Iranian Journal of Microbiology
Volume 7 Number 5 (Octobr 2015) 281-286

ORIGINAL ARTICLE

Evaluation of anti-bacterial effects of some novel thiazole and imidazole derivatives against some pathogenic bacteria

Behzad Ghasemi[1], Ghasem Sanjarani[1], Zahra Sanjarani[1], Hamidreza Majidiani[2*]

[1]Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.
[2]Department of Medical Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Received: May 2015**, Accepted:** August 2015

ABSTRACT

Background and Objectives: Bacterial resistance to antibiotics has motivated the researchers to evaluate the novel anti-bacterial compounds such as some thiazole and imidazole derivatives. Thereby, in this work, we investigated the anti-bacterial effects of one new thiazole and two new imidazole derivatives on *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli, Salmonella typhimurium*, *Proteus mirabilis* and *Shigella dysenteriae.*

Materials and Methods: The thiazole and imidazole derivatives were dissolved in DMSO. The disk diffusion method was utilized to measure the growth inhibition zone diameter values, and the broth micro-dilution method was applied to determine the minimum inhibitory concentration (MIC) values.

Results: The synthesized imidazole derivatives lacked any inhibitory effect against the tested bacteria. On the other hand, although the synthesized thiazole derivative showed no inhibitory effect against *Bacillus cereus, Salmonella typhimurium,* and *Escherichia coli,* it inhibited the growth of *Proteus mirabilis, Shigella dysenteriae,* and *Listeria monocytogenes* with the MIC values of 1000, 125, and 1000 µg/ml, respectively, and the growth inhibition zone diameter values of 9.3 ± 0.1, 15.6 ± 0.2, and 8.1 ± 0.0 mm, respectively.

Conclusion: The anti-bacterial effect of the synthesized thiazole derivative on *Shigella dysenteriae, Proteus mirabilis* and *Listeria monocytogenes* was proven. However, its inhibition effect against *Shigella dysenteriae* was more than that against the others. Many in-vitro and in-vivo experiments are required to evaluate the effects of this compound on the bacteria and the human body.

Keywords: Anti-bacterial effects, Thiazole, Imidazole.

INTRODUCTION

Pathogenic bacteria have caused serious diseases and a lot of mortality in many nations, especially in the developing countries. These agents usually spread quickly, and the most susceptibilities to them have been assigned to the immunocompromised persons, pregnant mothers, children, and older individuals (1). Along with the progressive resistance of bacteria to the current antibiotics as a result of irregular antibiotic consumption in medicine, the health and general hygiene of people are strongly at risk, and, therefore, to avoid this threat, identification and utilizing novel anti-bacterial compounds are required (2). In the recent years, experimental researches have introduced some thiazole derivatives as the multi-therapeutic

*Corresponding author:
Address: Tarbiat Modares University, Jalal Al-Ahmad highway, Nasr Bridge, Tehran, Iran.
Tel: +989306567151
P.O. Box: 14115-111
E-mail: Hamidreza.Majidiani@gmail.com

effect compounds including anti-cancer, anti-inflammatory, and inhibitor of the parasites like *Leishmania* and the fungi such as *Candida* (3-6). Moreover, the anti-bacterial effects of these compounds have been proven on a wide range of pathogens like *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Klebsiella aerogenes* so that their potency and broad spectrum activity have promised the researchers to replace them with out-dated drugs to which the bacteria are resisting (7). Also regarding the imidazole derivatives, many properties such as the anti-fungal, anti-viral, anti-parasitic, and in-vitro inhibition of cancer cells have been demonstrated, and studying the anti-bacterial effects of these compounds has emphasized their power to inhibit the bacteria such as *Enterococcus faecalis, Staphylococcus aureus*, and *Pseudomonas aeruginosa* (8-11). The potent and wide range anti-bacterial properties of the thiazole and imidazole derivatives have generally made the anti-bacterial test to be among the initial experiments that are studied after synthesizing these agents. In this study, we evaluated the in-vitro anti-bacterial effects of two novel imidazole and one new thiazole derivatives, which have recently been synthesized in Iran, against the bacterial pathogens that are often transferred to the human body by means of food, and cause disease.

MATERIALS AND METHODS

Synthesis of derivatives. The No. 6 thiazole derivative (Fig. 1) was incorporated into a three-phase process, and its chemical structure was verified by monocrystal X-ray diffraction, 1H NMR, 13C NMR, and IR spectroscopic techniques, element decomposition technique, and spectrophotometry. This synthesized derivative was then dissolved in DMSO (8000 µg/mL) (12).

The 3a-b imidazole derivatives were synthesized in a mono-phase process from malononitrile (10 mM, 1.7 g) and 2a-b diaminoalkanes (10 mM), and their chemical structures were confirmed by monocrystal X-ray diffraction, ^{1}HNMR, ^{13}CNMR, and IR spectroscopic techniques, element decomposition technique,and spectrophotometry. These compounds were then dissolved in DMSO (8000 µg/mL) (13).

