Volume 12, Issue 4, Page 54-62, 2023 https://doi.org/10.46810/tdfd.1301871 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Investigation of Anticancer and Antimicrobial Properties of Fluorinated Salicylaldimines

Fatma OZER[1*], Bestoon SHEKHANY [1,2], Mustafa Unal BOYRAZ[3] Hasan Ali Zafer SAK[4], Saduman ERSOZ[1], Ali Osman GÜROL[5,6], Faruk SUZERGOZ [1]

[1] Departmnet of Biology Science Art Faculty, Harran University, Sanliurfa, Türkiye
[2]Department of Medical Laboratory Technology, Shaqlawa Technical College, Erbil Polytechnic University, Erbil, Iraq
[3]Department of Histology and Embriology, Veterinary Faculty, Harran University, Sanliurfa, Türkiye
[4]Department of Chest Dissease, Medical Faculty, Harran University, Sanliurfa, Türkiye
[5]Department of Immunology, Aziz Sancar Institute of Experimental Medicine, Istanbul University, Istanbul-Türkiye.
[5]Department of Medical Pharmacology Istanbul Medical Faculty, Istanbul University, Istanbul-Türkiye.
Fatma OZER ORCID1 0000-0002-6424-7235
Bestoon SHEKHANY ORCID 0000-0001-8849-4153
Mustafa Unal BOYRAZ ORCID 0000-0002-5455-0353
Hasan Ali Zafer SAK ORCID 0000-0002-2388-2259
Saduman ERSOZ ORCID 0000-0001-5137-2814
Ali Osman GÜROL ORCID 0000-0001-6682-4289
Faruk SUZERGOZ ORCID 0000-0001-5000-0998

** Corresponding author Fatma OZER: fozer@harran.edu.tr*

(Received: 24.05.2023, Accepted: 14.11.2023, Online Publication: 28.12.2023)

Keywords
Anticancer, Antimicrobial, Fluor, Schiff base, Apoptosis

Abstract: Multidrug resistance (MDR) to anticancer and antimicrobial drugs has become a global health problem. Schiff bases are referred to as special ligands due to the functional azomethine (C=N) group in their structure and are used in the pharmaceutical industry. Approximately one-fourth of the drugs used in human and animal health contain fluorine atoms. In this study, the cytotoxic and antiproliferative effects of the synthesized fluorinated Schiff bases on the A549 cell line were investigated by MTT assay and 5 (6)-carboxyfluorescein succinimidyl ester (CFSE) staining technique, respectively. The ability of the compounds to induce apoptosis was investigated by Rhodamine 123 staining technique, the inhibitory effects on mitochondrial membrane potential, active caspase-3 analysis by immunofluorescence test, and morphological effects by hematoxylin-eosin (H&E), Giemsa and Papanicolaou (PAP) protocols. Antimicrobial effects of the compounds were investigated by MTT test on *P. aeruginosa*, *E. coli*, and *S. aureus*. The compounds used in the study were generally potent (IC_{50}:1,4-31,5 µM) and Compound 7 (IC_{50}:1,4 µM) exhibited a stronger cytotoxic effect than Doxorubicin (IC_{50}: 1.9 µM). The strongest antiproliferative effect was obtained with Compound 10 (PI:2,1). The strongest antiproliferative effect was obtained with Compound 10 (PI:2,1). The strongest ability to inhibit mitochondrial membrane potential was obtained with Compound 4 (1330). Immunofluorescence studies showed active caspase-3 and morphological studies showed findings indicating apoptosis such as chromatin condensation and DNA fragmentation. The antimicrobial effects of the compounds on *P. aeruginosa*, *E. coli*, and *S. aureus* (MIC_{50}:18,6-49,3 µM) were observed to be lower than Gentamicin (MIC_{50}:1,5-2,2 µM) used as the positive control. It can be said that the fluorinated Schiff Bases synthesized in the study especially Compound 7 are promising for the treatment of multidrug resistance to anticancer drugs and for in vivo studies.

Florlu Salisilaldiminlerin Antikanser ve Antimikrobiyal Özelliklerinin Araştırılması

Anahtar Kelimeler
Antikanser,
Antimikrobiyal,
Flor,
Schiff bazı,
Apoptozis

Öz: Antikanser ve antimikrobiyal ilaçlara karşı gelişen çoklu ilaç direnci (MDR) global bir sağlık sorunu haline gelmiştir. Schiff Bazları yapılarındaki fonksiyonel azometin (C=N) grubundan dolayı özel ligandlar olarak anılmakta ve ilaç endüstrisine kullanılmaktadır. İnsan ve hayvan sağlığı alanında kullanılan ilaçların yaklaşık dörtte biri flor atomu içermektedir. Bu çalışmada sentezlenen florlu schiff bazlarının A549 hücre hattında sitotoksik etkileri MTT testi ile, antiproliferatif etkileri; 5 (6)-karboksifluoresan süksinimidil ester (CFSE) boyama tekniği ile araştırıldı. Bileşiklerini apoptozu indükleme yetenekleri ise mitokondriyal membran potansiyeli üzerine olan inhibe edici etkileri Rhodamin 123 boyama tekniği ile, aktif kaspaz-3 analizi immünfloresan testi ile, morfolojik etkileri ise Hematoksilen-eozin (H&E), Giemsa ve Papanicolaou (PAP) protokolleri ile araştırıldı. Bileşiklerin antimikrobiyal etkileri*; P. aeruginosa, E. coli* ve *S. aureus* üzerinde MTT testi ile araştırıldı. Çalışmada kullanılan bileşiklerin genel olarak güçlü düzeyde (IC_{50}:1,4-31,5 µM) ve Bileşik 7'nin (IC_{50}:1,4 µM) Doxorubıcın' den (IC_{50}:1.9 µM) daha güçlü sitotoksik etki sergilediği saptandı. En güçlü antiproliferatif etki Bileşik 10 (PI:2,1) ile elde edilmiştir. En güçlü mitokondrial membran potansiyelini inhibe etme yeteneği Bileşik 4 (1330) ile elde edilmiştir. İmmünfloresan çalışmalarda aktif kaspaz-3, morfolojik çalışmalarda ise kromatin kondenzasyonu, DNA fragmantasyonu gibi apoptoza işaret eden bulgular gözlemlendi. Bileşiklerin P. aeruginosa, E. coli ve S. aureus üzerindeki antimikrobiyal etkileri ($MİK_{50}$:18,6-49,3 µM) pozitif kontrol olarak kullanılan Gentamisin ($MİK_{50}$:1,5-2,2 µM)'e göre düşük seviyede olduğu gözlemlendi. Çalışmada sentezlenen florlu Schiff Bazlarının ve özellikle Bileşik 7'nin antikanser ilaçlara karşı gelişen çoklu ilaç direnci tedavisinde ve in vivo çalışmalar için umut vaad ettiği söylenebilir.

