ORIGINAL ARTICLE AMHSR

Study of Antibacterial Effect of Novel Thiazole, Imidazole, and Tetrahydropyrimidine Derivatives against Listeria Monocytogenes

Behzad Ghasemi,[1]* Hamid Beyzaei,[2] Hadi Hashemi[3]

[1]*Department of Pathobiology, Faculty of Veterinary, University of Zabol, Zabol, Iran.*
[2]*Department of Chemistry, Faculty of Science, University of Zabol, Zabol, Iran.*
[3]*Department of Clinical Sciences, Faculty of Veterinary, University of Zabol, Zabol, Iran.*

ABSTRACT

Purpose: In this study, we have focused on antibacterial effect of newly synthesized thiazole, imidazole, and tetrahydropyrimidine derivatives in Iran on listeria monocytogenes.
Materials and Methods: For evaluation of antibacterial effect, the disk diffusion method was applied to measure the growth inhibition zone diameter and broth micro dilution was performed to determine the minimum inhibitory concentration.
Results: Assessing the antibacterial effect showed that only thiazole derivative 6d had inhibitory effect on listeria monocytogenes and the other thiazole, imidazole and tetrahydropyrimidine derivatives lacked any inhibitory clue on this organism. The inhibitory effect of thiazole derivative 6d was shown by minimum inhibitory concentration = 64 and growth inhibition zone diameter = 23 ± 0.1. In antibiogram test, also the most susceptibility was recorded for gentamicin and penicillin with minimum inhibitory concentration = 1 µg/mL.
Conclusion: The antibacterial effect of thiazole, imidazole and tetrahydropyrimidine derivatives differs from each other and cross connections such as linkage of oxygen to thiazole ring in derivative 6d, could reinforce this effect. By proving the *in vitro* antibacterial effect of the novel thiazole derivative on listeria monocytogenes, to more recognize this compound, next step is determining the toxicity and therapeutic effects in laboratory animals.

Keywords: listeriosis; drug therapy; treatment outcome; antifungal agents; chemistry; pharmacology.

AMHSR 2015;13:103-107
www.journals.ajaums.ac.ir

INTRODUCTION

Listeria monocytogenes (L. monocytogenes) is a gram-positive bacterium, zoonotic and food-born pathogen that infects a wide variety of animal species (including mammals, birds, fishes and crustaceans) and human.[1] This pathogen causes meningitis, encephalitis and abortion and old people, humans with weakened immune systems and pregnant women are most at risk.[1] Using antibiotics is of the cheapest and most popular way of controlling L. monocytogenes and their wide causes, which has vastly increased the drug resistance in this pathogen and consequently has led to an increase in the possibility of mortality, health care costs and has endangered the public hygiene in society. In recent years, researchers have recommended identification and use of novel antibacterial compounds to inhibit the drug-resistant strains of L. monocytogenes.[2]

Thiazoles have a crucial role in active biological compounds.[3] For instance, the thiazole ring exists in vitamin B_1, which is the important coenzyme of the carboxylase enzyme.[3] Some of the thiazole derivatives are applicable as drugs in treatment of cancer, lowering blood cholesterol, lowering blood pressure and treatment of infection with human immune deficiency virus.[3] Also *in vitro* high antioxidant potency, anti-inflammatory and inhibitory effects of thiazoles on parasites such as

anopheles mosquito or trypanosoma and on fungi such as candida albicans have been observed.[4-8] Scientists have proven the *in vitro* potency of thiazole derivatives to inhibit the bacterial pathogens like Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), staphylococcus epidermidis (S. eidermidis), streptococcus pyogenes, pseudomonas fluorescence and streptococcus fecalis.[9] Also in recent years the imidazoline derivatives have attracted researchers by inhibiting tumor cells, Leishmania parasite and aspergillus and fusarium fungi.[10-12] Studies have shown the antibacterial effect of imidazole derivatives on pathogens such as enterococcus fecalis, E. coli and *S. aureus* .[13]

Recent surveys have shown the effect of tetrahydropyrimidine derivatives to inhibit tuberculosis and fungi such as aspergillus niger and candida albicans.[14] Several derivatives of them are being developed for the treatment of Alzheimer's and infectious diseases.[15,16] Antibacterial effect of tetrahydropyrimidine derivatives has been proven *in vitro* on pathogens like Klebsiella pneumonia and pseudomonas aeruginosa.[17]

The potent and broad-spectrum activity of thiazole, imidazole and tetrahydropyrimidine derivatives has generally caused the antibacterial test to be among the first experiments which is studied by researchers after synthesis of these compounds. In this study, we have evaluated the antibacterial effects of novel thiazole, imidazole and tetrahydropyrimidine, which have recently been synthesized in Iran, on L. monocytogenes organism.

