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Research Article

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Antimicrobial, Antibiofilm and Anticancer Potentials of Glycine and Glycyl-Glycine; an in vitro study

Glisin ve Glisil-Glisinin Antimikrobiyal, Antibiyofilm ve Antikanser Potansiyelleri; in vitro Çalışma

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ABSTRACT

Globally, there is a significant demand for novel agents capable of providing protection against both pathogenic microorganisms and tumor cells. In this study, the antimicrobial, biofilm inhibitory, and anticancer effects of glycine and glycyl-glycine were investigated. The antimicrobial effects were determined using the broth dilution method, while the biofilm inhibitory effects were assessed through the crystal violet binding assay. Cytotoxic effects on HeLa cell and A549 cell line viability were measured using the MTT assay. Our results indicate that, although 100 mg/mL of glycine only inhibited *Staphylococcus epidermidis* W17 among the three tested isolates, 400 mg/mL of glycyl-glycine inhibited both *S. epidermidis* W17 and *Proteus mirabilis* U15 strains. Additionally, sub-MICs (concentrations below the Minimum Inhibition Concentration) of glycine inhibited biofilm formation by more than 70% in all tested clinical isolates and exhibited significantly more biofilm inhibition against *P. mirabilis* U15 and *S. epidermidis* W17 strains ($p<0.05$). A549 and HeLa cell types exhibited a significant reduction in cell viability at concentrations of 0.3 mM and 1 mM compared to the control after treatment with glycine and glycyl-glycine ($p<0.001$). The results obtained in this study will enhance the development of new strategies using amino acids and dipeptide-based synthesizable molecules, contributing to advancements in drug development research.

Key Words

Anticancer activity, cytotoxicity, antimicrobial activity, biofilm inhibition, glycine, glycyl-glycine.

ÖZ

Küresel anlamda, hem patojen mikroorganizmalara karşı koruma sağlayabilen hem de tümör hücrelerine karşı etkili olabilen yeni ajanlara karşı büyük bir talep bulunmaktadır. Bu çalışmada, glisin ve glisil-glisinin antimikrobiyal, biyofilm inhibe edici ve antikanser etkileri incelenmiştir. Antimikrobiyal etkiler, sıvı dilüsyon yöntemi kullanılarak belirlenmiş, biyofilm inhibisyonu etkileri ise kristal viyole tutunma testi ile değerlendirilmiştir. HeLa hücre canlılığındaki sitotoksik etkiler, MTT testi kullanılarak ölçülmüştür. Sonuçlarımız, test edilen üç izolat arasında 100 mg/mL glisinin sadece *S. epidermidis* W17'yi inhibe ettiği, ancak 400 mg/mL glisil-glisinin hem *S. epidermidis* W17 hem de *P. mirabilis* U15 klinik suşlarını inhibe ettiğini göstermektedir. Ayrıca, glisinin Minimum İnhibisyon Konsantrasyonu (MİK)'nun altındaki konsantrasyonlarının (sub-MİK), tüm test edilen klinik izolatlarda biyofilm oluşumunu %70'in üzerinde inhibe etmiş olduğu ve *P. mirabilis* U15 ile *S. epidermidis* W17 suşlarına karşı anlamlı olarak daha fazla biyofilm inhibisyonuna yol açtığı görülmektedir ($p<0,05$). 0,3 mM ve 1 mM glisin ve glisil-glisin konsantrasyonlarının ise, A549 ve HeLa hücre tiplerinde, kontrol grubuna kıyasla anlamlı bir hücre canlılığı azalmasına yol açmış olduğu gözlenmektedir ($p<0,001$). Bu çalışmadan elde edilen sonuçlar, amino asitler ve dipeptid bazlı sentezlenebilir moleküller kullanarak yeni stratejilerin geliştirilmesine ve ilaç geliştirme araştırmalarındaki ilerlemelere katkı sağlayacaktır.