1. INTRODUCTION

Patients with aggressive cancer exhibit high mortality rates, increasing the demand for new chemotherapeutic drugs with improved therapeutic effects and lower toxicity [1,2]. In cancer biology, controlling cancer remains a big challenge. Cancerous cells in patients are intrinsically resistant to standard chemotherapy, where resistant cells lead to treatment failure [3,4]. Thereby, treatment by traditional chemotherapeutic approaches is primarily limited due to acquired multidrug resistance (MDR) [5,6].

The development of resistance to at least one of three or more drugs with different mechanisms of action is defined as MDR [7,8]. MDR cancer cells and pathogenic microorganisms against drugs adversely affect the success of treatment and often cause deaths [9,10]. Due to the change in the drug target sites of cancer cells or bacteria and the increase in efflux pumps, drug entry into the cell is reduced, and at the same time, the drugs are pumped out of the cell. As a result of the weakening of the immune system caused by anticancer drugs, serious infections develop in about half of the patients. The infection of resistant bacteria with cancer worsens the prognosis in patients [11,12].

Cellular or bacterial MDR is tried to be treated with increasing drug doses or drug combinations, and therefore, undesirable conditions such as anemia, infections, nausea, and hair loss may occur due to the cytotoxic effects of drugs on cancer cells as well as on constant proliferating healthy cells [13]. The failure of treatments due to the development of MDR causes researchers to focus on new alternative compounds obtained from plants, microorganisms, and marine invertebrates, and newly synthesized synthetic chemicals are being tested to develop new alternatives to MDR [14,15,16].

Schiff Bases (SB) are known as special ligands due to the flexible, variable, and electronic properties of the functional azomethine bond (C=N) in their structures. They are used in many different fields from analytical chemistry to the pharmaceutical industry [17]. One of the most important areas of SB study is the biological activity of the compounds. The main goal is to find safe and effective therapeutic agents to treat bacterial infections and cancer [18].

Approximately 25% of the drugs used in the field of human and animal health contain one or more fluorine atoms. The fluorine atom increases the lipophilicity and thermal stability of drugs with its electronegative property and contributes to the formation of stronger bonds of drugs with enzymes and their receptors [19,20]. Apoptosis is a form of programmed death that requires energy and occurs in cells that become unable to sustain life as a result of internal and external effects. Caspase enzymes play an important role in the process of apoptosis. Apoptosis process; It occurs with the activation of initiating caspase (2,8,9,10), terminator caspase (3,6,7), and inflammatory (1,4,5,11,12,13,14) caspase enzymes that enable apoptosis. Situations such as irreparable DNA damage in the cell, exposure to cytotoxic drugs, and an increase in reactive oxygen species cause the internal apoptosis mechanism to be activated [21,22,23]. Cytochrome C is a structure located between the membranes of healthy cell mitochondria and plays an important role in obtaining energy. In the cell undergoing apoptosis, cytochrome c passes into the cytoplasm due to the decrease in the mitochondrial membrane potential [21]. The combination of the apoptosis-activating factor, cytochrome c, and caspase-9 forms apoptosome. The apoptosome binds to inactive caspase 3, enabling its activation. With the activation of Caspase-3 in the process of apoptosis, the cell enters the path of irreversible death. Active caspase- 3; Chromatin condensation, DNA fragmentation in cells causes the

formation of apoptotic bodies and the apoptosis process is completed [22,24,25].

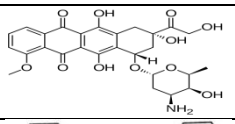
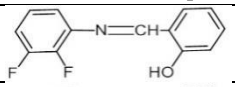
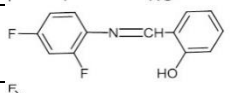
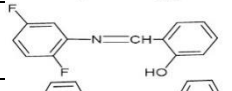
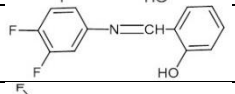
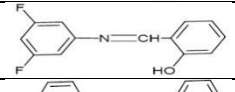
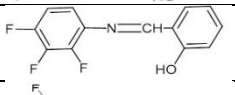
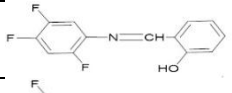
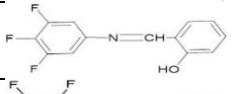
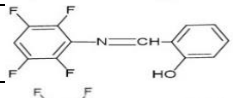
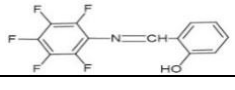
In this study, the cytotoxic, antiproliferative and apoptotic properties of fluorinated salicylamidine derivative (SB) ligands were investigated on A549 cells representing non-small cell lung cancer and some bacteria species *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus)* and *Pseudomonas aureginosa (P. aureginosa)* that are at risk for the development of drug resistance.