MATERIALS AND METHODS

Synthesis of Compounds

Thiazole derivatives **6a-d** were synthesized in a three-step process and their chemical structure was confirmed by single crystal X-ray diffraction and elemental analysis, proton nuclear magnetic resonance (^{1}H-NMR), carbon-13 nuclear magnetic resonance (^{13}C-NMR) and infrared (IR) spectroscopy (**Figure 1**).[3] Afterwards, these derivatives were solved in dimethyl sulfoxide (DMSO) with concentration of 8192 µg/mL.

6a: Ethyl 2-[(*E*)-cyano (thiazolidin-2-ylidene) methyl] thiazole-4-carboxylate

6b: (*E*)-2-(5-Acetyl-4-methylthiazol-2-yl)-2-(thiazolidin-2-ylidene) acetonitrile

6c: Ethyl 2-((*E*)-cyano (thiazolidin-2-ylidene)methyl)-4-methylthiazole-5 carboxylate

6d: (2*E*)-2-(4,5-Dihydro-4-oxothiazol-2-yl)-2-(thiazolidin-2-ylidene) acetonitrile

Imidazole and tetrahydropyrimidine derivatives **9a-g** were synthesized through a mono-step process from the

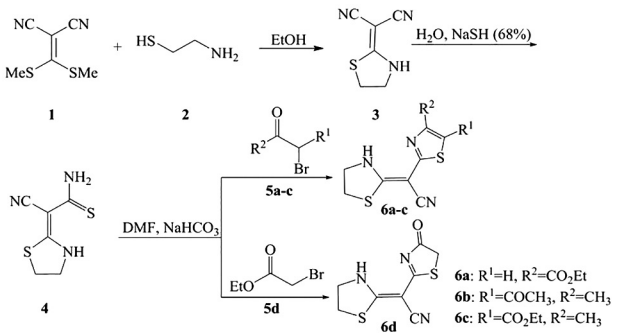

Figure 1. Steps of the synthesis of thiazole derivatives 6a-d (derivatives from reference No. 3)

reaction of 2-[bis (methylthio) methylene]malononitrile (**1**) and diaminoalkanes **8a-g**, and their chemical structure was confirmed by elemental analysis, ^{1}H-NMR, ^{13}C-NMR and IR spectrometry (**Figure 2**).[18] Thereafter, these derivatives were solved in DMSO with concentration of 8192 µg/mL.

9a: 2-(5,5-Dimethyltetrahydropyrimidin-2(1*H*)-ylidene)malononitrile

9b: 2-(4-Ethyltetrahydropyrimidin-2(1*H*)-ylidene) malononitrile

9c: 2-(5-Hydroxytetrahydropyrimidin-2(1*H*)-ylidene) malononitrile

9d: 2-(4,4-Dimethylimidazolidin-2-ylidene) malononitrile

9e: 2-(4-Methylimidazolidin-2-ylidene) malononitrile

9f: 2-(Octahydro-2*H*-benzo[*d*]imidazol-2-ylidene) malononitrile

9g: 2-(Tetrahydropyrimidin-2(1*H*)-ylidene) malononitrile

Preparation of Bacterial Suspension

L. monocytogenes bacteria (PTCC 1297) was obtained from Iranian Research Organization for Science and Technology. Then, the bacteria was cultured in Mueller-Hinton agar medium in 37 ºC for 24 hours. Henceforth in sterile conditions of Mueller-Hinton medium and in logarithmic growth phase, a concentration of 10^5 colony forming unit (CFU)/mL was obtained with

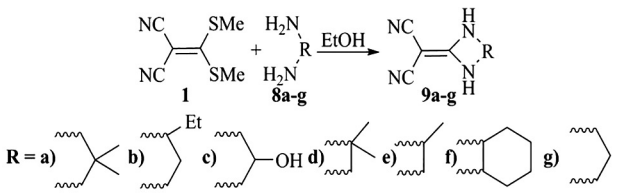

Figure 2. Steps of the synthesis of imidazole and tetrahydropyrimidine derivatives 9a-g (derivatives from reference No. 18).

spectrophotometer from each bacterium which was assigned as a stock solution.[19]