Anahtar Kelimeler

Antikanser aktivite, sitotoksisite, antimikrobiyal aktivite, biyofilm inhibisyonu, glisin, glisil-glisin.

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INTRODUCTION

Antimicrobial resistance, infectious diseases, and cancer pose significant challenges to the healthcare system worldwide and are extremely important problems for both human and animal health. Antimicrobial resistance is defined in the literature as a situation that occurs when microorganisms become resistant and do not respond to antibiotics with proven effectiveness used in the treatment of infections caused by these microorganisms [1]. It is stated in the literature that traditional antimicrobials rapidly lose their effectiveness due to microbial resistance. For this reason, interest in alternative treatment agents to combat drug-resistant microorganisms has recently increased. Additionally, as human cancer remains a leading cause of morbidity and mortality worldwide, there is also an urgent need for new, selective, and more efficient anticancer agents [2]. In these contexts, studies on amino acid-based substances in the literature show promise for the development of sustainable new compounds and therapeutic strategies [3]. Some amino acid-based compounds generally provide the desired activity due to their different biological effects and are also of interest from a pharmacological perspective [4,5].

Glycine is the most basic, small amino acid and is used as an antibacterial agent in foods due to its low toxicity in animals. It also has antibacterial potential and is known to inhibit the synthesis of a peptidoglycan component of the bacterial cell wall [6–9]. Furthermore, glycine concentrations of 1.5 to 6% resulted in 70 to 90% reductions in the growth of *Enterococcus faecalis* [10]. Glycyl-glycine is an amide-linked glycine derivative and a small peptide. Peptides are very effective and often specific ligands. They contain a range of potential donor atoms. They have multiple functions in biochemical processes and such small peptides are also remarkable in terms of their ease of synthesis and the discovery of new drugs. When the literature was examined, it was observed that there were studies on the antimicrobial and anticancer effects of peptides, but the number of studies on small molecules was limited. However, studies with small molecules such as prolyl-glycine, valyl-valine, and leucyl-valine have been reported to contribute to a better interpretation of the causes of biological effects occurring in amino acid-based drug structures in the future [11–15]. Therefore, this study aims to investigate the antimicrobial, biofilm inhibitory, and anticancer effects of glycine and glycyl-glycine. By testing these small molecules, our objective is to contribute to the development of new therapeutic agents and provide a better understanding of the biological effects of amino acid-based compounds.

MATERIALS and METHODS

Microorganisms

In this study, *Candida tropicalis* T26, *Proteus mirabilis* U15, and *Staphlococcus epidermidis* W17, which had been previously isolated and identified [16–18], were used. All strains were inoculated onto Brain Heart Infusion (BHI) Agar Media from stock cultures using the streak plate method and incubated at 37°C for 24 hours to obtain single colonies. The obtained cultures were stored at 4°C and were grown again once a month for future experiments.

Growth conditions and harvesting

Single colonies of microbial strains were initially grown in 10 mL of BHI Broth media at 37°C for 24 hours to obtain precultures. Following this, 1.5 mL of the precultures was inoculated into 30 mL of BHI broth and incubated at 37°C for 24 hours to obtain main cultures. The main cultures were harvested by centrifugation three times at 3220g at 5°C for 10 m (Eppendorf 5810R, with an Eppendorf Swing-bucket rotor A-4-62, Hamburg, Germany). A potassium phosphate buffer (pH 7) was used as the washing buffer. Following this, microbial cells were adjusted to MacFarland 2 turbidity standard and used in the further experiments.

Preparation of Glycine and Glycyl-Glycine

Glycine and glycyl-glycine were purchased from Sigma and 800 mg of them were dissolved in 1 mL sterile PBS buffer solution (pH 7) and vortexed for 2 minutes to obtain stock solutions. Stock solutions were sterilized under UV light for 1 h and further used in the following experiments.

