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Evaluation of Antifungal and Antiproliferative Effects of Two Different Organic Compounds with Cyclobutane Ring

Siklobütan Halkalı İki Farklı Organik Bileşiğin Antifungal ve Antiproliferatif Etkilerinin Değerlendirilmesi

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ABSTRACT

In the modern world, where there is a growing demand for new substances with anticancer and antifungal activities, Schiff bases and phthalimide derivatives, which exhibit a wide diversity of biological activity, have become the focus of new therapeutic research studies. Accordingly, this study examined the anti-proliferative effects of two distinct compounds synthesized by cyclobutane substitution on breast cancer and liver cancer cell lines, which are two major cancer types, were investigated with MTT method and their antifugal activities on *C. albicans* were evaluated with disk diffusion method. Minimum inhibitory concentrations of the compounds against *C. albicans* were also determined in the scope of the study. The results revealed that the synthesized Schiff base was more effective on the breast cancer cell line MCF7, whereas the phthalimide-derivative was more effective against the liver cancer cell line Mahlavu. Besides, according to the data related to the antifungal properties of the compounds, it can be inferred that both compounds are suitable for further investigation as potential building blocks for the creation of novel and efficient antifungal medications.

Key Words
Antifungal, anti-proliferative, schiff base, phthalimide derivative.

ÖZ

Antikanser ve antifungal etkinliklere sahip yeni bileşiklere duyulan gereksinimin giderek arttığı günümüzde, çeşitli biyolojik aktiviteler sergileyebilen bazı organik bileşiklerden Schiff bazlarının ve ftalimid türevlerinin yeni terapötik araştırma-geliştirme çalışmalarında önemli olduğu görülmektedir. Bu nedenle bu çalışmada, siklobütan sübstitüsyonu ile sentezlenen iki farklı bileşiğin, yaygın kanser türleri arasında olan meme kanseri ve karaciğer kanseri hücre hatları üzerindeki anti-proliferatif etkileri MTT yöntemi ile, *C. albicans* üzerindeki antifugal aktiviteleri ise disk difüzyon yöntemi ile incelenmiştir. Ayrıca bileşiklerin *C. albicans*'a karşı minimum inhibitör konsantrasyonları da çalışma kapsamında tespit edilmiştir. Sonuçlar, sentezlenen Schiff bazının meme kanseri hücre hattı MCF7 üzerinde daha etkili olduğunu, buna karşılık ftalimid-türevinin ise karaciğer kanseri hücre hattı Mahlavu'ya karşı daha etkili olduğunu ortaya koymuştur. Ayrıca, bileşiklerin antifungal özelliklerine ilişkin verilere dayanılarak; söz konusu bileşiklerin her ikisinin de yeni ve etkili antifungal ilaçların elde edilmesi için gerçekleştirilecek yeni çalışmalara potansiyel yapı taşları olarak katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler
Antifungal, anti-proliferatif, schiff bazı, ftalimid türevi.

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INTRODUCTION

Schiff bases, called azomethines or imines, are structures formed as a result of condensation reactions of primary amine groups with carbonyl compounds such as aldehydes or ketones, and they contain C=N double bonds in their structures. Schiff bases have become important and remarkable groups of substances in recent years due to their structural and biological properties. While Schiff bases have various biological activities in biological systems such as antibacterial, antifungal, antiviral, anticarcinogenic and anti-tumor, they are also important in the treatment of diabetes and AIDS by showing a protective effect on the hematopoietic system. Also, Schiff base compounds have been a widely used compound class in dyestuff production, technological polymer production, corrosion inhibitor, ion-selective electrode design and preparation of agricultural, pharmaceutical, medical and cosmetic products [1–8].

One of the most notable activities of Schiff bases is their role in amino acid biosynthesis. Their biological activities are due to the chelates they make with trace elements, and accordingly, they have been found to have a wide variety of pharmacological properties [9]. In addition, since Schiff bases have the ability to form complexes with metals, their use in the determination of metal amounts is increasing. These complex components, which are mostly used in the polymer and food industry, are used in pharmacy as a preservative that prevents the oxidation process [10].

