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Research Article

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Evaluation Of The Antimicrobial And Cytotoxic Activities Of Some Amino Acid Methyl Esters

Bazi Amino Asit Metil Esterlerinin Antimikrobiyal Ve Sitotoksik Aktivitelerinin Değerlendirilmesi

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ABSTRACT

In this study, the antimicrobial and cytotoxic activities of some amino acid methyl esters (L-methionine methyl ester, L-phenylalanine methyl ester, L-histidine methyl ester, L-lysine methyl ester, L-tryptophan methyl ester, and L-tyrosine methyl ester) were investigated. The antimicrobial effects of amino acid methyl esters were evaluated against Gram-negative and Gram-positive bacteria. The effect of amino acid methyl esters on cell viability was also investigated against cancerous and non-cancerous cell lines using the MTS method. The results showed the substances had low antimicrobial effect on both Gram-negative and Gram-positive bacteria. In addition, it was determined that the studied substances did not have a toxic effect on non-cancerous cells. For this reason, it is thought that the results obtained will contribute to research on new synthesizable compounds based on amino acid esters and studies on drug development.

Key Words

Amino acid esters, antimicrobial activity, antiproliferative activity, cytotoxicity.

ÖZ

Bu çalışmada bazı amino asit metil esterlerinin (L-metionin metil ester, L-fenilalanin metil ester, L-histidin metil ester, L-lisin metil ester, L-triptofan metil ester ve L-tirozin metil ester) antimikrobiyal ve toksik etkileri araştırılmıştır. Amino asit metil esterlerinin antibakteriyel etkileri, Gram-negatif ve Gram-pozitif bakterilere karşı değerlendirilmiştir. Amino asit metil esterlerinin hücre canlılığı üzerindeki etkisi de, MTS yöntemi kullanılarak kanserli ve kanserli olmayan hücre hatlarına karşı araştırılmıştır. Sonuçlar, maddelerin hem Gram negatif hem de Gram pozitif bakteriler üzerinde genel olarak düşük antimikrobiyal etkiye sahip olduğunu göstermiştir. Ayrıca, kanserli olmayan hücrelerde çalışılan maddelerin toksik etkisinin olmadığı belirlenmiştir. Bu nedenle, elde edilen sonuçların amino asit ester temelli yeni sentezlenebilir bileşikler üzerine yapılacak araştırmalara ve ilaç geliştirilmesine yönelik çalışmalara katkı sağlayacağı düşünülmektedir.

Anahtar Kelimeler

Amino asit ester, antimikrobiyal aktivite, antiproliferatif etki, sitotoksisite.

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INTRODUCTION

Organizations such as the US Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have accepted antimicrobial resistance (AMR), especially antibacterial resistance, as a global threat and have issued guidelines in order to take necessary measures in this regard [1].

Antimicrobial resistance by the World Health Organization is defined as a natural phenomenon that occurs when microorganisms become sensitized and no longer respond to previously active antibiotics in the treatment of infections caused by these microorganisms. As a result, the treatment of infections becomes difficult or impossible, and the risk of spread and death of serious infectious diseases increases [2].

This increase in resistance and the increase in infectious diseases that cause so many deaths worldwide have led to the use of modified substances and the design of new compounds [3-5]. In fact, the aim of new searches and the development of analogues of existing antimicrobial agents arises from the need to eliminate their known toxic effects.

When the literature is examined, it is seen that amino acid-based derivatives can be used for these purposes. In particular, some amino acid-based surfactants have been shown to have a low toxicity profile and good antimicrobial activity [6]. For example, it has been reported in the literature that amino acid-based substances derived from arginine and lysine show good antimicrobial activity with high biodegradability and low toxicity profile. Some amino acid-based surfactants are known to interact with the lipid bilayer of cell membranes. However, antimicrobial studies on amino acid-based substances are limited in the literature [7-9].

Therefore, in this study, in the light of this information, antimicrobial and toxicity effects of some amino acid methyl esters derivatives (L-methionine methyl ester, L-phenylalanine methyl ester, L-histidine methyl ester, L-lysine methyl ester, L-tryptophan methyl ester, and L-tyrosine methyl ester) were investigated and results were discussed.

