Original Article

The antibacterial effects of the new derivatives of Thiazole, Imidazole and Tetrahydropyridine against Proteus vulgaris: An in vitro study

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Abstract

Introduction:
Proteus vulgaris is a major opportunistic hospital pathogen whose antibiotic resistance has recently become a challenging topic for researchers exploring the effect of new antibacterial compounds on this bacteria. Thiazole, Imidazole, and Tetrahydropyridine derivatives are new antibacterial compounds with confirmed inhibitory effects on many pathogenic bacteria. The present study examines the antibacterial effects of 15 new derivatives of Thiazole, Imidazole and Tetrahydropyridine on Proteus vulgaris.

Materials and Methods:
Derivatives of Thiazole and Imidazole were first synthesized and prepared as DMSO solutions. To assess the antibacterial effects of the derivatives, the zone diameter of growth inhibition was measured using the disc diffusion method and the Minimum Inhibitory Concentration (MIC) was determined through a 96-well plate dilution.

Results:
The results obtained showed that the 9a-g derivatives of Imidazole and Tetrahydropyridine and the 6a-c, 10a and 10c derivatives of Thiazole had no inhibitory effects on Proteus vulgaris. The 6c, 9,and 10b derivatives of Thiazole had a zone diameter of growth inhibition of 1±0.2, 10.2±0.1 and 4.3±0.1 mm and a MIC of 128, 512 and 1024 µg/ml in Proteus vulgaris and were therefore found to have inhibitory effects against this pathogen.

Conclusion:
The present study confirms the antibacterial effects of some new derivatives of Thiazole (6d, 9 and 10b) on standard strains of Proteus vulgaris. Further studies are required for assessing the effects of these compounds on treatment-resistant strains of Proteus vulgaris.

Keywords: Antibacterial, Thiazoles, Imidazoles, P. Vulgaris

Introduction

Nosocomial infections are a serious problem in healthcare facilities in most developing countries and even developed countries, increase the mortality rate and medical costs, and threaten the public health (1). Proteus vulgaris is an opportunistic gram-negative pathogen of nosocomial infections from the Enterobacteriaceae family. These bacteria are normal flora of the human digestive system, colonized in the skin and oral mucosa and transmitted through medical

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equipment such as catheters and examination gloves. Urinary tract infections and kidney stone formation, particularly in immunocompromised patients, are of the most prevalent complications caused by this pathogen that infects not only humans but also animals such as cats and dogs (2 & 3). The antibiotic resistance to this bacterium has increased rapidly in recent years, and has resulted in higher medical costs, higher risk of deaths, and has become a threat to the public health (4). According to researchers, one of the main solutions for dealing with these treatment-resistant pathogens is to identify new antibacterial substances which can be used against resistant strains of proteus vulgaris (5).

Thiazoles play an important role in biologically active substances, for instance, thiazolium ring exists in vitamin B1, a major carboxylase coenzyme. Some thiazole derivatives are used as drugs for the treatment of cancer, blood lipids, blood pressure, and AIDS (6). Thiazoles have also shown in-vitro antioxidant and anti-inflammatory and inhibitory effects on parasites, such as anopheles or trypanosoma, and fungi, such as candida albicans (7 & 11). Researchers have proved the power of thiazole derivatives in the inhibition of bacterial pathogens, such as staphylococcus aureus, escherichia coli, staphylococcus epidermidis, streptococcus pyogenes, pseudomonas fluorescens, and streptococcus faecalis (12).

Imidazole derivatives with their ability to inhibit tumor cells, leishmania, aspergillus, and fusarium have received the researchers' attention in recent years (13-15). Studies have shown antibacterial effects of imidazole derivatives on pathogens, such as enterococcus faecalis, escherichia coli, and staphylococcus aureus (16).

Recent studies show the effect of tetrahydropyridine derivatives on the treatment of tuberculosis, Parkinson's, and diabetes and the inhibition of aspergillus niger and candida albicans (17-19). The antibacterial effect of tetrahydropyridine derivatives on pathogens, such as vancomycin-resistant enterococcus faecalis and methicillin-resistant staphylococcus aureus, has been proved in-vitro (20).