Minimum inhibitory and minimum microbicidal concentrations of Glycine and Glycyl-Glycine

100 µL of each stock solution (800 mg/mL) was added to the wells of 96-well plates, each containing 100 µL of BHI broth. Two-fold serial dilutions were conducted to obtain concentrations covering a range from 0.39 mg/mL to 400 mg/mL. Following this, 15 µL of microbial cells adjusted to the MF 2 turbidity standard in potassium phosphate buffer as described previously were inoculated into the wells. The 96-well plates were then incubated at 37°C for 24 hours. After incubation, growths

were visually observed, and the minimum concentrations at which no growth was observed were determined as the Minimum Inhibitory Concentrations (MICs). Subsequently, 10 µL samples were taken from the 96-well plates and inoculated onto BHI Agar plates to observe growth in media without amino acid/dipeptide inclusion. The minimum concentrations in the wells at which no growth was observed on the BHI media were determined as the Minimum Microbicidal Concentration (MMC). All experiments were conducted in triplicate at a minimum, and median values were presented.

Biofilm inhibitions by Glycine and Glycyl-Glycine

Biofilm inhibitions caused by sub-MIC values were determined by the modified method described by Sahal et al. [19]. Briefly, 0.5 MIC of glycine, glycyl-glycine dipeptide, and Pen-Strep-Amph B solution (control) against tested strains were prepared in 96-well plates. Following this, 15 µL of microbial cells adjusted to the MF 2 turbidity standard in potassium phosphate buffer (as described previously) were inoculated into the wells. The 96-well plates were then incubated at 37°C for 24 hours. After incubation, wells were rinsed with sterile distilled water three times and stained with crystal violet for 30 m. Afterwards, wells were rinsed with sterile distilled water again. Following this, the bound crystal violet which indicates biofilm formation was solubilized with 99.9% ethanol solution. Solubilized bound crystal violet was meaused with a microplate reader spectrophotometer at a wavelength 560 nm (BIO-TEK, µQuant, BIO-TEK Instruments, Inc). The wells without any microbial inoculation were set as a negative control; whereas wells without any ester inclusion were set as a positive control and their biofilm formation was set as 100%. The reduction (%) in biofilm formation relative to the positive control was calculated using the formula below:

% Decrease = [(Absorbance Control at 560 nm) - (Absorbance Treatment at 560 nm)] / (Absorbance Control at 560 nm) x 100%

All experiments were conducted in triplicate, with bacterial cells grown separately, and the mean values of percentage inhibitions were reported.

MTT Assay

The effects of glycyl-glycine and glycine on cell viability were measured with Tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma-Aldrich, Germany) assay based on our previous studies [20–22]. HeLa and A549 cells were used as cancer cell lines (HeLa cells 90061901, ŞAP Enstitüsü, Ankara, Turkey; A459 cells kindly gifted from Dr. Kılıç-Süloğlu lab Hacettepe University). The cells were cultured in DMEM: Ham's F12 (DMEM/F12) medium supplemented with 2 mM L-glutamine, 10% fetal bovine serum, and 100 IU/ml Penicillin-Streptomycin (Biochrom, Germany). For the MTT assay, briefly, each cell line was seeded in 96-well plates and incubated at 37 °C and 5% CO_2 in the air. Cells were treated with glycyl-glycine and glycine in different concentrations (0.003-1mM) while untreated cells were served as controls. The MTT assay was performed using culture media containing MTT after 24 and 48 hours of treatment. The absorbance (OD) values were collected using a UV-visible spectrophotometer (570 nm, EZ Read 400 Microplate Reader, Biochrom, UK). The cells were also visualized using inverted microscopy (IX70 Olympus, Japan). OD values were expressed as the mean±standard deviation of six biological replicates.

Statistical Analysis

Data of MTT assay were compared by an unpaired Student's t-test. Graph Pad Prism (Graph Pad Software, v 6.01, San Diego, CA, USA) was used for statistical analysis, and $p < 0.05$ was accepted as significant. The biofilm inhibitory data were statistically analyzed using IBM SPSS Statistics version 23 (IBM SPSS Statistics Inc., Chicago, IL, USA). To assess the normality of the data distribution, the Shapiro-Wilk test was employed. For normally distributed data, significant differences between different groups were determined using one-way ANOVA, Bonferroni, and Tukey HSD tests. In cases where the data did not exhibit normal distribution, non-parametric tests such as Kruskal-Wallis and Mann-Whitney U were applied. Significance was established for p values < 0.05.