2. MATERIAL AND METHOD

2.1. Chemicals

The compounds used in the study were synthesized from salicylaldehydes with anilines containing fluorine in different numbers and positions (Table 1). Their cytotoxic, antiproliferative, antiapoptotic, and antimicrobial properties were tested Doxorubicin (DOX) was used as a positive control for anticancer studies, and gentamicin (GEN) was used for antimicrobial studies. Compounds were dissolved in ethyl alcohol at a dose of 10 mM and sterilized by passing through syringe type 20 µm pore size filters and stored at 4ºC.

Table 1. Chemical properties of DOX and fluorinated SB ligands

Compound	Chemical name	Chemical Formula	Structure	MW(g/mol)
DOX	Doxorubicin	$C_{27}H_{29}N_{11}$		543
C 1	$F_{2,3}$-SAL	$C_{13}H_9NOF_2$		233
C 2	$F_{2,4}$-SAL	$C_{13}H_9NOF_2$		233
C 3	$F_{2,5}$-SAL	$C_{13}H_9NOF_2$		233
C 4	$F_{3,4}$-SAL	$C_{13}H_9NOF_2$		233
C5	$F_{3,5}$-SAL	$C_{13}H_9NOF_2$		233
C 6	$F_{2,3,4}$-SAL	$C_{13}H_8NOF_3$		251
C 7	$F_{2,4,5}$-SAL	$C_{13}H_8NOF_3$		251
C 8	$F_{3,4,5}$-SAL	$C_{13}H_8NOF_3$		251
C 9	F_4-SAL	$C_{13}H_7NOF_4$		269
C 10	F_5-SAL	$C_{13}H_6NOF_5$		287

2.2. Cell Line and Bacterial Strains

A549 cells (ATCC) a lung adenocarcinoma cell line derived from human non-small cell lung cancer cells were cultured in DMEM+F12 medium containing 10% fetal calf serum. Bacterial stock strains for *E. coli* (ATCC, 25922), *S. aureus* (ATCC, 29213), and *P. aureginosa* (ATCC, 27853) were cultured in Mueller-Hinton (MH) broth.

2.3. MTT Analysis for Cell Cytotoxicity

DOX and SB ligands (C1-10) were added to 96-well culture plates at 1, 10, 100, and 1000 µM concentration in triple order. A549 cells were suspended at 10^5 cells ml-1 and then added to each culture well in a volume of 100 µl. The culture plates were incubated at 37º C in a humidified environment containing 5% CO_2 for 72 h. At the end of culture period, 10 µl MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) at 250 µM concentration was added to each well and then incubated for more 4 h. MTT-formazan crystals were dissolved by adding 100 µl of dimethyl sulfoxide (DMSO, Sigma) to each well. Culture plates were read at an ELIZA microplate reader (MD Spectramax, M5) at a wavelength of 570 nm and optical density (OD) values were recorded for each well. IC_{50} values were calculated by nonlinear regression analysis using OD values.

2.4. CFSE Analysis for Cell Proliferation

A549 cells were incubated with CFSE (5(6)-Carboxyfluorescein diacetate N-succinimidyl ester, Sigma) at 1,5 µM concentration for 20 min in the dark at 37ºC under a humidified 5% CO_2 atmosphere. After incubation, CFSE was removed by washing twice with PBS and then analyzed at flow cytometry in FL-1 (green fluorescent channel in the range 10^0 - 10^4) to determine the maximum fluorescence intensity of the cells (Navios, Beckman Coulter). Unstained cells were used to determine autofluorescence. CSFE-stained cells were incubated at 10^5 ml-1 concentration with IC_{50} doses of DOX and SB ligands at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. After incubation, the fluorescent intensity of cells was determined by using an FL1 histogram (Figure 1). Proliferative indexes (PI) were calculated using LISTMOD data by FacsExpress flow cytometry software.

2.5. Rho123 Analysis for Determining Mitochondrial Potential

A549 cells were added to each well at 10^5 ml-1 doses and incubated with DOX and FSB ligands in IC_{50} doses at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. After incubation, cells were treated with 10 µl of Rho123 (1 mg ml-1, Sigma) at 37ºC for 60 min at 37°C in a humidified atmosphere containing 5% CO_2 in the dark. Rho123 accumulation levels of the cells were determined by flow cytometry using FL-1 histogram (Figure 2). Unstained cells were used for auto-fluorescence determination.

2.6. Cleaved Caspase-3 Expression Analysis for Detection of Apoptosis

A549 cells at 10_5 ml-1 doses were incubated with an IC_{50} dose of DOX and FSB ligands at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. After incubation, cells have been rinsed within PBS and then fixed with 4% paraformaldehyde at 37 °C for 20 min. Cells were incubated for 15 min with 1% Triton® X-100 for permeabilization. Cells then were treated for 30 min with 3% of BSA/PBS for blocking. Eventually, cells were incubated with anti-cleaved caspase-3 monoclonal antibody (Alexa Fluor® 488 conjugate, Cell Signaling Technology) at room temperature for 2 h in the dark. Cells were examined by using a fluorescent microscope to determine cleaved caspase-3 expressions.

2.7. Histopathological Morphology Analyses

A549 cells were added to each well at 105 ml-1 doses and incubated with DOX and FSB ligands in IC_{50} doses at 37°C in a humidified atmosphere containing 5% CO_2 for 72 h. After incubation, morphological cell analysis was performed using standard hematoxylin-eosin (H&E), Giemsa, and Papanicolaou (PAP) staining protocols. Cells were examined under a light microscope to determine the morphological changes indicating apoptosis formation in the cells.