Fig. 1. Steps forsynthesis of thiazole derivative No. 6 (derivative from reference No. 12).
6: (E)-2-(5-Acetyl-4-methylthiazol-2-yl)-2-[benzo[d]thiazol-2(3H)-ylidene]acetonitrile

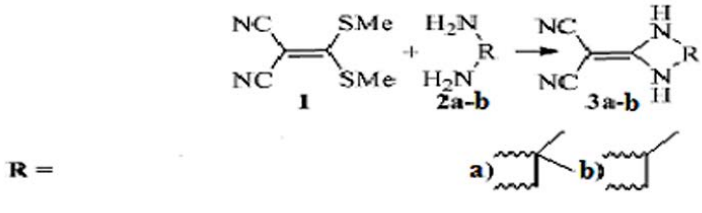

Fig. 2. Steps for synthesis of 3a-b imidazole derivatives (derivatives from reference No. 13).
3a: 2-(4-Methylimidazolidin-2-ylidene) malononitrile
3b: 2-(4,4-Dimethylimidazolidin-2-ylidene) malononitrile

Preparing bacterial suspension. The tested bacteria were *E. coli* (PTCC 1395), *S. dysenteriae* (PTCC 1188), *S. typhimurium* (PTCC 1596), *P. mirabilis* (PTCC 1076), *B. cereus* (PTCC 1665), and *L. monocytogenes* (PTCC 1297); they were obtained from the Iranian Research Organization for Science and Technology (IROST). Each bacterium was cultured in a Mueller-Hinton agar medium, and incubated at 37 °C for 24 h. Henceforth, in a sterile medium and in a logarithmic growth phase, a concentration of 0.5 McFarland (1.5×10^8 CFU/mL) was obtained using a spectrophotometer and a standard McFarland tube number 0.5 from each bacterium, which was assigned as the stock solution (14).

Minimum inhibitory concentration (MIC) determination. The MIC test was carried out in a sterile 96-well plate by broth microdilution as the CLSI (15) standard. First, 90 µL of the Muller-Hinton broth medium (Merck®, Germany) was added to each well. Then 100 µL of the thiazole and imidazole derivatives (in control groups, 100 µL of penicillin and gentamycin antibiotics (Sigma®) with the primary concentration of 256 µg/ml) were added to the first well (in which the concentration of derivatives was 4000 and concentration of antibiotics was 128 µg/ml). After mixing, 100 µL of this mixture was embedded into the second well. Similarly, the dilution procedure was carried out in the other wells. 10 µL of the bacterial suspension was added to each well. For a negative control, 100 µL of the Muller-Hinton broth, 100 µL of DMSO, and 10 µL of the bacterial suspension were added to the last well in each row. The incubation result was read after 24 h incubation at 37 °C. The lucidity and turbidity in each well indicated the lack and existence of the bacterial growth, respectively. The last well that did not show any turbidity by visual method was reported as MIC (14).

Growth inhibition zone diameter determination. Firstly, in the Muller-Hinton agar medium, the superficial bacterial culture was performed with a swab impregnated to the bacterial suspension. Then 20 µL of the MIC imidazole and thiazole derivatives obtained and also the antibiotics were shed on blank sterile disks. For the negative control, the DMSO-impregnated disk was used. After 24 h incubation at 37 °C, the growth inhibition zone diameter was measured using a particular ruler. The results of the growth inhibition zone diameter were provided as the average ± standard deviation, and for the aim of analyzing the data, the SPSS statistical software (version 22) was used (14).

RESULTS

In this study, the anti-bacterial effects of the 3a-b imidazole derivatives and compound 6 of thiazole were assessed for *E. coli, S. dysenteriae, S. typhimurium, P. mirabilis, B. cereus,* and *L. monocytogenes* by means of the growth inhibition zone diameter and MIC measurements. No inhibitory effect of the 3a-b imidazole derivatives against all of the tested bacteria was observed, and only compound 6 of thiazole had an inhibitory effect against *S. dysenteriae, P. mirabilis,* and *L. monocytogenes*, among the tested bacteria. The inhibitory effect of this compound was recorded with the MIC values of 1000, 125, and 1000 µg/mL, and the zone diameter values of 9.3 ± 0.1, 15.6 ± 0.2, and 8.1 ± 0.0 mm for *P. mirabilis, S. dysenteriae,* and *L. monocytogenes,* respectively. In the anti-biogram test performed, the most and least susceptibilities were recorded for *B. cereus* to gentamicin with the MIC value of 0.5 µg/mL, and for *E. coli* to penicillinwith the MIC value of 64 µg/mL (Tables 1 and 2).

Table 1. MIC values (µg/mL) for effects of thiazole and imidazole derivatives and antibiotics on tested bacteria.

Antibiotic/Derivative	*B. cereus*	*L. monocytogenes*	*E. coli*	*S. dysenteriae*	*S. typhimurium*	*P. mirabilis*
3a	-	-	-	-	-	-
3b	-	-	-	-	-	-
6	-	8.1 ± 0.0	-	15.6 ± 0.2	-	9.3±0.1
Penicillin	13.1 ± 0.3	22.5 ± 0.4	-	17.7 ± 0.1	17.3 ± 0.0	12.3 ± 0.2
Gentamicin	16.2 ± 0.5	15.3 ± 0.2	18.6 ± 0.3	23.1 ± 0.4	12.4 ± 0.2	14.7 ± 0.1

"-" indicates lack of effects at highest concentration.