Also, Phthalimides and N-substituted phthalimides are a fascinating class of chemicals since they have important biological activities [11,12]. Recently, these compounds have also received more attention for their androgen-antagonistic activities and antihyperlipidemic activities, research is ongoing in these areas [13,14]. Although there are several medications that lower blood triglycerides and cholesterol, it is critical to identify even more potent and nontoxic ones. Also, phthalimides are able to inhibit acetyl-CoA carboxylase activity. Furthermore, phthalimide derivatives have anti-cancer properties, making them useful for treating tumors such Kaposi's sarcoma, renal cell carcinoma, and erythema nodosum leprosum. [15,16]. Therefore, research on this group of substances is extremely important.

In this study, we synthesized cyclobutane substituted two different compounds; *Schiff Bases*: 4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-(2-hydroxy-1-naphthylideneimino) thiazole, and Phthalimide derivative: 1-Methyl-1-phenyl-3-(phthalimidoacetyl) cyclobutane. Then, the antifungal activities of the compounds were tested against *C. albicans* (NCPF 3179), and their antiproliferative effects were evaluated on two different cancer cell lines, Mahlavu and MCF7.

MATERIALS and METHODS

Chemicals

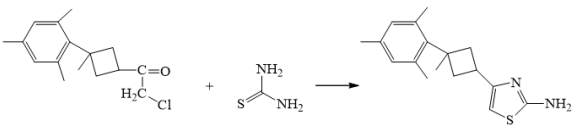
All chemicals, and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), Serva (Heidelberg, Germany) and Merck (Darmstadt, Germany). Cell culture media and media supplements were purchased from Serana (Brandenburg, Germany) and Lonza (Verviers, Belgium). 96-well microtiter plates were purchased from Nest Scientific (Jiangsu, China).

Synthesis of studied compounds

The synthesis steps are given below, respectively.

1. Synthesis of 4-(1-Methyl-1-mesitylcyclobutane-3-yl)-2-aminothiazole

The compound 1 was synthesized according to our previous paper [17]. Briefly, to a solution of 10 mmol (0.76 g) of thiourea in 50 mL absolute ethanol, a solution of 10 mmol (2.645 g) of 1-methyl-1-mesityl-3-(2-chloro-1-oxoethyl) cyclobutane in 20 mL absolute ethanol was added dropwise at 60–70 ˚C. Once the reaction was completed, 5% aqueous solution of NH_3 was used for neutralization, and 4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-aminothiazole was precipitated.



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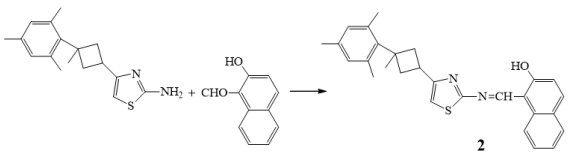
4-(1-Methyl-1-mesitylcyclobutane-3-yl)-2-aminothiazole

2. Synthesis of 4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-(2-hydroxy-1-naphthylideneimino) thiazole

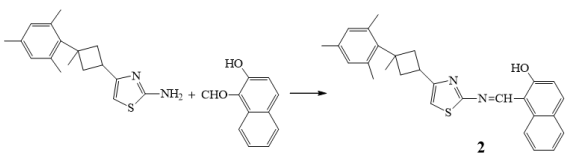
The compound 2 similarly synthesized and characterized [17]. Briefly, 10 mmol (1.43 g) of Compound 1 and 10 mmol (0.86 g) of 2-hydroxy-1-naphthaldehyde were mixed in 50 mL absolute ethanol. The mixture was refluxed for 2 hours and permitted to stand overnight to precipitate target compound.



4-(1-methyl-1-mesitylcyclobutane-3-yl)-2-(2-hydroxy-1-naphthylideneimino) thiazole

3. Synthesis of 1-Methyl-1-phenyl-3-(phthalimidoacetyl) cyclobutane

The compound 3 was synthesized and characterized according to our previous papers [18,19]. Briefly, a mixture of 1- methyl -1-phenyl -3-(2-chloro-1-oxoethyl) cyclobutane (0.01 mol, 2.225 g), which was synthesized using the protocol defined by Akhmedov et al.[20], triethylamine (0.01 mol, 1.0 g) and phthalimide (0.01 mol, 1.471 g) in acetonitrile was stirred for 4 hours. After purification the product obtained was crystallized from ethanol.