2.8. MTT Analysis for Determining Antibacterial Effects

1 ml of each pre-culture suspension of bacterial strains was transferred into 50 ml Mueller Hinton (MH) broth for sub-culturing. Gentamycin (GEN) and SB ligands were added to 96-well culture plates in triple order as 1, 10, 100, and 1000 µM. The bacteria suspension in MH medium was adjusted to 0.5 McFarland standards and 100 µl of suspension was added to each well and then incubated at 37ºC for 18 h. After incubation, 10 µl of 250 µM MTT solution was added to each well and incubated for another 4 h 100 µl of DMSO was added to the culture wells to dissolve the MTT-formazan crystals. Culture plates were read at 570 nm in the ELIZA microplate reader and OD values were recorded for each culture plate well. MIC_{50} values were calculated by linear regression analysis using OD values.

2.9. Statistical Analysis

Analysis of data, determination of IC50 values, and MIC50 values of each compound were performed using GraphPad Prism version 9.1.1 (GraphPad Software, La Jolla California USA). The ANOVA test was used to compare the dose-dependent cytotoxic effects of each compound on A549 cells. SPSS (IBM AMOS VERSION) was used for the ANOVA test. The reliability of the antibacterial data was obtained by performing the homogeneity test from the OD values. Differences between data sets were considered statistically significant when the p-value was less than 0.05.

3. RESULTS

3.1. Cytotoxicity

IC_{50} (half-maximal inhibitory concentration) is an informative measure of the efficacy of a drug [26]. The IC_{50} values are used to determine the potency of DOX and SB ligands to inhibit the biological functions of A549 cells. The strongest cytotoxic effect on A549 cells was obtained with C7 (F2,4,5-SAL, IC_{50}: 1.4 µM), which has 3 fluorine atoms in its structure, showed stronger cytotoxic activity than DOX (IC_{50}: 1.9 µM) which is a standard chemotherapy drug (Table 2).

3.2. Antiproliferative Effects

PI gives information about the proliferation ability of cells [27] The lowness of this index determines the power of DOX or SB ligands to inhibit the proliferation of A549 cells. It was observed that C8 (F3,4,5-SAL) and C10 (F5-SAL) showed the strongest antiproliferative activity (respectively PI: 2.1 and 2.2) according to the PI values giving information about the antiproliferative capacity of SB ligands on A549 cells. DOX (PI: 1.5) was found to have stronger antiproliferative capacity than SB ligands in terms of antiproliferative capacity (Table 2).

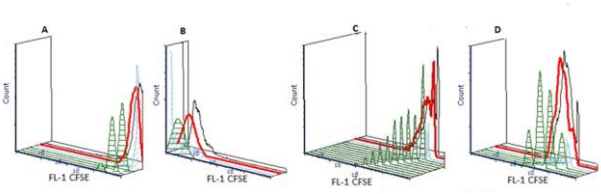


Figure 1. CFSE analysis by flow cytometry (A) Fluorescent intensity of the cells zero h CFSE staining (B) Fluorescent intensity of the cells 72 h after CFSE staining (C) Fluorescent intensity of the cells after 72 h with DOX (D) Fluorescent intensity of the cells after 72 h with Salicylaldimine compound

Table 2. Cytotoxic, antiproliferative, apoptotic effects of DOX and fluorinated SB ligands on A549 cells

	Bacteria	***E. coli***	***S. aureus***	***P. aeruginosa***
MIC_{50} (µM)	**GEN**	1.5	1.7	2.2
	C1	35.0	49.3	42.8
	C2	32.6	50.1	40.4
	C3	33.7	27.3	41.2
	C4	33.0	24.3	36.2
	C5	33.1	24.1	37.3
	C6	36.5	38.5	43.5
	C7	35.8	26.9	46.2
	C8	32.2	54.5	36.5
	C9	32.8	18.6	42.2
	C10	32.8	23.04	43.0

3.3. Mitochondrial Membrane Potential

Changes in mitochondrial membrane potential can be determined by flow cytometry analysis of Rho123 accumulation in cells. This loss of potency is associated with the development of apoptosis in cells. In flow cytometric analysis which has been performed after the application of DOX and SB ligands, it was observed that there were decreases in Rho123 accumulation at varying levels in the cells. Among the SB compounds, the lowest

accumulation of Rho123 was found in cells treated with C5 (F3.5-SAL, mean fluorescent channel: 1330), and this level was higher than in cells treated with DOX (mean fluorescence channel: 1574), (Table 2).

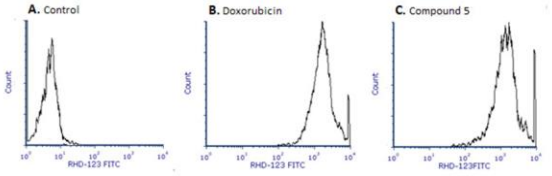


Figure 2. Rho123 analysis by flow cytometry. A) Unstained cells used for determining auto-fluorescent B) DOX-treated cells stained with Rho123 C) Salicylaldimine compound (C5) treated cells stained with Rho123.

3.4. Cleaved Caspase-3 Expression

Caspase-3 is a critical implementer of apoptosis, as it is partially or completely responsible 58ort he proteolytic cleavage of many important key proteins. Active caspase-3 is expressed in the cells when a cell is driven to apoptosis [22]. Determination of cleaved caspase-3 expression in A549 cells treated with DOX and SB ligands indicates that the cytotoxic effect in cells occurs via apoptosis (Figure 3).

