiff base were highly effective against Gram-positive bacteria *S. aureus* and *Micrococcus luteus*, and their MIC values were comparable to the standard antibiotic ampicillin. In addition, in the same study, it was shown that the high antifungal potential of compound against the fungus *Aspergillus niger* was comparable to the standard antifungal drug nystatin, and the MIC values determined for Schiff base and nystatin were reported to be identical (MIC=12.5 µg/mL) [29]. Considering the studies above, it is clear that compounds 1 and 2 have limited antimicrobial activity.

To evaluate the cytotoxic activities of the synthesized compounds, tests were performed using the MCF7 breast cancer cell line and the Mahlavu liver cancer cell line. Figure 1 presents the results of the MTT assay, which involves the reduction of yellow tetrazolium dye (MTT) to a purple and water-insoluble formazan by metabolically active cells, and is one of the most frequently applied methods used to assess cell viability [31]. Through this approach, the cytotoxic activities of compounds 1 and compounds 2 were tested at different concentrations, ranging from 400 µM to 1 µM, in Mahlavu and MCF7 cell lines. For comparison, a control group (Ctrl), representing 100% cell viability without exposure to the compounds, is also given.

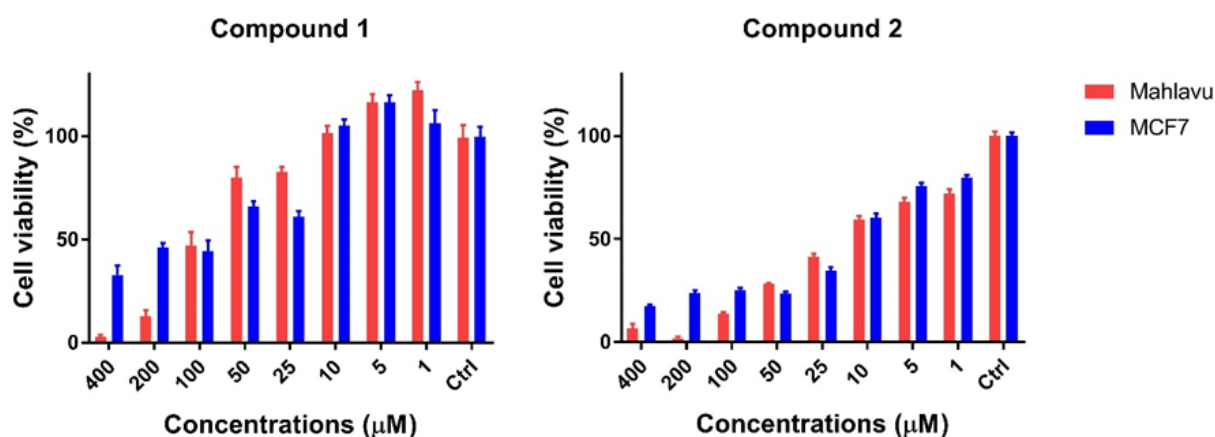


Figure 1. Viability of cells exposed to various concentrations of compounds for 72 hours.

The results demonstrate that higher doses of the compounds more prominently inhibited cell growth in both cell lines and led to a dose-dependent reduction in cell viability. This finding highlights the potential anti-proliferative effects of the compounds. On the other hand, although cell viability increases with decreasing concentrations of the compounds, a marked recovery in viability of MCF7 cells is observed for Compound 1 at concentrations of 25  $\mu\text{M}$  and below. In contrast, increasing concentrations of Compound 2 generally lead to a more gradual reduction in cell viability.

The median inhibitory concentration ( $\text{IC}_{50}$ ), which represents the concentration needed to achieve a 50% reduction in cell viability is another important parameter in evaluation of anti-proliferative effects of test

compounds. The  $\text{IC}_{50}$  values of compounds 1 and 2 are summarized in the Table 3. According to the results; Compound 1 is more effective at inhibiting the growth of MCF7 cells ( $\text{IC}_{50} = 25.96 \mu\text{M}$ ) than Mahlavu cells ( $\text{IC}_{50} = 55.81 \mu\text{M}$ ). This indicates that MCF7 cells are more susceptible to Compound 1, requiring a lower concentration to reduce cell viability by 50%. Similarly; MCF7 ( $\text{IC}_{50} = 8.89 \mu\text{M}$ ) cells show a slightly higher susceptibility to Compound 2 compared to Mahlavu cells ( $\text{IC}_{50} = 11.36 \mu\text{M}$ ). These results suggest that Compound 2 is more cytotoxic than Compound 1 with lower  $\text{IC}_{50}$  values for both cell lines. The lower  $\text{IC}_{50}$  values suggest that both Mahlavu and MCF7 cells are more sensitive to Compound 2.