1-Methyl-1-phenyl-3-(phthalimidoacetyl) cyclobutane

Determination of Antifungal Activity Preparation of Inoculum for Antifungal Activity Evaluation

The antifungal effects of cyclobutane ring-bearing compounds 2 and 3 were tested against *C. albicans* (NCPF 3179). The stock culture of the microorganism was maintained at +4 °C on Sabouraud Dextrose Agar medium. In order to prepare the inoculum used in the studies, a single colony selected from the stock culture plate was incubated in Sabouraud Dextrose broth for 16-18 hours at 30 °C with shaking. At the end of the incubation period the fungal suspension was standardized by matching the turbidity to that of 0.5 McFarland standard [(1 – 2 x 10^6 colony forming units (CFU)/mL)] [21] and the antifungal activity evaluations were performed using a 1/10 (1 – 2 x 10^5 CFU/mL) dilution of this suspension.

Antifungal Susceptibility Test via Disk Diffusion Method

Antifungal susceptibility testing was conducted using the agar diffusion technique. 20 ml of Sabouraud Dextrose Agar was poured into petri dishes and allowed to solidify. Then, 0.1 ml of standardized inoculum of *C. albicans* was evenly dispersed throughout the plate's surface. Then, 6mm diameter sterile disks loaded with 20 µl of Compound 2 and 3 solutions with a concentration of 1400 µM were applied and the plates were incubated at 30 °C for 16 hours. The diameters of the inhibition zones were measured following the incubation.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations of Compound 2 and Compound 3 were tested by the two-fold serial dilution method adapted for 96-well microtiter plates. The test compounds were dissolved in 5% DMSO to obtain 2800 µM stock solutions. 0.1 ml of the prepared stock chemicals were mixed with an equal amount of 1/5 dilution of standardized *C. albicans* inoculum to get a concentration of 1400 µM. Values of 700, 350, 175, 87.5, 43.75 µM were obtained by two-fold serial dilution of this suspension with 1/10 diluted standardized inoculum of *C. albicans*. Control wells were prepared with untreated *C. albicans* inocula, test compound dilutions without microorganisms and uninoculated Sabouraud Dextrose Broth. Eventually, the lowest concentrations that did not result in any growth of the tested microorganism detectable with unaided eye were determined as the MIC value after the microtiter plates were incubated for 16–18 hours at 30 °C.

Evaluation of Antiproliferative Effect

Mahlavu, a human hepatocellular carcinoma cell line, and the estrogen receptor positive (ER+) breast cancer cell line MCF7 were cultured in Dulbecco's modified Eagle medium supplemented with 10 % FBS and 1 % penicillin/streptomycin (Pen/Strep). A humidified chamber with 5% CO_2 and a temperature of 37 °C were used to grow the cells in monolayers. Using the previously described MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay [22], Compounds 2 and 3's effects on cell proliferation and viability were examined. Briefly, Mahlavu and MCF7 cells were plated in 96-well microtiter plates in a final volume of 100 µL of growing media, at a density of $2x10^3$ and $5x10^3$ cells/

well, respectively. The growth media was changed the following day, and the cells were cultured for 72 hours with a fresh medium that was supplemented with one of the organic compounds in varying concentrations (0-400 µM). The cells were then treated for 4 hours with 10 µL of 5 mg/ml MTT solution prepared in Dulbecco's phosphate buffered saline. The cells were subsequently incubated for an additional 24 hours with 10% sodium dodecyl sulfate (SDS) in 0.01 M hydrochloric acid (HCl) and a microplate spectrophotometer (Multiskan GO; Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the absorbance at 570 nm. Each experiment was carried out in triplicate with five technical replicates and the percentage of cell viability was estimated using the formula below:

$$\%\ Cell\ via\ bility = \frac{A(Treated\ Cells) - A(Blank)}{A(Control\ Cells) - A(Blank)} \times 100$$

RESULTS and DISCUSSION

Evaluation of Antifungal Activity

Fungal pathogens and infections pose an increasing burden on public health. People who have underlying medical issues or a compromised immune system are especially vulnerable to fungal infections. Invasive fungal disease (IFD) cases are rising along with the size of the population at risk and this is a result of a variety of causes, including the improvements in modern medicine and the availability of immune-suppressing drugs and procedures like chemotherapy, immunotherapy for cancer, and solid organ transplantations [23]. Furthermore, the rapidly developing antifungal resistance worsens the under recognized threat that invasive fungal diseases pose to public health. Antifungal resistance frequently leads to prolonged therapies, extended hospital stays, and a greater requirement for pricy and often extremely toxic second-line antifungal medications. However, while being a growing hazard to human health, fungal infections generally get little global attention and funding [24]. The urgency of the situation is actually demonstrated by the "fungal priority pathogens list" that the World Health Organization published in 2022 for the first time and had 19 fungal pathogens [23].