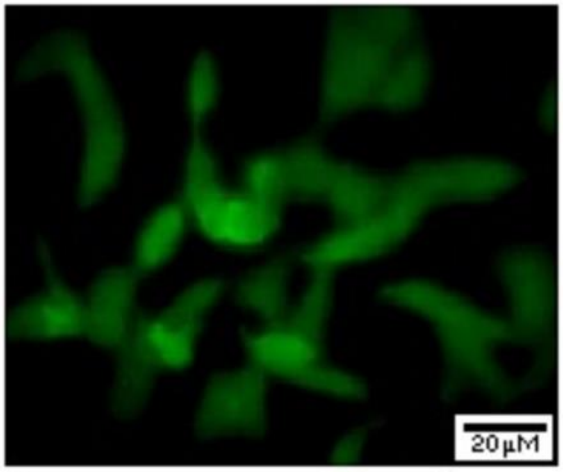


Figure 3. Cleaved caspase-3 expression of A549 cells treated with florosalicyaldimines. Cells were stained with anti-cleaved caspase-3 after 72 h incubation with synthesized fluorinated florosalicyaldimines and then examined under a fluorescent microscope (Mag.

3.5. Cell Morphology

The occurrence of apoptosis in cells can be characterized by a series of typical morphological features that can be followed by staining the cells with dyes used in histopathology and then microscopic examinations [25]. Chromatin condensation and marginalization, bubbling and shrinkage on the cell surface, and the presence of apoptotic bodies and ghost cells in microscopic evaluation after Giemsa, H&E, and PAP staining are important evidence that DOX and SB ligands triggered apoptosis in cells (Figure 4A, B and C)

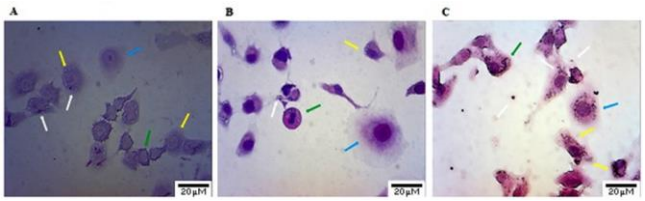


Figure 4. A) Giemsa, B) H&E, and C) PAP staining of the cells after cultured 72 h with chemicals. Green arrows: chromatin condensation and marginalization, white arrows: apoptotic bodies, blue arrow: ghost cell, yellow arrows: cell membrane blebbing and blisteri

3.6. Antibacterial Activity

The MIC value is the minimum dose of antimicrobial agent concentration that is needed to inhibit the growth of a target microorganism. MIC_{50} more specifically indicates the intrinsic potency of an antimicrobial and is used in studies to describe how effective a particular antimicrobial is on a species [28]. GEN used as a positive control showed strong antimicrobial capacity on *E. coli*, *S. aureus*, and *P. aeruginosa* (MIC_{50}: 1.5, 1.7, and 2.2 µM, respectively), but the antimicrobial effects of SB ligands on these bacterial species (MIC_{50}: ranged 32.2-36.5, 18.6-54.5, 36.2-46.2 µM, respectively) were not found remarkable.

Table 3. Antibacterial effects of GEN and fluorinated SB ligands on the bacteria species

	Parameters	IC_{50} (µM)	Proliferative Index (PI)	Rho123 (FL-1*)
Compounds	DOX	1.9	1.5	1574
	C1	11.6.	2.4	1571
	C2	12.3	2.4	2700
	C3	6.1	2.3	2305
	C4	2.1	2.4	1444
	C5	5.2	3.9	1330
	C6	2.5	4.6	2986
	C7	1.4	2.6	2609
	C8	4.1	2.2	2521
	C9	31.5	2.4	3615
	C10	13.9	2.1	2031

4. DISCUSSION AND CONCLUSION

The development of new effective anti-tumor agents based on new chemical compositions with novel structures is important to cope with this drug resistance [29]. In this respect, SB compounds are of great interest in the field of new alternative drug research [30]. The discovery of compounds with both anticancer and antimicrobial effects is of great importance as cancer cells and bacteria have similar resistance mechanisms, such as increasing the extracellular excretion of drugs and changing drug target sites [31,32].

Replacing the Hydrogen (H) atom with Fluorine (F) is a frequently used strategy in new drug studies to increase the efficacy of existing drugs. F atom in drugs prevents enzymes involved in metabolic events such as Krebs cycle and glycolysis in cells, prevents protein synthesis, damages lysosomes, and reduces cell membrane potential by inhibiting sodium ATP'ase [33,34,35]. It is sometimes stated that Fluorinated SB derivates exhibit stronger effects than anticancer and antimicrobial drugs used as positive controls [36,37,38] In addition, it can be said that fluorine-containing SB derivatives exhibit

strong cytotoxic effects on cancer cells, while they show low cytotoxicity on normal cells with high proliferation rates [39,40].

It has been reported in some studies that Schiff bases containing fluorine or trifluoromethyl in the phenyl ring exhibit potent cytotoxic effects on A549 cancer cells [40,41]. The biological activities of fluorine-substituted SB compounds are affected by the number and position of fluorine atoms, and it has been reported that the compound containing 5 fluorine atoms has the strongest cytotoxic effect on A549 cells [42]. In a study that investigated the anticancer effects of SB compounds carrying fluorine atoms on A549, Hep G-2, and HeLa cells, it is emphasized that the compound carrying two fluorine atoms has stronger anticancer effects than cisplatin and the position of the fluorine atom is important in the activities of the compounds [38]. In this study, we can state that the fluorine atom numbers and positions of the SB ligands are effective in cytotoxicity and that the compounds containing 3 fluorines show stronger cytotoxic effects 2, 4, and 5, and the difference in the cytotoxic effects of the 3-fluorinated ligands varies depending on the positions of the fluorine atoms.

Determining the antiproliferative capacities of a compound on cancer cells is of great importance in the discovery of new chemotherapeutic agents. In a recent study, aminophenylhydrazine SB compounds were tested for their antiproliferative capacity on A549 cells and showed that those containing two fluorine atoms had the strongest antiproliferative effect compared to five fluorine atoms [42]. Another study with SB compounds carrying fluorine atoms in the ortho and para positions showed that the antiproliferative effects of the compounds on K562 cells were lower than that of DOX [43]. In our study, although the lowest PI value among SB ligands was obtained with 5 fluorinated compounds, this antiproliferative capacity lagged behind DOX, which is the standard chemotherapeutic drug.