Table 3.  $\text{IC}_{50}$  Values for Mahlavu and MCF7 cells exposed to the compounds 1 and 2 ( $\mu\text{M}$ )

| Cell lines | Compound 1         | Compound 2            | Doxorubicin       |
|------------|--------------------|-----------------------|-------------------|
| Mahlavu    | $55.81 \pm 5.90^c$ | $11.36 \pm 1.37^{ab}$ | $0.377 \pm 0.005$ |
| MCF7       | $25.96 \pm 3.53^b$ | $8.89 \pm 0.90^a$     | $0.265 \pm 0.040$ |

Superscript letters (a, b, c) indicate statistically significant differences between groups within the same column ( $p < 0.05$ )

According to literature, Schiff bases have garnered significant attention in cancer research due to their potent anticancer, anti-proliferative, and cytotoxic activities. These compounds, often complexed with metals, have demonstrated remarkable efficacy against various cancer cell lines, including colon cancers, lung, liver and breast [32–34]. For instance, in a recent study conducted to test the cytotoxic effects of a 5-fluorouracil-based Schiff base and its some metal complexes on cancer cells, the  $\text{IC}_{50}$  value of the Schiff base for MCF7 cells was found to be  $83.77 \mu\text{M}$ . For the Ni (II) complex of the same Schiff base, which had the highest antiproliferative effect, the  $\text{IC}_{50}$  value dropped to  $9.43 \mu\text{M}$  [35]. The results obtained from another study carried out with a similar approach revealed that the in vitro cytotoxic effects of the metal

complexes of a 4(3H)-Quinazolinone derived Schiff base on MCF7 cell line were higher compared to the Schiff base itself. In fact, it was shown that the synthesized Cu and Zn complexes were even more effective than the standard chemotherapy drug doxorubicin [36]. In a different study where the effects of a morpholine-based Schiff base and its metal complexes on different cell lines were tested, it was found that the activity of different metal complexes on MDA-MB231, MCF7, PC-3 and WI-38 cells varied. The results showed that the complex containing silver atoms in its structure had the highest anti-proliferative effect on cancer cell lines, whereas the complexes containing Zn, Mn and Ni atoms in their structure had lower activity on normal cell lines than their activity against cancer cells [37]. Similarly, in another study in which the anti-

proliferative effects of 5 different Schiff bases were tested with different cell lines, no IC<sub>50</sub> value could be determined at the concentrations tested for MCF7 cells, while the IC<sub>50</sub> values of two compounds, named L2 and L5, in HeLa cells were determined as 56.7 μM and 20.8 μM, respectively [38]. These data suggest that the mechanisms of action and the degree of effectiveness of different Schiff bases may differ depending on their structure and the genetic makeup of the cell lines in which they are tested, emphasizing the importance of studies to be carried out with different cell lines and different Schiff bases. Of the Schiff bases examined, Compound 2 (IC<sub>50</sub> = 11.36 μM for MHLV and 8.89 μM for MCF7) – although less effective than the reference drug – highlights its significant antiproliferative potential due to its low range of activity.

When we make a general assessment, the literature emphasizes that the activity of Schiff bases can be increased through various complexation studies. Researchers also state that biologically active metal complexes carrying Schiff base ligands can be promising candidates for antimicrobial and anticancer activities thanks to the chelation process which greatly affects the overall biological performance of the synthesized compound. Given that Schiff base-metal complexes exhibit higher activity than the original Schiff base, we believe that the synthesis of metal complexes of compounds 1 and 2 and the re-evaluation of their activities will provide valuable data and contribute to the literature [39].

#### 4. Conclusion

Today, widespread drug resistance to infectious bacterial pathogens and its negative impact on disease treatment has become extremely important. Furthermore, cancer remains one of the leading causes of death worldwide, and similarly, treatment methods remain inadequate. In this context, Schiff bases are privileged ligands due to their adaptability, fundamental properties, and pharmacological activities. In this study, the antimicrobial effects of two Schiff bases on a group of microorganisms including Gram-positive and Gram-negative bacteria and the fungus *C. albicans* and their capacity to inhibit cell proliferation in liver cancer and breast cancer cell lines were analyzed. The data obtained highlight the properties of the bases we are investigating in this field and form the basis of various experimental methods. Moreover, various forms such as gels, metal-organic structures, and nanocomposites can be designed using these compounds for further research.

#### Conflict of Interest

There are no conflicts of interest in this work.

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