In compliance with this criterion, the disk diffusion method was used in this investigation to assess the antifungal activity of the synthesized Compound 2 and Compound 3 on *C. albicans*. The findings are shown in Table 1. According to the results on zone inhibition, Compound 2 has a stronger antifungal impact in comparison to Compound 3.

The present study's scope also included determining the minimal inhibitory concentrations of the synthesized Schiff bases for *C. albicans*. The evaluations revealed that Compound 2 has a lower MIC value than Compound 3 (700 µM and 1400 µM, respectively). Consequently, the results from disk diffusion experiments and MIC evaluations are congruent with one another and emphasize the higher potential of Compound 2 as an antifungal agent in compared to Compound 3. On the other hand, it is obvious that the relatively high MIC values obtained for both of the compounds can be attributed to the limited antifungal activities. However, given that the literature emphasizes that metal complexes of Schiff bases have much higher biological activities than the original compounds [25], it is believed that the obtained Schiff bases can be used as a starting point for the synthesis of new complexes with greater antifungal activity.

Determination of Antiproliferative Activity

Cancer is among the leading causes of death worldwide. Additionally, it is predicted that cancer cases and related deaths will increase rapidly as a result of population growth, population aging, and lifestyle changes [26]. As a result of these changes in population structure, the distribution of cancer cases by cancer type also shifted. For instance, according to data from 2020, female breast cancer has surpassed lung cancer as the most common type of cancer worldwide [27]. Additionally, primary liver cancer has also been identified as the third most common cancer-related cause of death worldwide, with an anticipated 830.000 deaths in 2020 [27]. These findings unequivocally demonstrate the significance

Table 1. Inhibition zones obtained in disk diffusion assays.

	Inhibition Zones (mm)[¥]
Compound 2	8.33 ± 0.27
Compound 3	6.67 ± 0.54

[¥]Results are expressed as the Mean ± SEM of 3 independent measurements.

Table 2. IC_{50} values of compounds detected for Mahlavu MCF7 cell lines

	IC_{50} Values (µM)[¥]	
Compound 2	158.45 ± 3.35	26.87 ± 5.84
Compound 3	37.11 ± 4.72	145.29 ± 10.95

[¥]Results are expressed as the Mean ± SEM of 3 independent measurements.

of finding novel compounds with potential therapeutic value, particularly for the treatment of breast and liver cancers.

In accordance with this need, in this study, the antiproliferative effects of two different compounds on the liver cancer cell line Mahlavu and the breast cancer cell line MCF7 were tested with the MTT method. As is well known, the MTT test, which is a simple, robust, fast and cost-effective method, is one of the most popular methods used to determine cell proliferation and viability.

The percentage of cells that survived 72 hours of incubation with various test compound concentrations (1 – 400 µM) was used to gauge the effectiveness of compounds as antiproliferative agents. Table 2 provides the IC_{50} values determined for Compound 2 and Compound 3 as a result of the analyses carried out. The data obtained show that both compounds have promising antiproliferative potential and revealed that Compound 2 was more effective against MCF7 cell line, whereas Compound 3 was more potent on Mahlavu cells.

Additionally, Figure 1 depicts the dose-dependent effects of tested compounds on cell survival. As is evident, the chemicals have an increasing cytotoxic effect on cells depending on their concentration. As seen in the figure, relatively low doses of Compound 2, such as 25 µM, cause the viability of MCF7 cells to fall below 50%. On the other hand, for the viability of Mahlavu cells to fall below 50%, Compound 3 had to be applied at a concentration of 50 µM. Besides, it has also been found that high concentrations of Compound 3 (100 to 400 µM) have very drastic effects particularly on the viability of the Mahlavu cell line, and Compound 3 applied at the aforementioned concentrations reduces the viability of the cell line in question by more than 95%

The results showed that the tested compounds were promising in terms of both antifungal activity and antiproliferative effect. The discovered MIC values of 700 µM (for Compound 2) and 1400 µM (for Compound 3) are known to imply that the compounds are less effective than currently available antifungal medications.