Determination of mitochondrial membrane potential is considered an important criterion in determining the apoptotic effect of compounds on cells. In a study that tested the apoptotic effects of N-phenyl carbazole triphenyl amine modified semi-sandwich (III) SB compounds and their complexes with iridium on A549, HeLa, BEAS cells, it was emphasized that the decrease in mitochondrial membrane potential indicates that apoptosis is effective in the death of cells [35]. In our study, it was observed that SB ligands with difluorine atoms had a stronger effect on mitochondrial membrane potential in A549 cells than DOX, whereas ligands containing three, four, and five fluorine atoms had a lower capacity than DOX. We understand that there is a negative correlation between the number of fluorine atoms of ligands and their ability to reduce mitochondrial membrane potential, thereby driving cells into apoptosis.

Cleaved caspase-3 expression is one of the most important molecular biomarkers that indicate the occurrence of apoptosis in cells. Cell death is a process with many types; It occurs in different ways such as apoptosis, pyroptosis, and autophagy. The role of apoptosis in cancer treatment and prognosis is very important. Recently, it has been suggested that caspase-3 may associate apoptosis with pyroptosis which causes inflammatory cell death It is stated that the induction of both apoptosis and pyroptosis with the effectiveness of chemotherapeutics is an important modality for cancer treatment and caspase-3 is an important stimulator for both [44]. The observation of cleaved caspase-3 expression in A549 cells in our study indicates that the cytotoxic effects of DOX and SB ligands occur through the induction of apoptosis in the cells.

Cell morphology analyses provide useful data for obtaining information about cell cytotoxicity. Morphological changes indicative of apoptosis such as cytoplasmic shrinkage, membrane overflow, and DNA fragmentation were observed in cells treated with SB copper coordinated compound [45]. Observation of cellular morphological changes such as chromatin condensation and marginalization, presence of apoptotic bodies, ghost cells, and cell membrane swelling and shrinkage, which indicate apoptosis in A549 cells in our study, is another important finding that apoptosis is effective in cell cytotoxicity of FSB ligands.

Many studies show that SB compounds have antimicrobial properties. Fluorinated SB containing F or CF3 at the meta position on the N-aryl ring has been found to exhibit potent antimicrobial activity on *S. aureus*, *B. subtilis*, *E. coli*, and *K. pneumoniae* [46]. It has been determined that the fluorine-bearing compound in the para position of the phenol ring shows strong antimicrobial activity and has a stronger antifungal effect than fluconazole, which is currently used in the treatment of fungal infections [47]. In a study that investigated the antimicrobial properties of six SB compounds derived from 3-fluoro salicylaldehyde on *K. pneumonia, S. typhi, S. aureus, B. subtilis, P. aeruginosa, E. coli bacteria* and *P. chrysogenum, C. albicans, A. niger* and *A. flaves*. It has been determined that most of the compounds have antibacterial and antifungal activity on these bacteria [48]. In this study, contrary to the other presented studies, it was observed that the antimicrobial effects of fluorinated salicylaldehyde SB compounds on *E. coli, S. aureus*, and *P. aeruginosa* were lower than gentamicin, a standard antibacterial drug, and none of the compounds had significant effects on the tested bacteria.
Salicylaldimine derivative Schiff Base compounds containing fluorine atoms in different numbers and positions show promising anti-cancer properties such as cytotoxicity, inhibiting proliferation, and directing A549 lung cancer cells to apoptosis but, they remained unremarkable in terms of their antibacterial effect on *E. coli, S. aureus* and *P. aeruginosa*.

In this study, the anticancer and antimicrobial properties of Schiff base derivative (C1-C10) containing fluorine atoms in different numbers and positions were investigated. Cytotoxic, antiproliferative, and apoptotic properties of the synthesized compounds were examined

on A549 cells. Its antimicrobial properties were investigated on *E. coli*, *P. aureginosa*, and *S. aureus* bacterial strains. It was observed that these synthesized compounds exhibited strong cytotoxic effects on A 549 cells. It was determined that Compound 7 (IC_{50}: 1.4 µM), which has fluorine atoms attached at the –middle, -meta, and –para positions, exhibited a stronger cytotoxic effect than DOX (IC_{50}: 1.9 µM), which was used as a positive control. Compound 4 (2.1 µM) and Compound 6 (2.5 µM) showed cytotoxic effects close to DOX. The synthesized compounds showed high (Compound 3, 4, 5, 6, 7, and 8) and moderate (1, 2, 9, and 10) cytotoxic effects. Compounds 1, 2, 3, 4, 5, 7, 8, 9, and 10 (PI values 2,4, 2.4, 2.3, 2.4, 2.6, 2.2, 2.4, and 2.1, respectively) were used as positive control near DOX (PI: 1.5). It has been determined that it exhibits antiproliferative effects. Compounds 1, 4, and 5 (Rho 123 values 1571, 1444, and 1330, respectively) were found to be more potent in reducing mitochondrial membrane potential than Dox (Rho 123:1574). It was determined that the compounds caused decreases in mitochondrial membrane potential, thus the release of active caspase-3. After the release of active caspase-3 in cells, DNA fragmentation, apoptotic bodies, ghost cells, etc. occur. By observing the structures microscopically after Hematoxylin-Eosin, Giemsa, and PAP staining, it can be said that the synthesized compounds are successful in directing A549 cells to apoptosis. When evaluated as antimicrobials, the MIC_{50} values of the synthesized compounds on *E. coli*, *P. aureginosa*, and *S. aureus* strains were found to vary between 18.50 and 49.3. It can be said that the compounds showed moderate antimicrobial effects compared to Gentamicin (MIC_{50} values of 1.5, 1.7, and 2.2, respectively), which was used as a positive control. It can be said that the results of the study are promising for the use of the synthesized compounds in vivo studies.