MATERIALS and METHODS

Chemicals

All chemicals, reagents, and media supplements were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany), and Becton Dickinson and Company (Franklin Lakes, NJ, USA). CellTiter 96® AQueous One Solution Cell Proliferation (MTS) Assay was obtained from Promega (Madison, WI, USA). Healthy human embryonic kidney cells (HEK293T) and liver cancer cell lines (PLC/PRF/5 and HEP3B) were sourced from ATCC.

Antimicrobial Assay of Amino Acid Methyl Esters

An investigation of the *in vitro* antimicrobial activity of the amino acid methyl esters was performed using the modified microdilution broth method [10] in Luria-Bertani (LB) medium [1 % (w/v) nutrient broth, 0.5 % (w/v) yeast extract, and 0.8 % (w/v) NaCl] with a twofold dilution of the compounds. The 96-well microplates were used to test Gram-positive *Staphylococcus aureus* ATCC 29213 and Gram-negative *Escherichia coli* ATCC 53323. Using sterile distilled water, the stock solution for the amino acid esters was prepared. To get a final concentration of ranged from 4 mM to 0.00049 mM, serial dilutions were made with the growth medium. A drug-free control groups were also used. The second well to the 12^{th} well in each column of a 96-well microplate was filled with 100 µl of LB medium. After that, 200 µl of the stock solution was added to each of the first wells in each column, and 100 µl of the stock solution was transferred from the first well to the 11^{th} well to prepare serial dilutions. Growth control was conducted in the 12th wells, which were drug-free. To achieve 10^5 cells/ml, 100 µl of the bacterial suspension was inoculated into each well. After 24 hours of incubation at 37°C in a humid environment, the antimicrobial effect was determined as an optical density (OD_{600}) growth reduction compared to the control using the Fluostar Omega Microplate Reader (BMG Labtech, Ortenberg, Germany).

Cytotoxicity Analysis

To determine the effect on cell viability of amino acid esters, tetrazolium compound [3-(4,5dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] method was used by

the manufacturer's instructions. For this purpose, each of the healthy HEK293T cell line and liver cancer cell lines PLC/PRF/5 and HEP3B cells were seeded in 96-well culture dishes (respectively 4x103 and 8x103 cell/well) and were kept confluent for 24 hours. The cells were treated for 72 hours with amino acid esters at different concentrations (prepared at 250 µM, 500 µM, 1 mM, 2 mM, and 4 mM concentrations in water). At the end of the incubation, the medium containing amino acid esters were removed and 20ul of MTS in 100ul of fresh medium was added to the cells. Cells were incubated with MTS at 37°C for 3 hours. After incubation, the medium containing MTS was withdrawn and discarded. Then, 100 ul of fresh medium was added and absorbance values were measured at 490 nm with a Varioskan Flash Multiplex Microplate Reader (Thermo Fisher Scientific, Waltham, MA USA).