Besides, studies carried out to reveal the biological properties of Schiff bases and their complexes demonstrated to exhibit a wide variety of biological activities including but not limited to; antiviral, antiproliferative, anti-inflammatory, antimalarial, analgesic, antipyretic, antifungal and antibacterial properties [25,28,29]. In fact, a study examining the anticancer effects of platinum (II) complexes of reduced amino acid Schiff bases found that one of the investigated compounds had activity against BGC-823 and HL-60 cell lines that was even higher than the chemotherapeutic drug cisplatin [30]. In another study that examined the antifungal activity of 23 different cinnamyl Schiff bases, it was discovered that two of the compounds exhibited a minimum inhibitory concentration against *Cryptococcus neoformans*

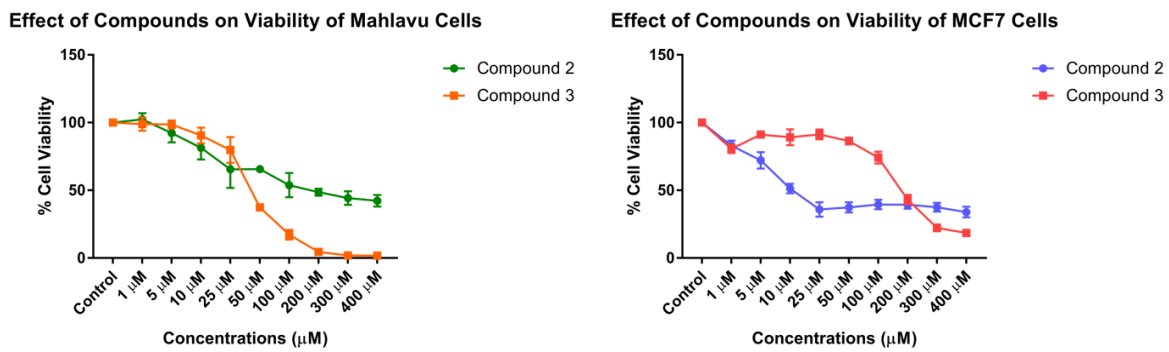


Figure 1. Data on the viability of cells exposed to different concentrations of compounds for 72 hours.

that was two times lower than fluconazole [31]. These findings demonstrate the high potential of Schiff bases to provide the antiproliferative and antifungal medicines required worldwide. In addition, it has been recently reported in the literature that many Phthalimide derivatives have antimicrobial activity. But a limited number of these are studies with phytopathogenic fungi [32]. They have also been used as inhibitors of tumor necrosis factor alpha (TNF-alpha) that plays a vital role in different physiological immune systems. Due to its wide range of medical applications, it also has an important place in medicinal chemistry [33]. For this reason, there is a need for studies on both schiffbases and derivatives and Phthalimides.

Conclusion

A sizable portion of scientific research nowadays is motivated by the global need for novel substances with antifungal and/or anticancer properties. This study, which was carried out to contribute to meeting these requirements, analyzed the effects of two distinct compounds, on the capacity to inhibit cell proliferation in breast cancer and liver cancer cell lines, as well as their antifungal effects on *Candida albicans*. However, it is supported by literature data that metal complexes to be developed by using these compounds as a base can be much more effective and our future studies will be planned by taking this situation into account. On the other hand, the IC_{50} values of the compounds detected against cancer cell lines seem much stronger compared to their antifungal potentials, but it is still believed that metal complexes which will be synthesized in future studies may also be superior in terms of their anticancer activity and we will carry out our upcoming studies in alignment with this conviction.