Derivatives of thiazole, imidazole, and tetrahydropyridine are new antibacterial substances whose power and broad spectrum in the inhibition of various pathogenic bacteria have made researchers prioritize the study of antibacterial effects of these derivatives after synthesis. The spread of treatment-resistant strains of proteus vulgaris as a nosocomial infection led to the examination of the antibacterial effects of eight thiazole and four tetrahydropyridine derivatives, which have all been recently synthesized, and their chemical structure was confirmed through single-crystal X-ray diffraction, H NMR[1], C NMR[13], IR, elemental analysis, and mass spectrometry in Iran by Beyzaei et al., on the standard strain of proteus vulgaris (6, 21, & 22).

Materials and Methods

Synthesis and preparation of derivatives

The 6a-d derivatives were synthesized through a 3-step process and then were prepared in solutions using DMSO solvent at the concentration of 9011 µg/ml (6).

Thiazole 9 and 10a-c derivatives were synthesized through a 3-step process and then were prepared in solutions using DMSO solvent at the concentration of 9011 µg/ml (21).

Imidazole and tetrahydropyridine 9a-g derivatives were synthesized through a single step process and then were prepared in solutions using DMSO solvent at the concentration of 9011 µg/ml (22).

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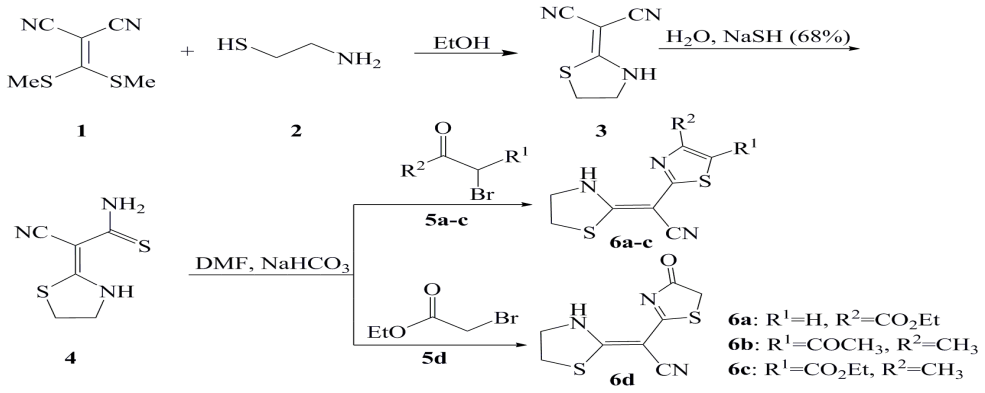

Figure 1: Synthesis steps of thiazole 6a-d derivatives

6a: Ethyl 2- ((E)-cyano (thiazolidin-2-ylidene)methyl)thiazole-4-carboxylate
6b: (E) -2- (5- acetate-4-methyl thiazole-2-yl) -2- thiazolidin-2-ylidene) acetonitrile
6c: Ethyl 2- ((E)-cyano(thiazolidin-2-ylidene)methyl)-4-methyl thiazole-5-carboxylate
6d: (2E)-2-(4, 5-dihydro-4-oxothiazole-2-yl)-2-(thiazolidin-2-ylidene) acetonitrile