RESULTS and DISCUSSION

Antimicrobial resistance and the uncontrolled spread of abnormal cells, known as cancer, constitute two primary challenges in human healthcare [2,23]. The escalating incidence of antimicrobial resistance and cancer, coupled with the inadequacy of existing medications, has prompted the exploration of natural substances [24]. Over the past century, the discovery of various small-molecule antibiotics, including amino acids and antimicrobial peptides (AMPs) with short amino acid sequences, has revolutionized healthcare and contributed

to the treatment of life-threatening bacterial infections and cancerous tissues [25]. Among these, glycine, utilized as a metabolic product in certain bacteria, is reported to exhibit antimicrobial effects and is employed as a nonspecific antiseptic agent due to its low level of toxicity in animals [26,27]. In addition, the glycyl-glycine dipeptide has been reported to enhance the surface activity of the antibacterial drug domiphen bromide [28]. Nevertheless, studies demonstrating not only the antimicrobial effects but also the antibiofilm and anticancer effects of glycine and glycyl-glycine do not exist in the literature. Therefore, this study investigated the antimicrobial, antibiofilm, and anticancer effects of glycine and glycyl-glycine. The data obtained from these two dipeptides constitute novel contributions to the literature.

Minimum inhibitory and minimum microbicidal concentrations of Glycine and Glycyl-Glycine

In the initial part of this study, we determined the minimum inhibitory concentrations (MIC) and minimum microbicidal concentrations (MMC) of glycine and glycyl-glycine. Our results indicate that, in comparison to glycyl-glycine, glycine exhibits stronger antimicrobial activity against *S. epidermidis* W17, with a MIC of 100 mg/mL, compared to glycyl-glycine, which requires a higher concentration (400 mg/mL) for antimicrobial efficacy (Table 1). In addition, glycyl-glycine at a concentration of 400 mg/mL not only inhibited *S. epidermidis* W17 but also demonstrated an inhibitory effect against *P. mirabilis* U15 (Table 1). Although the MMC caused by Pen-Strep-Ampho B was > 500 mg/mL against *P. mirabilis* U15, it was determined to be 400 mg/mL for glycyl-glycine, indicating its higher bactericidal effect. However, none of the tested agents demonstrated antimicrobial activity against the *C. tropicalis* T26 strain. Among all the tested clinical isolates, *S. epidermidis* W17 showed greater susceptibility, with lower MIC and MMC values, in response to the glycine amino acid and glycyl-glycine (Table 1).

When AMPs are examined in terms of their antimicrobial effect, they are reported to interact with components of bacterial membranes, leading to the disruption of the lipid bilayer and cell death due to their positive (cationic) net charge [29,30]. Apart from this, amino-acid based antimicrobials are reported to primarily target enzymes responsible for the biosynthesis of peptidoglycan (murein) [26], an essential component of the bacterial cell wall [31], and there are no homologs of these enzymes in fungal cells [26]. Therefore, the reason of amino acid-based antibacterials tested in this study are insufficient to inhibit the yeast strain *C. tropicalis* T26 might be due to their mechanism of action being primarily effective against bacterial cell composition. Additionally, the observation that the amino acid-based antibacterials in our study were more effective against *S. epidermidis* may be related to the higher peptidoglycan biosynthesis in gram-positive bacteria.