References

1. E.Ö. Karaca, Yeni Schiff Bazı Bileşiklerinin Sentezi ve Yapılarının Aydınlatılması, J. Polytech., 21 (2018) 245–249.
2. V.M. Barot, S.A. Gandhi, U.H. Patel, M.C. Patel, Synthesis, X-Ray Powder Diffraction Studies and Antimicrobial Activities of Novel Chalcone Derivatives, Chem. Sci. Trans., 4 (2015) 642–648.
3. A. Rani, M. Kumar, R. Khare, H.S. Tuli, Schiff bases as an antimicrobial agent: A review Anti-Neoplastic effects of Garcinol View project Schiff bases as an antimicrobial agent: A review, J. Biol. Chem. Sci., 2 (2015) 62–91.
4. M.M. Abd-Elzaher, A.A. Labib, H.A. Mousa, S.A. Moustafa, M.M. Ali, A.A. El-Rashedy, Synthesis, anticancer activity and molecular docking study of Schiff base complexes containing thiazole moiety, Beni-Suef Univ. J. Basic Appl. Sci., 5 (2016) 85–96.
5. K.N. Jean-Baptiste, K.C. Guillaume, O.Z. Adama, K.A.L. Claude, D.K. Jacques, K.B. Antoine, Z. Nahosse, Synthesis, Characterization and Biological Evaluation of New Series of Schiff Bases Derived from Hexamethylenediamine as Potential Antibacterial and Antifungal Agents, IRA-International J. Appl. Sci. (ISSN 2455-4499), 7 (2017) 69–74.
6. N. Al-Lami, Z. Amer, R.A. Ali, Pryparation, characterization and biological activity of new derivatives of 2-biphenyl-3-aminomethylimidazo(1,2-a)pyrimidine, J. Pharm. Sci. Res., 10 (2018) 3344–3350.
7. A.P. King, H.A. Gellineau, S.N. Macmillan, J.J. Wilson, Physical properties, ligand substitution reactions, and biological activity of Co(iii)-Schiff base complexes, Dalt. Trans., 48 (2019) 5987–6002.
8. I. Bernadette Amali, M.P. Kesavan, V. Vijayakumar, N. Indra Gandhi, J. Rajesh, G. Rajagopal, Structural analysis, antimicrobial and cytotoxic studies on new metal(II) complexes containing N 2 O 2 donor Schiff base ligand, J. Mol. Struct., 1183 (2019) 342–350.
9. B.A. Ansell, Juvenile chronic arthritis, in Drug Treat. Rheum. Dis., 2nd ed., ADIS Health Science Press, (1982): p. 186.
10. B. Shivarama Holla, B. Veerendra, M.K. Shivananda, B. Poojary, Synthesis characterization and anticancer activity studies on some Mannich bases derived from 1,2,4-triazoles, Eur. J. Med. Chem., 38 (2003) 759–767.
11. K. Van Derpoorten, J. Balzarini, E. De Clercq, J.H. Poupaert, Anti-HIV activity of N-1-adamantyl-4-aminophthalimide, Biomed. Pharmacother., 51 (1997) 464–468.
12. H. Miyachi, A. Ogasawara, A. Azuma, Y. Hashimoto, Tumor necrosis factor-alpha production-inhibiting activity of phthalimide analogues on human leukemia THP-1 cells and a structure-activity relationship study, Bioorganic Med. Chem., 5 (1997) 2095–2102.
13. R. Antunes, H. Batista, R.M. Srivastava, G. Thomas, C.C. Araujo, New phthalimide derivatives with potent analgesic activity: II, Bioorganic Med. Chem. Lett., 8 (1998) 3071–3076.
14. H. Miyachi, A. Azuma, T. Kitamoto, K. Hayashi, S. Kato, M. Koga, B. Sato, Y. Hashimoto, Potent novel nonsteroidal androgen antagonists with a phthalimide skeleton, Bioorganic Med. Chem. Lett., 7 (1997) 1483–1488.
15. E.P. Sampaio, G. Kaplan, A. Miranda, J.A.C. Nery, C.P. Miguel, S.M. Viana, E.N. Sarno, The Influence of Thalidomide on the Clinical and Immunologic Manifestation of Erythema Nodosum Leprosum, J. Infect. Dis., 168 (1993) 408–414.
16. T. Eisen, C. Boshoff, I. Mak, F. Sapunar, M.M. Vaughan, L. Pyle, S.R.D. Johnston, R. Ahern, I.E. Smith, M.E. Gore, Continuous low dose Thalidomide: A phase II study in advanced melanoma, renal cell, ovarian and breast cancer, Br. J. Cancer, 82 (2000) 812–817.
17. A. Çukurovali, I. Yilmaz, H. Özmen, M. Ahmedzade, A new mesitylenic cyclobutane substituted schiff base ligand and its Co(II), Cu(II), Ni(II), and Zn(II) complexes, Heteroat. Chem., 12 (2001) 42–46.
18. N. Özdemir, M. Dinçer, I. Yilmaz, A. Çukurovali, 1-Methyl-1-phenyl-3-(phthalimidoacetyl)-cyclobutane, Acta Crystallogr. Sect. E Struct. Reports Online, 60 (2004) 14–16.
19. A. Çukurovali, I. Yilmaz, M. Ahmedzade, Synthesis and characterization of a new cyclobutane-substituted Schiff base ligand and its Co(II), Cu(II) and Ni(II) complexes, Synth. React. Inorg. Met. Chem., 30 (2000) 843–853.