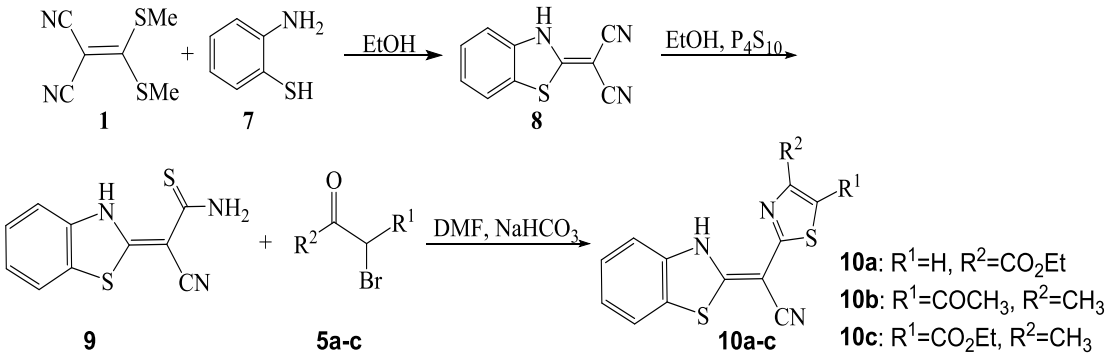

Figure 2: Synthesis steps of thiazole 9 and 10a-c derivatives

9: (E)-2-(benzo(d)thiazole-2(3H)- ylidene)-2- cyano ethan thioamide
10a: Ethyl 2- ((E)-benzo(d) thiazole-2(3H)-ylidene)(cyano)methyl)thiazole-2-carboxylate
10b: (E) -2- (5- acetate-4-methyl thiazole-2-yl) -2- (benzo(d) thiazole-2(3H)-ylidene) acetonitrile
10c: Ethyl 2- ((E)- benzo(d) thiazole-2(3H)-ylidene(cyano)methyl) -4-methyl thiazole-5-carboxylate

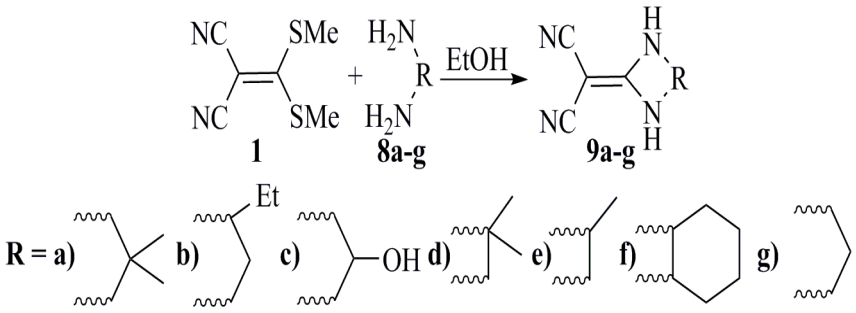

Figure 3: Synthesis steps of imidazole and tetrahydropyridine 9a-g derivatives

9a: 2-(5, 5-dimethyl tetrahydropyridine-2(1H)- ylidene) malononitrile
9b: 2-(4-ethyl tetrahydropyridine-2-(1H)- ylidene) malononitrile
9c: 2-(5-hydroxy tetrahydropyridine-2(1H)- ylidene) malononitrile
9d: 2-(4, 4-dimethyl imidazolidine-2- ylidene) malononitrile
9e: 2-(4-methyl imidazolidine-2- ylidene) malononitrile
9f: 2-(octahydro-2H-benzo(d)imidazole-2- ylidene) malononitrile
9g: 2-(tetrahydropyridine-2-(1H) - ylidene) malononitrile

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Preparation of bacterial suspensions

To examine the antibacterial effects of the above-mentioned derivatives, the standard strain of proteus vulgaris (PTCC 1079) was purchased from the bacterium collection of the Iranian Research Organization for Science and Technology (IROST). Then, the bacteria were cultured in Mueller-Hinton agar medium at 37 °C for 24 hours. Under sterile conditions in Mueller-Hinton broth medium, the 1×10^6 CFU/ml concentration of the bacteria was eventually obtained using a spectrophotometer to be used as a reservoir (23).

Determination of minimum inhibitory concentration (MIC)

MIC was assessed in a sterile 96-well plate using broth microdilution method based on CLSI standard. Firstly, 100 µl of the Mueller-Hinton broth medium (Merck, Germany) was added to each well. Then, 100 µl of thiazole, imidazole, or tetrahydropyridine derivatives (100 µl of tetracycline and gentamicin antibiotics manufactured by Sigma Co. in control groups) was added to the first well. Once the substances were mixed, 100 µl of this mixture was added to the second well, and dilution was performed in other wells likewise. At the end, 10 µl of the bacterial suspension was added to each well. As a negative control, 100 µl of the Mueller-Hinton broth medium, 100 µl of DMSO, and 100 µl of bacterial suspension were added to the last well of each row. The results were read after 24 hours of incubation at 37 °C. The transparency of the well was an indicator of the absence of bacterial growth, and its opacity was an indicator of the bacterial growth. The well with a minimum concentration of derivatives or antibiotics with no opacity was reported as the MIC (23).