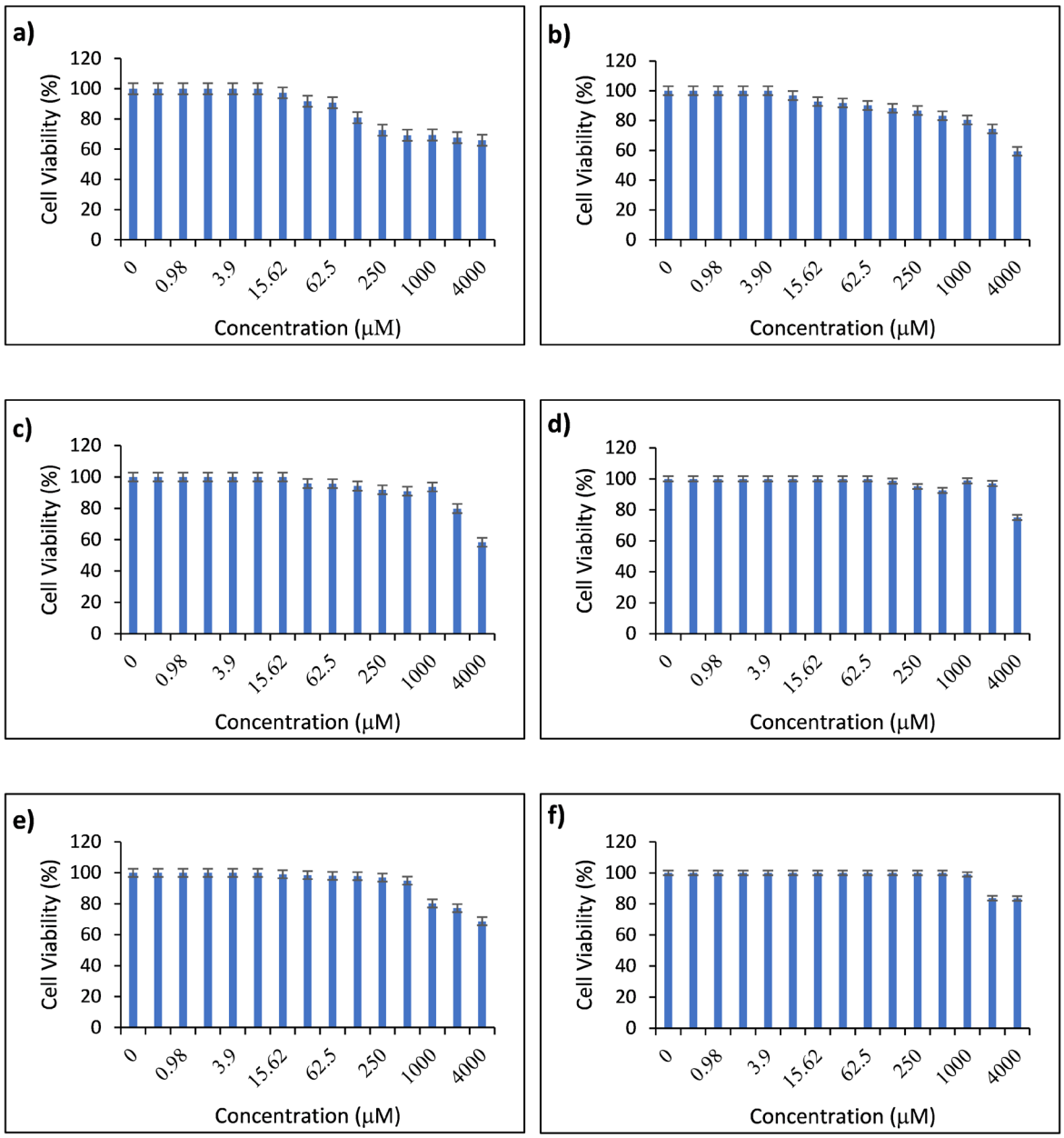

Figure 1. Antimicrobial effect of the indicated methyl ester concentrations (0.49 to 4000 µM) on E. coli after 24 hours of incubation. **a)** L-Methionine Methyl Ester. **b)** L-Phenylalanine Methyl Ester. **c)** L-Histidine Methyl Ester. **d)** L-Lysine Methyl Ester. **e)** L-Tryptophan Methyl Ester. **f)** L-Tyrosine Methyl Ester. The data shown are the most representative of 3 separate experiments. Error bars represent the standard deviations.

RESULTS and DISCUSSION

Antimicrobial assay

To determine and describe the antimicrobial activity of amino acid methyl esters, we studied the growth of two co mMon bacterial pathogens, *E. Coli,* and *S. aureus*, in the presence of various concentrations of methyl esters. The results given in Figs. 1 and 2 show that the methyl esters cause a dose-dependent reduction in growth in both bacteria, although they do not completely inhibit the growth of either bacteria even at the highest concentration tested (4000 µM). At the highest methyl ester concentration tested (4000 µM), L-phenylalanine and L-histidine were found to reduce the growth of *E. coli*, by approximately 41% and 42%, respectively (Fig. 1). In *S. aureus*, the greatest reduction in cell growth was caused by L-histidine and L-lysine methyl esters, 26% and 27%, respectively (Fig. 2).