Glycine, utilized as an antibacterial agent in foods due to its low toxicity in animals, has been reported to inhibit the synthesis of a peptidoglycan component of the bacterial cell wall [27]. Additionally, it has been observed that a medium containing more than 2% and 6% glycine inhibits the growth of *Lactococcus lactis* and *Enterococcus faecalis*, respectively [27]. While some studies report the antimicrobial effects of glycine, there is currently no research demonstrating the antimicrobial effect of the glycyl-glycine. Glycyl-glycine has only been reported to facilitate the aggregation of Domiphen bromide, an antimicrobial drug targeting oral malodor, dental plaque, and gingivitis, in aqueous solutions [28].

Biofilm Inhibitions by Glycine and Glycyl-Glycine

When examining the effect of glycine on biofilm formation, a concentration of 400 mg/mL inhibited the biofilm formation of both *C. tropicalis* T26 and *P. mirabilis* U15 (by more than 70%) (Figure 1). On the other hand, 0.5 MIC (50 mg/mL) of glycine resulted in a 91.33% inhibition of biofilm formation by *S. epidermidis* W17. This

Table 1. MICs and MMCs of glycine and glycyl-glycine against clinical isolates of C. tropicalis T26, P. mirabilis U15 and S. epidermidis W17 strains.

	Glycine		Glycyl-Glycine		Pen-Strep-Ampho B	
	MIC (mg/mL)	MMC (mg/mL)	MIC (mg/mL)	MMC (mg/mL)	MIC (µL/mL)	MMC (µL/mL)
C. tropicalis T26	> 400	> 400	> 400	> 400	500	>500
P. mirabilis U15	> 400	> 400	= 400	= 400	250	>500
S. epidermidis W17	= 100	= 200	= 400	> 400	0.49	0.49

inhibition was significantly higher than the inhibition caused by 0.5 MIC of Pen-Strep-Amph B against *S. epidermidis* W17 (Figure 1, Table 4). In terms of the biofilm inhibitory effect of glycyl-glycine, the highest effect observed at 400 mg/mL (Sub-MIC), caused 82.05% biofilm inhibition against *C. tropicalis* T26. However, 0.5 MIC (200 mg/mL) of glycyl-glycine resulted in 47.52% and 52.87% inhibition of biofilm formation of *P. mirabilis* U15 and *S. epidermidis* W17 strains, respectively (Figure 1). Our results indicate that, compared to glycyl-glycine, glycine exhibited significantly more biofilm inhibition against *P. mirabilis* U15 and *S. epidermidis* W17 strains (Figure 1, Tables 3 and 4). Moreover, although neither of the tested agents showed antimicrobial activity against the *C. tropicalis* T26 strain, both demonstrated over 78% biofilm inhibition against *C. tropicalis* T26 (Figure 1). One of the studies conducted on the effects of amino acids (arginine, valine, leucine, glycine, lysine, phenylalanine, threonine, and proline) on the biofilm formation of the *Escherichia coli* BL21 strain reported that glycine inhibited the biofilm formation of *E. coli* BL21 [32]. Glycyl-glycine, reported for its potential use as a drug carrier [33], has not been examined for its antimicrobial and biofilm inhibitory potentials alone until now. However, its silver complexes have been reported to display antimicrobial and medium biofilm inhibitory effects against *Staphylococcus aureus* strain [25].

AMPs are generally divided into groups based on their ability to inhibit, reduce, or eradicate biofilms [34]. Glycyl-glycine is one of the water-soluble dipeptides. Therefore, its hydrophilic nature can potentially impact yeast biofilm formation by interfering with adhesion, disrupting the biofilm matrix, and affecting cell-cell interactions more than it affects bacterial biofilm formation. This explains why, in our study, the inhibition of biofilm formation of *C. tropicalis* T26 caused by glycyl-glycine was greater compared to bacterial species. Although it does not display antifungal effects against *C. tropicalis* T26 in this study, it inhibits the biofilm formation of it. Therefore, it might be selected as a vehicle in biomedical applications to prevent *Candida*-associa-