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC test was done in a sterile 96-well plate by broth microdilution as Clinical and Laboratory Standards Institute (CLSI) 2014 standard. First, 100 µL of Mueller-Hinton broth medium (Merck®, Darmstadt, Germany) was added to each well. Then, 100 µL of thiazole, imidazole and tetrahydropyrimidine derivatives (in control groups, 100 µL of penicillin and gentamicin antibiotics (with 512 µg/mL) (Sigma®) were added to the first well and after mixing, 100 µL of this mixture was embedded into the second well. Similarly, dilution procedure was done in other wells. Ten µL of bacterial suspension was added to each well. For negative control, 100 µL of Mueller-Hinton broth, 100 µL DMSO and 10 µL of bacterial suspension were added to last well in each row. The result of incubation was read after 24 hours of incubation in 37°C. The lucidity and turbidity in each well indicated lack or existence of bacterial growth, respectively. The last well that didn't show any turbidity, was reported as MIC.[19]

Determination of the Growth Inhibition Zone Diameter

First, the superficial bacterial culture was performed in Mueller-Hinton agar medium with a swab impregnated to bacterial suspension. Then, 20 µl of obtained MIC for derivatives and antibiotics (20 µL DMSO for negative control) were shed on blank sterile disks and after 24 hours of incubation in 37°C, the growth inhibition zone diameter was measured with coulisse. The results of the growth inhibition zone diameter have been provided as the average ± standard deviation; also in order to analyze data, the Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 22.0 was used.[1]

RESULTS

The results showed that imidazole and tetrahydropyrimidine compounds **9a-g** and thiazole derivatives **6a-c** don't have inhibitory effects on L. monocytogenes bacteria, only the inhibitory effect of thiazole derivative **6d** was recorded on L. monocytogenes with halo diameter = 23 ± 0.1 mm and MIC = 64 µg/mL. In the antibiogram test, the highest susceptibility of L. monocytogenes was measured for gentamicin and penicillin with MIC = 1 µg/mL, respectively. The results confirmed no inhibitory effect of DMSO on L. monocytogenes which was used as solvent for derivatives (**Tables 1** and **2**).

DISCUSSION

In this study, four tetrahydropyrimidine derivatives lack any inhibitory effects on tested L. monocytogenes bacteria. Evaluation of antibacterial effects of tetrahydropyrimidine derivatives on some bacterial pathogens by Vishwakarma and colleagues showed that among the tested bacteria such as E. coli, S. aureus, Staphylococcus epidermidis, Bacillus subtilis (B. subtilis) and Bacillus mycoides, only some of the examined derivatives had inhibitory effect and this research indicates that tetrahydropyrimidine derivatives do not have broad-spectrum activity on different bacteria.[20]

Also, in this study, three derivatives of imidazoline didn't have inhibitory effect on L. monocytogenes, meanwhile some of imidazoline derivatives have the ability to inhibit bacteria like S. aureus and E. coli and this variation in bacterial inhibition is due to compounds such as chlorine.[21] One of the reasons for no effect activity of derivatives **9d-f** is methyl nitroimidazole and experiments have proven the potency of this substance to inhibit Micrococcus luteus, S. aureus and Pseudomonas aeruginosa and have shown that this derivative could damage bacteria and lead it to death by producing free radicals, the advantage that isn't seen in derivatives **9d-f**.[22]

Table 1. Growth inhibition zone diameter (mm) of thiazole, imidazole and tetrahydropyrimidine derivatives and antibiotics on L. monocytogenes (PTCC 1297).

Derivatives/ Antibiotics	6a	6b	6c	6d	9a	9b	9c	9d	9e	9f	9g	DMSO	Gentamicin	Penicillin
Growth inhibition zone diameter	–	–	–	23 ± 0.1	–	–	–	–	–	–	–	–	17 ± 0.3	21 ± 0.1

Abbreviation: DMSO, dimethyl sulfoxide.
– indicates no inhibitory effect at maximum concentration

Table 2. Minimum Inhibitory Concentration (µg/mL) of thiazole, imidazole and tetrahydropyrimidine derivatives and antibiotics on L. monocytogenes (PTCC 1297).