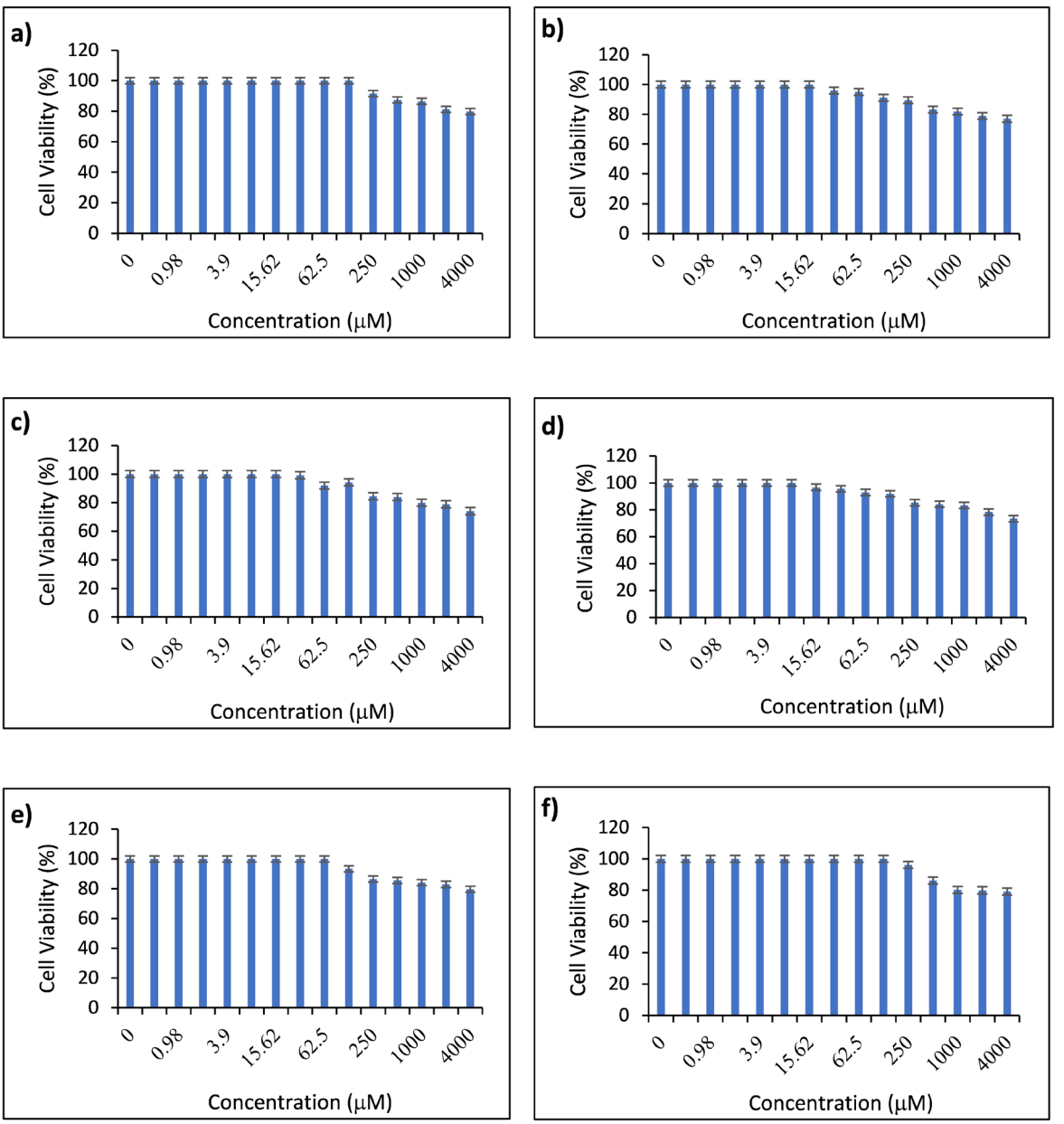

Figure 2. Antimicrobial effect of the indicated methyl ester concentrations (0.49 to 4000 µM) on S. aureus after 24 hours of incubation. **a)** L-Methionine Methyl Ester. **b)** L-Phenylalanine Methyl Ester. **c)** L-Histidine Methyl Ester. **d)** L-Lysine Methyl Ester. **e)** L-Tryptophan Methyl Ester. **f)** L-Tyrosine Methyl Ester. The data shown are the most representative of 3 separate experiments. Error bars represent the standard deviations.

Overall, methyl esters have been found to have an antimicrobial effect on both Gram-negative and Gram-positive bacteria, albeit a mild one. This could be because of the interaction of methyl esters with cell membranes. As a result, increased cell membrane permeability, depolarization, lysis, and cell death may have occurred [11].

Compared to *E. coli*, *S. aureus* showed slightly greater resistance to the amino acid esters. The differences in cell wall composition between Gram-positive and Gram-negative bacteria may have resulted in less interaction of amino acid esters with *S. aureus* [12,13].