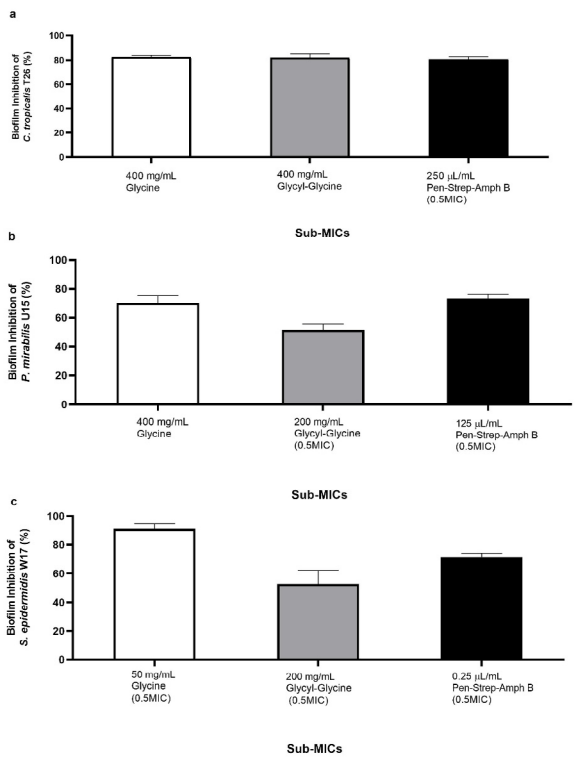

Figure 1. Biofilm inhibitory effect (%) of a. Sub-MIC of glycine (400 mg/mL) and glycyl-glycine (400 mg/mL) against *C. tropicalis* T26; b. Sub-MIC of glycine (400 mg/mL) and glycyl-glycine (200 mg/mL) against *P. mirabilis* U15; c. 0.5MIC glycine (50 mg/mL) and glycyl-glycine (200 mg/mL) against *S. epidermidis* W17. All data were calculated relative to the control; the biofilms formed in wells without the inclusion of any agent. Error bars indicate standard deviations over at least three independent experiments.

Table 2. Descriptive statistics and analysis results for biofilm inhibitory effect of glycine and glycyl-glycine against *C. tropicalis* T26.

Multiple Comparisons

	(I) Agents	(J) Agents	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Glycine	Glycyl-Glycine	.58333	1.88962	.949	-4.9817	6.1484
		Pen-Strep-Amph B	1.97917	1.76758	.533	-3.2265	7.1848
	Glycyl-Glycine	Glycine	-.58333	1.88962	.949	-6.1484	4.9817
		Pen-Strep-Amph B	1.39583	1.76758	.721	-3.8098	6.6015
	Pen-Strep-Amph B	Glycine	-1.97917	1.76758	.533	-7.1848	3.2265
		Glycyl-Glycine	-1.39583	1.76758	.721	-6.6015	3.8098
Bonferroni	Glycine	Glycyl-Glycine	.58333	1.88962	1.000	-5.3266	6.4932
		Pen-Strep-Amph B	1.97917	1.76758	.899	-3.5490	7.5074
	Glycyl-Glycine	Glycine	-.58333	1.88962	1.000	-6.4932	5.3266
		Pen-Strep-Amph B	1.39583	1.76758	1.000	-4.1324	6.9240
	Pen-Strep-Amph B	Glycine	-1.97917	1.76758	.899	-7.5074	3.5490
		Glycyl-Glycine	-1.39583	1.76758	1.000	-6.9240	4.1324

Dependent Variable: Biofilm Inhibition (%)

Table 3. Descriptive statistics and analysis results for biofilm inhibitory effect of glycine and glycyl-glycine against *P. mirabilis* U15.