Table 2. Growth inhibition zone diameter values (mm) for effects of thiazole and imidazole derivatives and antibiotics on tested bacteria.

Antibiotic/Derivative	*B. cereus*	*L. monocytogenes*	*E. coli*	*S. dysenteriae*	*S. typhimurium*	*P. mirabilis*
3a	-	-	-	-	-	-
3b	-	-	-	-	-	-
6	-	1000	-	125	-	1000
Penicillin	32	1	64	8	8	16
Gentamycin	0.5	2	2	2	2	1

"-"indicates lack of effects athighestconcentration.

DISCUSSION

Thiazole and imidazole derivatives with high potencies of anti-bacterial activity could be some novel compounds that are used as antibiotics, and in many countries, are in focus of research fields. According to the results obtained for this work, the 3a-b compounds (two imidazole derivatives) lacked any inhibitory effect on the tested bacteria, whereas some of the imidazoline derivatives had the ability to inhibit *Pseudomonas* and *Escherichia coli*. This difference in effect and inhibition potency of these substances is due to the presence of chlorine and phenyl compounds (15). Beside the lack of effects of the 3a-b derivatives, experiments proved the power of methylnitroimidazole to inhibit the growth of the *Enterobacteriaceae* family such as *Proteus vulgaris* and *Proteus mirabilis*, and this compound could release free radicals that harm bacteria and kill them; this ability was not observed for the 3a-b derivatives (16).

Compound 6 of thiazole could only inhibit *S. dysenteriae, P. mirabilis*, and *L. monocytogenes* among all the tested bacteria, and its inhibitory effect was much more on *S. dysenteriae* rather than *P. mirabilis* and *L. monocytogenes*. In the recent studies, inhibition of the bacterial DNA or enzymes has been proposed to be the influential mechanism for the inhibitory action of the thiazole derivatives. Inhibition of the bacterial enzyme ecKASIII (or FabH) (that is essential for the synthesis of fatty acids in Gram negative and gram positive bacteria) and the enzyme DNA-gyrase (that is needed to replicate the bacterial DNA) have been studied in some research works (17, 18). Noting that the quinolone-family antibiotics and the thiazole derivatives could inhibit the subunits A and B in DNA-gyrase, respectively, is promising for the inhibition of the quinolone-resistant bacteria by the thiazole derivatives (18).

An important feature in the structure of compound 6 of thiazole is the presence of benzothiazole, and researchers like Maddila et al. have paid attention to the anti-bacterial effects of the benzothiazole derivatives against *Escherichia coli*; the MIC values in the range of 12.5-200 µg/mL have been evaluated for these compounds, suggesting that their possible potent activities are due to the presence of the chlorophenyl or pyrimidine ring in their structures (19). In the experiment performed by Patel et al. the effects of the benzothiazole derivatives on *Escherichia coli, Salmonella typhi, Bacillus cereus*, and *Shigella flexneri* have been substantiated; the chlorine and benzoimidazole connections are possibly involved in increasing the anti-bacterial effects of these substances (20). In the study carried out by Gilani et al. the MIC values in the range of 12.5-200 µg/mL have been reported, indicating the inhibitory effects of the benzothiazole derivatives against *Escherichia coli* and *Staphylococcus aureus*, in which the chlorine and chlorphenole connections are likely to be involved in increasing the anti-bacterial effects of these compounds (21). Alizadeh et al. have shown the inhibitory effects of the benzothiazole derivatives bonded to Cu and Zn against *Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis,* proving the potency of these benzothiazole derivatives by attaching to the DNA molecules of the bacteria, inhibiting their activities (22).

There has been a focus on the anti-bacterial activities of the thiazole derivatives in many research works, in which the growth inhibition zone diameter or MIC or both have been measured. In this regard, we can refer to the research work carried out by Jagani et al. in which they have shown the in-vitro anti-bacterial potency of the thiazole derivatives against *Bacillus subtilis, Bacillus megaterium, Escherichia coli,* and *Pseudomonas aeruginosa* by measuring the growth inhibition zone diameter (23). Bharti et al. have

proved the in-vitro potency of the thiazole derivatives to inhibit *Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Vibrio cholera,* and *Staphylococcus aureus* by measuring the values for the growth inhibition zone diameter and MIC (24). Juspin et al. have carried out an experiment, in which the growth inhibition zone diameter and MIC values were measured, and the in-vitro power of the thiazole derivatives to inhibit the activities of *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* was confirmed (25).

CONCLUSION

With respect to the inhibitory effect of the thiazole derivatives and their synergistic effect with other antibiotics, it is suggested to assess their therapeutic and toxic effect in laboratory animals, and is recommended to do more researches in order to better recognize these compounds and their possible functionality.

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