Generally, in this study, amino acid methyl esters were found to have an antimicrobial activity, albeit low. Therefore, it is very likely that new antimicrobial compounds with higher antimicrobial activity will be obtained by synthesizing different analogs from their methyl esters. There are several studies in the literature showing that the addition of different side chains to amino acid esters increases their antimicrobial properties [14-16]. Joondan et al [11] synthesized new cationic substrate analogs from L-phenylalanine and L-tyrosine esters and reported that the newly synthesized esters were more effective in Gram-positive bacteria than in Gram-negative bacteria and that the activity increased with increasing chain length. By using the data obtained as a result of this preliminary study with methyl esters, new ester analogs with increased antimicrobial effects will be synthesized in further studies.

Antiproliferative Actions of the Amino Acid Esters

The cytotoxicity test determines whether a product or compound will have a toxic effect on living cells. In this study, the cytotoxic effects of L-methionine methyl ester, L-phenylalanine methyl ester, L-histidine methyl ester, L-lysine methyl ester, L-tryptophan methyl ester, L-tyrosine methyl ester on the non-cancerous embryonic kidney cell line HEK293T and well-differentiated liver cancer cell lines PLC/PRF/5 and HEP3B were analyzed by in vitro MTS assay.

All other methyl esters, except L-tryptophan showed a similar profile in the cell growth plot compared to the untreated negative control. It has been shown that methyl esters other than L-tryptophan do not affect cell growth of the HEK293T healthy cell line and the PLC/PRF/5 and HEP3B liver cancer cell lines. It is noteworthy that it does not significantly affect cell growth, especially at increasing doses. Especially at some concentrations, cell viability was above 100%. However, compared to the untreated negative control, L-tryptophan methyl ester showed no adverse effects on cell growth of healthy cell lines HEK293T and liver cancer cell lines PLC/PRF/5 and HEP3B at low concentrations, whereas both healthy and liver cancer cell lines showed no adverse effects. Cell growth was significantly reduced at 4 mM L-tryptophan concentration. In fact, cell viability of all cells is around 20-30% in 4 mM L-tryptophan methyl ester (Fig. 3)

As emphasized in the results obtained from the analyzes conducted within the scope of the study, all methyl esters except L-tryptophan showed similar effects on the cell viability of both healthy and liver cancer cell lines. It reduced cell viability to around 90% by showing little cytotoxicity at some concentrations. However, it is not possible to say that there is a dose-dependent pattern. Increasing concentrations of the same substance do not appear to have a continuous positive or negative effect on cell viability. Fortunately, the analyzed substances do not show toxic effects on cell growth. The only important point among them is that L-tryptophan is toxic at 4 mM concentration. However, the fact that other low doses of the same substance did not show any toxic effects shows that the toxic effect of the substance can be eliminated. The findings determined that the analyzed methyl ester groups do not show high selective anti-proliferative effects on healthy and cancer cell lines. The support of these compounds to cell viability is probably due to the bioactive property of the methyl group.

Methionine is important for cancer cell growth and metabolism. Studies indicated that methionine restriction inhibits cancer cell growth and can increase the effectiveness of chemotherapeutic agents and liver cancer cells evade the i mMune system through methionine metabolism. However, another study showed that liver cancer cells escape from the i mMune system mediated by methionine metabolism [17,18].

Studies have shown that L-phenylalanine methyl ester (PME) has the ability to increase the growth of human hematopoietic colonies [19].