Multiple Comparisons

	(I) Agents	(J) Agents	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Glycine	Glycyl-Glycine	18.27417*	3.30855	.001	8.8202	27.7282
		Pen-Strep-Amph B	-3.53750	3.06312	.510	-12.2902	5.2152
	Glycyl-Glycine	Glycine	-18.27417*	3.30855	.001	-27.7282	-8.8202
		Pen-Strep-Amph B	-21.81167*	3.30855	.000	-31.2657	-12.3577
	Pen-Strep-Amph B	Glycine	3.53750	3.06312	.510	-5.2152	12.2902
		Glycyl-Glycine	21.81167*	3.30855	.000	12.3577	31.2657
Bonferroni	Glycine	Glycyl-Glycine	18.27417*	3.30855	.002	8.2964	28.2520
		Pen-Strep-Amph B	-3.53750	3.06312	.844	-12.7751	5.7001
	Glycyl-Glycine	Glycine	-18.27417*	3.30855	.002	-28.2520	-8.2964
		Pen-Strep-Amph B	-21.81167*	3.30855	.001	-31.7895	-11.8339
	Pen-Strep-Amph B	Glycine	3.53750	3.06312	.844	-5.7001	12.7751
		Glycyl-Glycine	21.81167*	3.30855	.001	11.8339	31.7895

*. The mean difference is significant at the 0.05 level..
Dependent Variable: Biofilm Inhibition (%).

Table 4. Descriptive statistics and analysis results for biofilm inhibitory effect of glycine and glycyl-glycine against *S. epidermidis* W17.

Multiple Comparisons

	(I) Agents	(J) Agents	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Glycine	Glycyl-Glycine	38.71583*	5.22599	.000	23.3250	54.1067
		Pen-Strep-Amph B	19.76000*	5.58682	.023	3.3065	36.2135
	Glycyl-Glycine	Glycine	-38.71583*	5.22599	.000	-54.1067	-23.3250
		Pen-Strep-Amph B	-18.95583*	5.22599	.020	-34.3467	-3.5650
	Pen-Strep-Amph B	Glycine	-19.76000*	5.58682	.023	-36.2135	-3.3065
		Glycyl-Glycine	18.95583*	5.22599	.020	3.5650	34.3467
Bonferroni	Glycine	Glycyl-Glycine	38.71583*	5.22599	.000	22.3713	55.0604
		Pen-Strep-Amph B	19.76000*	5.58682	.029	2.2869	37.2331
	Glycyl-Glycine	Glycine	-38.71583*	5.22599	.000	-55.0604	-22.3713
		Pen-Strep-Amph B	-18.95583*	5.22599	.025	-35.3004	-2.6113
	Pen-Strep-Amph B	Glycine	-19.76000*	5.58682	.029	-37.2331	-2.2869
		Glycyl-Glycine	18.95583*	5.22599	.025	2.6113	35.3004

*. The mean difference is significant at the 0.05 level.

Dependent Variable: Antibiofilm

ted biofilm infections [30].

Cytotoxicity effects of Glycine and Glycyl-Glycine

The cytotoxic effects of glycine and glycyl-glycine on A549 and HeLa cell viability at 24 and 48 hours were lower than those of the control group at higher concentrations. The most effective incubation time was 48 hours for both cell types (Figure 2). After treatment with glycine and glycyl-glycine, both cell types (A549 and HeLa) exhibited a significant reduction in cell viability at concentrations of 0.3 mM and 1mM compared to the control with a *p*-value of < 0.0001 (Figure 2). When comparing the viability results of glycine and glycyl-glycine at higher concentrations, both peptides showed similar cytotoxicity with a *p*-value of > 0.05. Our results indicate that glycine and its dipeptide form caused a similar decrease in cell viability in both A549 and HeLa cells.

The MTT is a well-known method for measuring the cytotoxicity of materials [35]. It is widely used for assessing drug cytotoxicity, the biocompatibility of materials, and in cancer research [22,36]. In our study, we selected cancer cells from two different origins: adenocarcinomic human alveolar basal epithelial cells (A549 cells) and a cervical cancer cell line (HeLa cell). Both cell lines are aggressive cancer cells commonly used for drug research [37,38]. Qi et. al. showed that Cu (II) dipeptide complexes suppressed cancer cell lines (A549, HeLa, PC-3) at concentrations ranging from 17-101µM and suggested that these complexes induce apoptosis in HeLa cells through mitochondrial dysfunction [39]. Additionally, researches have shown that cyclic dipeptides possess anticancer activity on various cancer cell lines at concentrations ranging from micromolar to millimolar levels [40]. Parallel to these studies our results indi-