Acknowledgement

This study is supported by the Harran University Scientific Research Project Coordination Unit (Project No: 20160). Supplying chemical compounds, Prof. Dr. Thank you Veli KASUMOV.

There is no need for an ethics Committee Document for our study.

REFERENCES

[1] Chabner BA, Roberts TG Jr. Chemotherapy and the war on cancer. Nat Rev Cancer.2005;5: 65–72.
[2] Kamb A, Wee S, Lengauer C.Why is cancer drug discovery so difficult? Nat Rev Drug Disc. 2007; 6: 115–120.
[3] Shilpa BS, Padmamma S, Kumara AT. Mapping of scientific articles on Leukemia: A scientometric study. Libr Philos Pract. 2019; 1: 1-24.
[4] Aktimur SH, Gunes AK, Akidan O. Efficacy of the Combination of Venetoclax and Azacitidine in Elderly of Frail Relapsed/Refractory Patients with Acute Myeloid Leukemia, First Multi-Institutional Real World Experience from Türkiye. J Hematol Oncol. 2020; 31(4): 213.
[5] Kankala RK, Liu CG, Yang DY. Ultrasmall platinum nanoparticles enable deep tumor penetration and synergistic therapeutic abilities through free radical species-assisted catalysis to combat cancer multidrug resistance. Chem Eng J. 2020;383: 123.
[6] Sonbol, H., Ameen, F., AlYahya, S., Almansob, A., & Alwakeel, S. Padina Boryana mediated green synthesis of crystalline palladium nanoparticles as potential nanodrug against multidrug-resistant bacteria and cancer cells. Scientific reports. 2021; 11(1), 1-19.
[7] Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Monnet, D. L. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012;18(3), 268-281.
[8] Moitra K. Overcoming Multidrug Resistance in Cancer Stem Cells. Biomed Res Int. 2015:635745.
[9] Wang, X., Zhang, H., & Chen, X. Drug resistance and combating drug resistance in cancer. Cancer Drug Resistance. 2019; 2(2), 141.
[10] Orsi, G. B., Falcone, M., & Venditti, M. (2011). Surveillance and management of multidrug-resistant microorganisms. Expert Review of Anti-Infective Therapy. 2019; 9(8), 653-679.
[11] Peraman, R., Sure, S. K., Dusthackeer, V. N., Chilamakuru, N. B., Yiragamreddy, P. R., Pokuri, C.,et.al. Insights on recent approaches in drug discovery strategies and untapped drug targets against drug resistance. Future Journal of Pharmaceutical Sciences. 2021; 7(1), 1-25.
[12] WC Soo, V., Kwan, B. W., Quezada, H., Castillo-Juárez, I., Pérez-Eretza, B., Julieta García-Contreras, S., García-Contreras, R. Repurposing of anticancer drugs for the treatment of bacterial infections. Current topics in medicinal chemistry. 2017; 17(10), 1157-1176.
[13] Can, G. Side effects of antineoplastic drugs and nursing approaches. Journal of Education and Research in Nursing. 2005; 2(2), 8-15.
[14] Majolo, F., Delwing, L. K. D. O. B., Marmitt, D. J., Bustamante-Filho, I. C., & Goettert, M. I. Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. Phytochemistry Letters. 2019; 31, 196-207.
[15] Temel, M. K. The development of cytotoxic chemotherapeutics in the twentieth century. Turkish Journal of Oncology. 2015; 30(2).
[16] Bukowski, K., Kciuk, M., & Kontek, R. Mechanisms of multidrug resistance in cancer chemotherapy. International journal of molecular sciences. 2020; 21(9), 3233.
[17] Uddin, M. N., Ahmed, S. S., & Alam, S. R. Biomedical applications of Schiff base metal complexes. Journal of Coordination Chemistry. 2020; 73(23), 3109-3149.