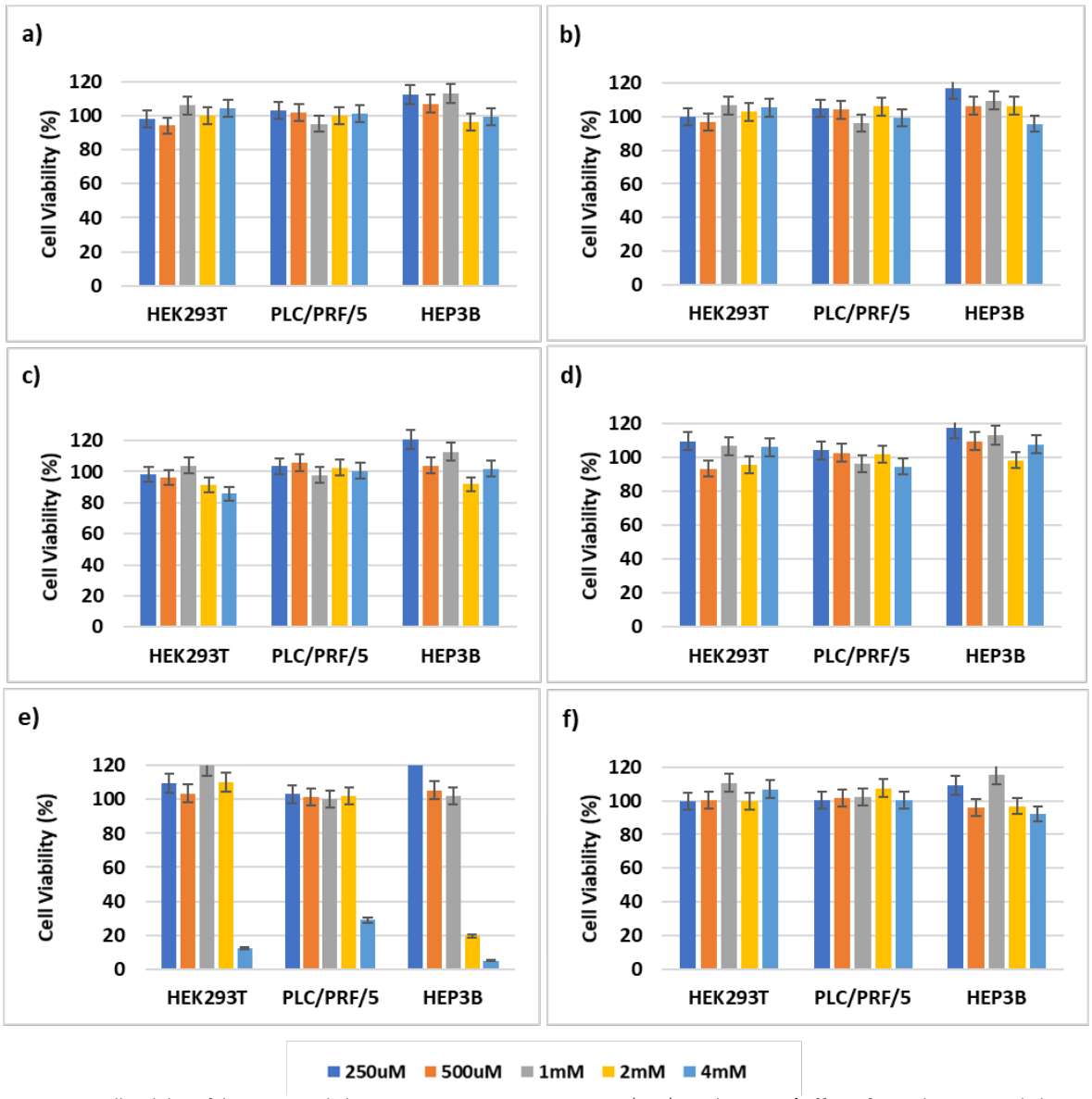

Figure 3. %Cell Viability of the some methyl ester groups against HEK293T, PLC/PRF/5, and HEP3B. **a)** Effect of L-Methionine Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. **b)** Effect of L-Phenylalanine Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. **c)** Effect of L-Histidine Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. **d)** Effect of L-Lysine Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. **e)** Effect of L-Tryptophan Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. **f)** Effect of L-Tyrosine Methyl Ester on cell viability of HEK293T, PLC/PRF/5, and HEP3B cell lines. MTS assay data showing the optical density (OD), after incubation of different substance concentrations at 72 of culture. The data shown are the most representative of 3 separate experiments. Error bars represent the standard deviations. Experimental groups are normalized to negative control. Dark blue bar (250 µM), orange bar (500 µM), grey bar (1 mM), yellow bar (2 mM), light blue bar (4 mM).

In the literature, it is stated that amphotericin B (AmB) used in the treatment of lung cancer has antineoplastic effects and as an alternative, L-Histidine methyl ester hydrochloride of Amphotericin B is used and can be used pharmacologically cause of it has less toxic effects. Preclinical studies have shown that the L-Histidine methyl ester derivative has the same antifungal pharmacological activity, but less toxicity than AmB. From this point of view, cell toxicity evaluation of L-histidine methyl ester derivatives of chemotherapy drugs can be made [20].