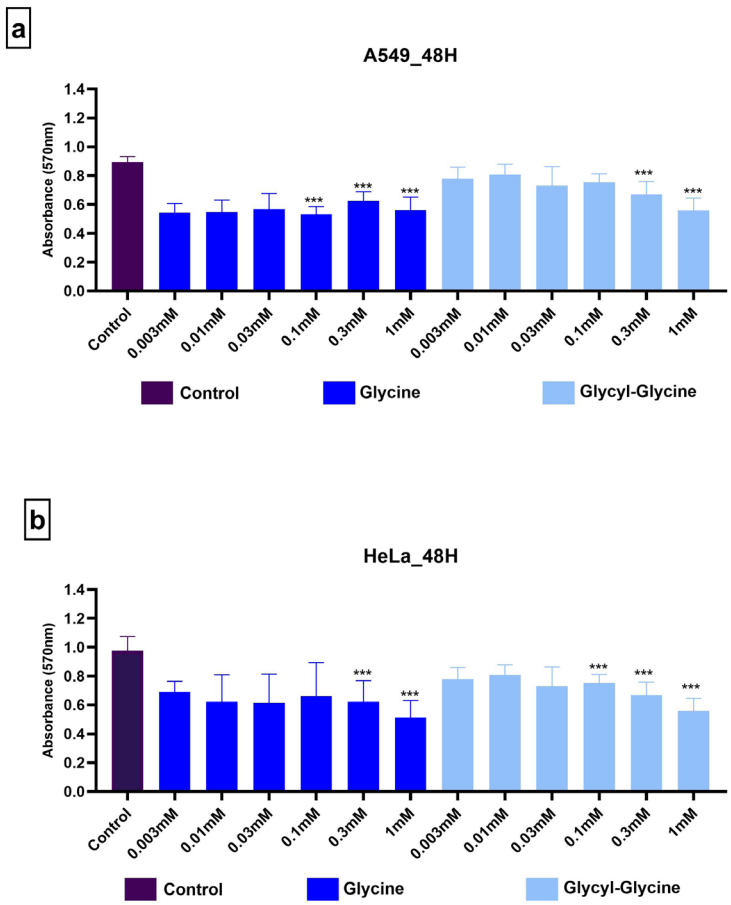

Figure 2. MTT results after 48 hours of incubation with glycine and glycyl-glycine. Absorbance results showed a dose-dependent viability decrease for both cancer cell lines. The observed difference between the higher concentrations and the control group is considered statistically significant with a high level of confidence. (** p<0.0001). Student t-test was used to compare the groups, and GraphPad Prism was used for analysis (n=6).

cate that glycine and its dipeptide form suppress A549 and HeLa cell viability.

In conclusion, our results indicate that both glycine and glycyl-glycine display antimicrobial effects against *S. epidermidis* W17 and exert cytotoxic effects on A549 and HeLa cell viability. Moreover, both molecules demonstrate biofilm inhibition across all tested clinical isolates, with glycine showing a stronger effect. These findings emphasize the antimicrobial, biofilm inhibitory, and anticancer potentials of glycine and glycyl-glycine. Therefore, this study provides valuable data for future investigations in the development of peptide-based synthesizable molecules and drugs, offering useful insights into their biological activities. The comprehensive testing of multiple clinical isolates belonging to different microbial groups, which provides results on the antimicrobial and biofilm inhibitory effects of glycine and glycyl-glycine, is a strength of this study. The observed strong biofilm inhibition by glycine underscores its potential in combating biofilm-associated infections. Additionally, the cytotoxic effects demonstrated against A549 and HeLa cells suggest promising applications in anticancer therapy. However, further research is needed to validate these findings in more clinically relevant settings before translating these peptides into therapeutic applications.

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