Determining the diameter of the growth inhibition zone

A small volume of bacterial suspension of the reservoir was firstly transferred into the plates containing Mueller-Hinton agar medium using a swab, and the bacteria were cultured on the surface of the plates. Sterile blank disks were placed on the surface of agar medium at appropriate intervals. Then, 15 µl of the MIC obtained for the derivatives and antibiotics (15 µl of DMSO in the negative control) was poured on the medium. After 24 hours of incubation, the diameter of the growth inhibition zone was measured using a caliper and the results were shown as mean ± standard deviation values (23).

Data Analysis

The MIC and diameter of the growth inhibition zone for each derivative and antibiotic were measured three times. Mean and standard deviation of the data were determined using SPSS 22 software.

Results

The analysis of the antibacterial effects of the new derivatives of thiazole, imidazole, and tetrahydropyridine on proteus vulgaris showed that imidazole and tetrahydropyridine 9a-g derivatives and thiazole 6a-c, 10a derivatives, and 10c lacked an inhibitory effect on proteus vulgaris. The only inhibitory effect on proteus vulgaris was related to three thiazole derivatives, 6d, 9, and 10b with the diameter of the growth inhibition zone of 22.1± 0.2 mm, 10.2 ± 0.1 mm, and 4.3 ± 0.1 mm and MIC of 128 µg/ml, 512 µg/ml, and 1024 µg/ml. Based on the data analyses shown in Tables 1 and 2 and also Figures 1 and 2, thiazole 6d derivative showed a maximum inhibitory effect on this pathogen compared with other tested derivatives. The diameter of the growth inhibition zone of this derivative was even higher than that of gentamicin and tetracycline although it was not repeated in the MIC test, and its MIC was higher than that of antibiotics. The data also showed the higher inhibitory effect of gentamicin than that of tetracycline in both tests.

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Table 1: Mean diameter of the growth inhibition zone (mm) of 15 derivatives of thiazole, imidazole, and tetrahydropyridine and 2 antibiotics on proteus vulgaris

Derivatives / Antibiotics	Mean diameter of the growth inhibition zone (mm)
6a	-
6b	-
6c	-
6d	22.1±0.2
9	10.2±0.1
10a	-
10b	4.3±0.1
10c	-
9a	-
9b	-
9c	-
9d	-
9e	-
9f	-
9g	-
DMSO	-
Gentamicin	19.5±0.2
Tetracycline	16.1±0.3

No inhibitory effect was observed at maximum concentration

Table 2: MIC (µg/ml) of 15 derivatives of thiazole, imidazole, and tetrahydropyridine and 2 antibiotics on proteus vulgaris

Derivatives / Antibiotics	MIC (µg/ml)
6a	-
6b	-
6c	-
6d	128
9	512
10a	-
10b	1024
10c	-
9a	-
9b	-
9c	-
9d	-
9e	-
9f	-
9g	-
DMSO	-
Gentamicin	1
Tetracycline	8