The L-lysine methyl ester is an amino acid derivative used for drug delivery of doxorubicin, which is a drug used in many types of cancer in the literature. It is preferred because it is less toxic and biocompatible [21].

L-Tryptophan methyl ester dilates small mesenteric arteries by inhibiting voltage-operated calcium channels in smooth muscle. In Hartnup's disease, studies of bypassing defective neutral amino acid transport by using tryptophan ethyl ester and dilation of small mesenteric arteries by inhibiting voltage-operated calcium channels in smooth muscle with L-tryptophan are noteworthy [22,23]. From this point of view, toxicity studies of the L-tryptophan methyl ester group will give a different perspective to the literature.

Amino acid-based drugs generally have low bioavailability due to low intestinal permeability. Therefore, derivatization of amino acid-based drugs by chemical means, a temporary modification of the physicochemical properties, was the preferred approach for such drugs. In addition to the use of L-Tyrosine carboxylic esters as prodrugs, L-tyrosine derivatives have been suggested in the treatment of Parkinson's, a neurodegenerative disease [24,25].

Promising and versatile nanoscale drug delivery systems include nanocapsules, nanoparticles, nanotubes, nanogels and dendrimers. They can be used to deliver both small molecule drugs and various classes of biomacromolecules such as peptides, proteins, plasmid DNA. In addition, amino acid derivatives can be integrated into these systems due to their biocompatibility [26]. The key features of most of these transporters are to target and increase the cellular uptake of drugs without affecting healthy cells. However, it is important to investigate the effects of methyl ester derivatives on the cell, since amino acids are indispensable building blocks in human metabolism and play a role in the treatment of many diseases, including cancer metabolism and neurodegenerative diseases. Within the scope of this study, toxicity analysis of 6 amino acid derivatives on both healthy and cancer cells was made and an important contribution was made to the literature.

Conclusion

In recent years, human health is under major threat because of the different deadly bacterial, viral and fungal infections worldwide. Excessive use of antibiotics have led to the emergence and spread of antibiotic resistant bacteria. Therefore, there is an urgent need for new antibiotics against resistant bacteria. Scientists have been working on new alternative agents in recent years. In this regard, amino acid, amino acid derivatives and peptides are among the important substance groups. In this study, the data obtained clearly show that some amino acid methyl ester derivatives had no more effective toxic effects on healthy cells HEK293T and PLC/PRF/5 and HEP3B carcinoma. The high cell viability of the amino acid esters studied in this study highlights the ideal biocompatibility of these substances. In the light of the information we presented in our study, it can be said that the results of amino acid esters, which we aimed to determine the antimicrobial activity and toxicity, will guide further in vitro and in vivo research studies on drug development.

References

1. A.K. Nanayakkara, H.W. Boucher, V.G. Fowler Jr, A. Jezek, K. Outterson, D.E. Greenberg, Antibiotic resistance in the patient with cancer: Escalating challenges and paths forward, CA Cancer J. Clin., 71 (2021) 488-504.
2. G. Mancuso, A. Midiri, E. Gerace, C. Biondo, Bacterial antibiotic resistance: The most critical pathogens, Pathogens, 10 (2021) 1310.
3. R.J. Fair, Y. Tor, Antibiotics and bacterial resistance in the 21st century, Perspect. Med. Chem., 6 (2014) 25-64.
4. J. Song, What's new on the antimicrobial horizon?, Int. J. Antimicrob. Agents, 32 (2008) 207-13.
5. S. Chen, L. Li, C. Zhao, J. Zheng, Surface hydration: Principles and applications toward low-fouling/nonfouling biomaterials, Polym. J., 51 (2010) 5283–5293.
6. L. Sanchez, V. Martinez, M.R. Infante, M. Mitjans, M.P. Vinardell, Haemolysis and antihaemolysis induced by amino acid-based surfactants, Toxicol. Lett., 169 (2007) 177-84.
7. R. Sarma, K.S. Alva, K.A. Marx, S.K. Tripathy, J.A. Akkara, Kaplan, D.L., Enzymatic polymer-ization of amphiphillic alkyl tyrosine derivatives from emulsions, Mater. Sci. Eng. C., 4 (1996) 189–92.
8. L. Perez, A. Pinazo, M.T. Garcia, M.C. Moran, M.R. Infante, Monoglyceride surfactants from arginine: synthesis and biological properties, New J. Chem., 28 (2004) 1326-34.
9. Perez, L., Pinazo, A., Tereza, Garcia M., Lozano, M., Manresa, A., Angelet, M., et al. Cationic surfactants from lysine: synthesis, micellisation and biological evaluation, Eur J Med Chem, 44 (2009) 1884-1892.
10. M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity, A review. J. Pharm. Anal., 6 (2016) 71-79.
11. N. Joondan, S. Jhaumeer-Laulloo, P. Caumul, A study of the antibacterial activity of l-Phenylalanine and l-Tyrosineesters in relation to their CMCs and their interactions with1,2-dipalmitoyl-sn-glycerol-3-phosphocholine, DPPC as model membrane, Microbiol. Res., 169 (2014) 675-685.
12. N. Malanovic, K. Lohner, Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides, Biochim. Biophys. Acta - Biomembr., 1858 (2016) 936-946.