20. M.A. Akhmedov, I.K. Sardarov, I.M. Akhmedov, R.R. Kostikov, A. V. Kisin, N.M. Babaev, ChemInform Abstract: Formation of Substituted Cyclobutanes in the Reaction of 2-(2-Methyl-2-propenyl)-3-chloromethyloxirane with Aromatic Hydrocarbons, ChemInform, 23 (2010) no-no.
21. J. Guinea, S. Recio, P. Escribano, M. Torres-Narbona, T. Peláez, C. Sánchez-Carrillo, M. Rodríguez-Créixems, E. Bouza, Rapid Antifungal Susceptibility Determination for Yeast Isolates by Use of Etest Performed Directly on Blood Samples from Patients with Fungemia, J. Clin. Microbiol., 48 (2010) 2205.
22. B.E. Tefon Öztürk, B. Eroğlu, E. Delik, M. Çiçek, E. Çiçek, Comprehensive Evaluation of Three Important Herbs for Kombucha Fermentation, Food Technol. Biotechnol., 61 (2023).
23. World Health Organization, WHO fungal priority pathogens list to guide research, development and public health action, Licence CC BY-NC-SA 3.0 IGO, 1 (2022) 1–48.
24. F. Bongomin, S. Gago, R.O. Oladele, D.W. Denning, Global and multi-national prevalence of fungal diseases—estimate precision, J. Fungi, 3 (2017).
25. J. Ceramella, D. Iacopetta, A. Catalano, F. Cirillo, R. Lappano, M.S. Sinicropi, A Review on the Antimicrobial Activity of Schiff Bases: Data Collection and Recent Studies, Antibiotics, 11 (2022).
26. L.A. Torre, R.L. Siegel, E.M. Ward, A. Jemal, Global cancer incidence and mortality rates and trends - An update, Cancer Epidemiol. Biomarkers Prev., 25 (2016) 16–27.
27. H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA. Cancer J. Clin., 71 (2021) 209–249.
28. A. Hameed, M. al-Rashida, M. Uroos, S. Abid Ali, K.M. Khan, Schiff bases in medicinal chemistry: a patent review (2010-2015), Expert Opin. Ther. Pat., 27 (2017) 63–79.
29. K.S. Munawar, S.M. Haroon, S.A. Hussain, H. Raza, Schiff Bases: Multipurpose Pharmacophores with Extensive Biological Applications, J. Basic Appl. Sci., 14 (2018) 217–229.
30. L.J. Li, C. Wang, Y. Qiao, X.Y. Yang, X.X. Hua, J.L. Du, Platinum(II) complexes of reduced amino acid ester Schiff bases: Synthesis, characterization, and antitumor activity, Res. Chem. Intermed., 40 (2014) 413–424.
31. T.F.F. Magalhães, C.M. da Silva, L.B.F. dos Santos, D.A. Santos, L.M. Silva, B.B. Fuchs, E. Mylonakis, C.V.B. Martins, M.A. de Resende-Stoianoff, de Fátima, Cinnamyl Schiff bases: synthesis, cytotoxic effects and antifungal activity of clinical interest, Lett. Appl. Microbiol., 71 (2020) 490–497.
32. L. Pan, X. Li, C. Gong, H. Jin, B. Qin, Synthesis of N-substituted phthalimides and their antifungal activity against Alternaria solani and Botrytis cinerea, Microb. Pathog., 95 (2016) 186–192.
33. H. Jelali, L. Mansour, E. Deniau, M. Sauthier, N. Hamdi, An Efficient Synthesis of Phthalimides and Their Biological Activities, Polycycl. Aromat. Compd., 42 (2022) 1806–1813.