No inhibitory effect was observed at maximum concentration

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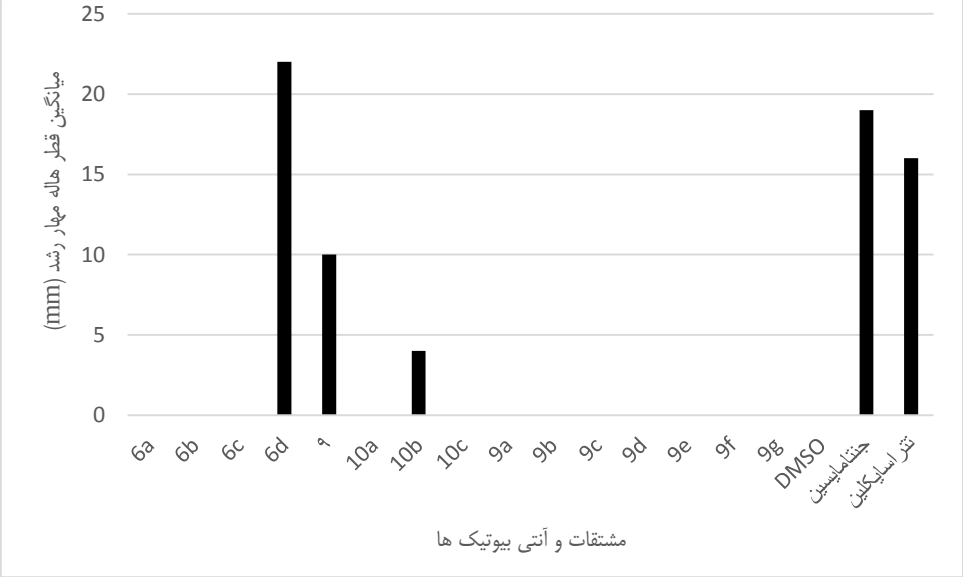

Figure 1: Comparison of the derivatives and antibiotics in terms of their growth inhibition zone

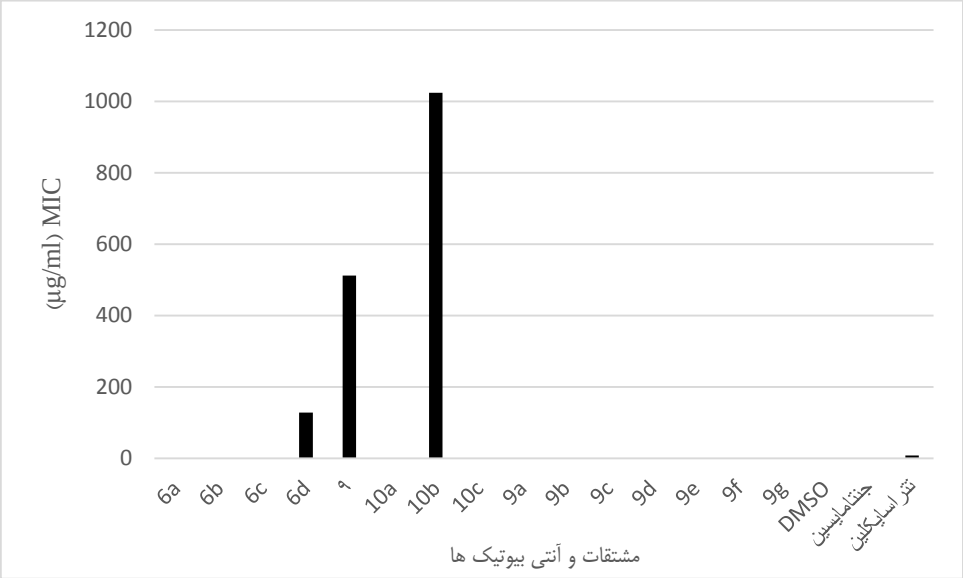

Figure 2: Comparison of the derivatives and antibiotics in terms of their MIC

Discussion

The four derivatives of tetrahydropyridine in this study lacked an inhibitory effect on proteus vulgaris. Prachayasittikul et al.'s study on the antibacterial effect of tetrahydropyridine derivatives on a large number of pathogenic bacteria also showed that only one derivative with MIC of 128 µg/ml and 256 µg/ml of the studied bacteria, including escherichia coli, aeromonas hydrophila, pseudomonas aeruginosa, shigella dysenteriae, salmonella typhi, moraxella catarrhalis, vibrio cholerae, staphylococcus aureus, staphylococcus epidermidis, staphylococcus pyogenes, and bacillus subtilis, had an inhibitory effect respectively on moraxella catarrhalis and staphylococcus pyogenes. Their study showed a limited range of the effects of all tetrahydropyridine derivatives on various bacteria (24).