13. T.J. Silhavy, D. Kahne, S. Walker, The bacterial cell envelope, Cold Spring Harb. Perspect. Biol., 2 (2010) 414.
14. A. Pinazo, M.A. Manresa,A.M. Marques, M. Bustelo, M.J. Espuny, L. Pérez, Amino acid–based surfactants: New antimicrobial agents, Adv. Colloid Interface Sci., 228 (2016)17-39.
15. A. Hameurlaine, W.A. El-Sayed, A.H. Abdel-Rahman, Amino acid derivatives, IX [1]: synthesis and antimicrobial evaluation of α-amino acid esters bearing a tryptophane side chain, Monats. Chem., 139 (2008) 1507–1511.
16. A. Siebert, M. Wysocka, B. Krawczyk, G. Cholewiński,J. Rachoń, Synthesis and antimicrobial activity of amino acid and peptide derivatives of mycophenolic acid, Eur. J. Med. Chem., 143 (2018) 646-655.
17. D. Wanders, K. Hobson, X. Ji, Methionine restriction and cancer biology, Nutrients, 12 (2020) 684.
18. M.H. Hung, J.S. Lee, C. Ma, et al., Tumor methionine metabolism drives T-cell exhaustion in hepatocellular carcinoma, Nat. Co mMun., 12 (2021) 1455.
19. W.K. Chau, P. Law, Effect of L-Phenylalanine methyl ester on the colony formation of hematopoietic progenitor cells from human bone marrow, Int. J. Cell Cloning, 9 (1991) 211–219.
20. R. Escobar-Resendiz, J. Reyes-Esparza, I. Ortega,Lourdes, L. Rodriguez-Fragoso, Evaluation of cytotoxic effect of l-histidine methyl ester hydrochloride of amphotericin-B (A21) on tumoral cells, FASEB J., 33 (2019) 515.
21. U. Kandekar, R. Pujari, P. Chaudhari, et al, Nanocarriers for breast cancer: Advanced perspective, Hacet. Univ. J. Fac. Pharm., 41 (2021) 179-195.
22. A.J. Jonas, I.J. Butler, Circumvention of defective neutral amino acid transport in Hartnup disease using tryptophan ethyl ester, J. Clin. Invest., 84 (1989) 200-4.
23. A. Jadhav, W. Liang, J. Balsevich, G. Bastin, J. Kroetsch, S. Heximer, P.H. Backx, V. Gopalakrishnan, L-tryptophan ethyl ester dilates small mesenteric arteries by inhibition of voltage-operated calcium channels in smooth muscle, Br. J. Pharmacol., 166 (2012) 232-42.
24. R. Oliyai, V.J. Stella, Prodrugs of peptides and proteins for improved formulation and delivery, Annu. Rev. Pharmacol. Toxicol., 32 (1993) 521–544.
25. P.A.E. Carlsson, H.R. Corrodi, Aktiebolaget Hassle, Treatment of Parkinson's disease, US Patent, (1975) 19730820.
26. K. Sztanke, A. Maziarka, A. Osinka, M. Sztanke, An insight into synthetic Schiff bases revealing antiproliferative activities in vitro, Bioorg. Med. Chem., 21 (1975) 3648-3666.