[18] Matela, G. Schiff bases and complexes: a review on anti-cancer activity. Anti-Cancer Agents in Medicinal Chemistry. 2020; 20(16), 1908-1917.
[19] Al-Harthy, T., Zoghaib, W., & Abdel-Jalil, R.Importance of fluorine in benzazole compounds. Molecules. 2020; 25(20), 4677.
[20] Inoue, M., Sumii, Y., & Shibata, N. Contribution of organofluorine compounds to pharmaceuticals. ACS omega. 2020;5(19), 10633-10640.
[21] Bildik A, Bayar İ. Kanserde apoptotik Yolakların İnhibisyonu. Turkiye Klinikleri J Vet Sci. 2018;9(2):42-51.
[22] Kaya C, Çalışkan Y, Yönden Z. Apoptozis. The Medical Journal of Mustafa Kemal University. 2012; 3(11); 26-37.
[23] Atagün G, Zafer, E, Gürkanlı İ. Apoptoziste mitokondrinin rolü. Türk Bilimsel Derlemeler Dergisi. 2011;4(2): 9-53.
[24] Dağdeviren T. Programlı hücre ölümü; Apoptoz. Gaziosmanpaşa Üniversitesi Tıp Fakültesi Dergisi. 2021; 3(3), 120-135.
[25] Coşkun G, Özgür H. Apoptoz ve nekrozun moleküler mekanizması. Arşiv Kaynak Tarama Dergisi. 2012; 20(3): 145-158.
[26] Berrouet C, Dorilas N, Rejniak K A, Tuncer N. Comparison of drug inhibitory effects (IC 50) in monolayer and spheroid cultures. Bulletin of Mathematical Biology,2020; 82(6) 68.
[27] Elias J M. Cell proliferation indexes: a biomarker in solid tumors. Biotechnic & histochemistry, 1997; 72(2): 78-85.
[28] Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson A P., et al. (Assessing the antimicrobial susceptibility of bacteria obtained from animals. Journal of antimicrobial chemotherapy. 2010; 65(4): 601-604.
[29] Rice, L. B. Unmet medical needs in antibacterial therapy. Biochemical pharmacology. 2006; 71(7), 991-995.
[30] Fekri R, Salehi M, Asadi A. Synthesis, characterization, anticancer and antibacterial evaluation of Schiff base ligands derived from hydrazone and their transition metal complexes. Inorganica Chim Acta. 2019; 484: 245-254.
[31] Kumar, A., Chakma, U., Chandra, A., Howlader, D., Akash, S., Kobir, M., et.al. Modified D-glucofuranose computationally screening for inhibitor of breast cancer and triple breast cancer: Chemical descriptor, molecular docking, molecular dynamics, and QSAR. Journal of the Chilean Chemical Society. 2022; 67(3), 5623-5635.
[32] Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & et.al. Drug resistance in cancer: an overview. Cancers. 2014; 6(3), 1769-1792.
[33] Strunecká, A., Patočka, J., & Connett, P.Fluorine in medicine. J Appl Biomed. 2004; 2, 141-150.
[34] Kurtdede, E. Türkiye'de florozis sorunu ve florun biyokimyasal etkileşimi. Atatürk Üniversitesi Veteriner Bilimleri Dergisi. 2017; 2(3), 320-326.
[35] Chen, S., Liu, X., Ge, X., Wang, Q., Xie, Y., Hao, Y., et.al. Lysosome-targeted iridium (III) compounds with pyridine-triphenylamine Schiff base ligands: Syntheses, antitumor applications and mechanisms. Inorganic Chemistry Frontiers. 2020; 7(1), 91-100.
[36] Gupta, D., & Jain, D. K. Synthesis, antifungal and antibacterial activity of novel 1, 2, 4-triazole derivatives. Journal of advanced pharmaceutical technology & research. 2015; 6(3), 141.
[37] Avila-Sorrosa, A., Bando-Vázquez, A. Y., Alvarez-Alvarez, V., Suarez-Contreras, E., Nieto-Meneses, R., Nogueda-Torres, B., et. al. Synthesis, characterization and preliminary in vitro trypanocidal activity of N-arylfluorinated hydroxylated-Schiff bases. Journal of Molecular Structure. 2020; 1218, 128520.
[38] Wu, R., Bi, C., Zhang, X., Zong, Z., Fan, C., Zhang, X., et.al. Syntheses, crystal structure and biological evaluation of three novel Cu (II) complexes with Schiff base derived from fluorinated amino acid and Salicylaldehyde. Applied Organometallic Chemistry. 2020; 34(1), e5264.
[38] Würtenberger, I., Follia, V., Lerch, F., Cwikla, C., Fahrner, N., Kalchschmidt, C., et. Al. Fluorinated Fe (III) salophene complexes: optimization of tumor cell-specific activity and utilization of fluorine labeling for in vitro analysis. Journal of Medicinal Chemistry. 2015; 58(2), 588-597.
[40] Zeng, L., Deng, Y., Weng, L., Yang, Z., Chen, H., Liu, Q. Synthesis and Anti-Tumor Activities of Fluoride-Containing Gossypol Derivatives Compounds. Natural Science. 2017;9(9), 312-318.
[41] Mahal, A., Wu, P., Jiang, Z. H., & Wei, X. Schiff bases of tetrahydro curcumin as potential anticancer agents. Chemistry Select. 2019; 4(1), 366-369.
[42] Sak, Z. H. A., Süzergöz, F., Kasumov, V. T., Gürol, A. O. Anticancer Properties of Fluorinated Aminophenylhydrazines on A549 Lung Carcinoma Cell Line. Iranian Journal of Public Health. 2021; 50(3), 550-556.
[43] Gurol, A. O., Kasim, V., Suzergoz, F. Antiproliferative effects of fluorine substitute 3, 5-di-tert-butylphenol bearing Schiff bases using CFSE-based cell proliferation assay. Current Science. 2017;619-624.
[44] Li, L., Wang, S., & Zhou, W.Balance. Cell Apoptosis and Pyroptosis of Caspase-3-Activating Chemotherapy for Better Antitumor Therapy. Cancers. 2022; 15(1), 26.
[45] Xia, Y., Liu, X., Zhang, L., Zhang, J., Li, C., Zhang, N., Li, Y. A new Schiff base coordinated copper (II) compound induces apoptosis and inhibits tumor growth in gastric cancer. Cancer Cell International. 2019; 19, 1-11.
[46] Avila-Sorrosa, A., Hernández-González, J. I., Reyes-Arellano, A., Toscano, R. A., Reyes-Martínez, R., Pioquinto-Mendoza, J. R., et.al. Synthesis, structural characterization and biological activity of fluorinated Schiff-bases of the type [C6H4-1-(OH)-3-(CHNArF)]. Journal of Molecular Structure. 2015; 1085, 249-257.
[47] Carreño A, Rodríguez L, Páez-Hernández D, Martin-Trasanco R, Zúñiga C, Oyarzún DP, et.al. Two New Fluorinated Phenol Derivatives Pyridine

Schiff Bases: Synthesis, Spectral, Theoretical Characterization, Inclusion in Epichlorohydrin-β-Cyclodextrin Polymer, and Antifungal Effect. Front Chem.2018;30; 6:312.

[48] Shanmugam M, Narayanan K, Mahalakshmi M, Kabilan S, Chidambaranathan V.Synthesis, characterization and biological studies of some novel 3-fluorosalicylaldehyde based amine derivatives. Spectrochim Acta A Mol Biomol Spectrosc. 2013; 116:394-400.

.