The three imidazole derivatives in this study lacked any inhibitory effect on proteus vulgaris. However, some

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imidazole derivatives could inhibit gram-negative bacteria, such as pseudomonas and Escherichia coli, probably due to the presence of some substances, such as chlorine, in their structure (25). A reason for the lack of inhibitory effects in 3a-b derivative is the presence of methyl nitroimidazole whose effect in the inhibition of the bacteria from Enterobacteriaceae family, especially Proteus vulgaris and Proteus mirabilis has been proved in experiments. The above derivative causes injuries and death of the bacterium by producing free radicals (26), a feature non-existent in the imidazole derivatives examined in this study.

The results of this study showed that only 3 out of 8 derivatives of thiazole had an inhibitory effect on proteus vulgaris, and the analysis of the structure of these derivatives can clarify this difference. The 6d derivative, which had a maximum inhibitory effect with MIC of 128 µg/ml compared to other derivatives will be studied first. This derivative has two effective structures besides having its thiazole ring, the thiazolidin ring and an oxygen bond to the thiazole ring. Thiazolidin derivatives are of the most recent antibacterial substances that have been reported to have inhibitory effects on many gram-negative bacteria from Enterobacteriaceae family, such as proteus mirabilis, salmonella typhi, escherichia coli, and shigella flexneri (27). In effect, 6a-c derivatives have thiazole and thiazolidin rings and structurally differ from the 6e derivative because of the presence of oxygen bond with the thiazole ring and the formation of the oxo-thiazole compounds whose inhibitory effect on gram-negative bacteria from Enterobacteriaceae family, such as Escherichia coli, has been proved in experiments (28). The derivative 9 among other derivatives with MIC 512 µg/ml had the highest inhibitory effect on proteus vulgaris after the derivative 6d. This derivative has one thiazole ring, but 10a-c derivative has two thiazole rings. Derivative 9 has an important bond with the thiazole ring, thioamide's bond (-C=S (NH_2)) with the thiazole ring, which is likely the cause of the inhibitory effect on Proteus vulgaris. It should be noted that the thioamide functional group is present in the structure of many antibacterial drugs such as prothionamide which is used to inhibit the mycobacterium (29).

Studies conducted in recent years have shown that thiazole derivatives inhibit the bacterial growth through the inhibition of the synthesis of vital enzymes. The enzymes ecKASIII or FabH, which are necessary for the synthesis of fatty acids in both gram-positive and gram-negative bacteria, and DANgyras enzyme, which is essential for DNA replication, are enzymes whose synthesis is blocked in the presence of thiazole derivatives. Considering the fact that the antibiotics of Quinolone family inhibit the A subunit of DANgyras enzyme, and thiazole derivatives inhibit its B subunit, the possibility of the inhibition of Quinolone antibiotics-resistant bacteria by thiazole derivatives has increased (30 & 31).

The antibacterial effect of thiazole derivatives with pyridine binding on proteus vulgaris was examined in a study by measuring the growth diameter inhibition zone, and the diameter of 12-20 mm was recorded. The values of the diameter are very similar to those obtained for the thiazole derivatives 6d and 9 in the present study and show the importance of a side bond with the thiazole ring (32). The MIC was measured as 4-16 µg/ml for thiazole derivatives against proteus vulgaris, and the major difference between the inhibitory effects of these derivatives and those examined in the present study is likely the double effect of groups binding with the thiazole ring, such as chlorine and fluorine (33).

The comparison of the antibacterial effect of thiazole derivatives, especially 6d, on proteus vulgaris in vitro with inhibitory effects of substances like the alcoholic extract of pistachio hull, urotropin (from

[DOI: 10.29252/jmj.13.4.47]

Methenamine family used to disinfect the urinary tract), alcoholic and aqueous extracts of seaweed, and aqueous and alcoholic extract of sorrel on proteus vulgaris, shows that the antibacterial effect of thiazole derivatives was much higher in vitro (34-37).

Conclusion

Considering that the ultimate objective of the study of all new antibacterial substances is to deal with treatment-resistant strains, and the inhibitory effect of the three new thiazole derivatives on the standard strain of Proteus vulgaris was proved, it is necessary to examine the inhibitory effect of the these derivatives on resistant drug strains of this bacterium in future studies.

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Conflicts of Interest

The authors did not express any conflicts of interest.

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