Derivatives/ Antibiotics	6a	6b	6c	6d	9a	9b	9c	9d	9e	9f	9g	DMSO	Gentamicin	Penicillin
MIC	–	–	–	64	–	–	–	–	–	–	–	–	1	1

Abbreviations: MIC, Minimum Inhibitory Concentration; DMSO, dimethyl sulfoxide.
– indicates no inhibitory effect at maximum concentration

The only inhibitory effect in this research was related to thiazole derivative **6d** and no inhibitory effects were seen from thiazoles **6a-c**. Study of the structure of this compound shows that besides existence of thiazole ring, there are two major structures; one of which is the thiazolidine ring. The thiazolidine derivatives are known as compounds with broad-spectrum activities on bacteria; recent works have indicated the inhibitory effect of these derivatives on bacteria like E. coli and S. aureus and we could provethe potent effect of these compounds on E. coli and S. aureus with MIC = 6.25-25 µg/mL.[23] The other major structure is oxygen linked to thiazole ring which has established the oxothiazole, whichis only present in compound **6d**among derivatives **6a-d**; also the inhibitory power of oxothiazole-containing compounds has been proven on E. coli.[24] Liaras have shown the inhibitory effect of thiazole derivatives on L. monocytogenes with MIC = 30.58–38.75 µg/mL and possibly due to existence of chlorine as trichlorophenyl, the inhibitory potency of these derivatives has been increased in comparison to derivative **6d** in our research.[19]

Studies on antibacterial effects of thiazoles have suggested that thiazole derivatives act by inhibiting enzymes like DNA gyrase B (quinolone antibiotics inhibit DNA gyrase A and possibly we can use thiazoles against quinolone-resistant bacteria orhave synergistic effects along with quinolone antibiotics) or inhibiting genes such as *fabH* (which has a vital role in fatty acid metabolism of bacteria).[25,26]

Many researches have indicated the inhibitory potency of thiazole derivatives on E. coli by measuring growth inhibition zone diameter or MIC or both, which is a confirmation to our study. We can briefly indicate the following works; Cheng and colleagues in 2013 showed the *in vitro* potency of thiazole derivatives to inhibit E. coli, S. aureus and B. Subtilis bacteria by measuring MIC.[27] Shah and colleagues in 2012 proved the *in vitro* power of thiazole compounds to inhibit Pseudomonas aeruginosa, S. aureus and B. subtilis by means of growth inhibition zone diameter.[28] Bondoc in 2007 reported the *in vitro* activity of thiazole derivatives on E. coli and B. megaterium organism by measuring MIC.[29] Juspin and colleagues in 2010 proved the *in vitro* activity of thiazole derivatives by means of MIC and growth inhibition zone diameter on Pseudomonas aeruginosa, S. aureus and Enterococcus hirae.[30] Sarojini and colleagues, in 2010, reported the *in vitro* power of thiazole compounds to inhibit E. coli, Klebsiella pneumoniae and S. aureus.[31]

CONCLUSIONS

The L. monocytogenes bacteria is one of the most important pathogens in human being and we have recently observed the spread of antibiotic-resistant strains of this pathogen globally,which shows the necessity of identification and the use of novel antibacterial compounds against *L. monocytogenes*. In this study, among the thiazole, imidazole and tetrahydropyrimidine derivatives were tested, only thiazole **6d** showed inhibition effects on L. monocytogenes bacteria, of course the inhibitory effect of this deravetive is higher than gentamicin and penicillin. More tests can be led to the use of this compound as an antibacterial agent.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Adzitey F, Rahmat Ali G, Huda N, Cogan T, Corry J. Prevalence, antibiotic resistance and genetic diversity of Listeria monocytogenes isolated from ducks, their rearing and processing environments in Penang, Malaysia. Food Contr. 2013;32:607-14.
2. Paul D, Steele C, Donaldson JR, Banes MM, Kumar R, Bridges SM, Arick M 2nd, Lawrence ML. Genome comparison of Listeria monocytogenes serotype 4a strain HCC23 with selected lineage I and lineage II L. monocytogenes strains and other Listeria strains. Genom Data. 2014;2:219-25.
3. Bakavoli M, Beyzaie H, Rahimizadeh M, Eshghi H, Takjoo R. Regioselective synthesis of new 2-(E)-cyano(thiazolidin-2-ylidene)thiazoles. Molecules. 2009;14:4849-57.
4. Jaishree V, Ramdas N, Sachin J, Ramesh B. In vitro antioxidant properties of new thiazole derivatives. J Saudi Chem Soc. 2012;16:371-6.
5. Helal MH, Salem MA, El-Gaby MS, Aljahdali M. Synthesis and biologicalevaluation of some novel thiazole compounds as potential anti-inflammatoryagents. Eur J Med Chem. 2013;65:517-26.
6. Venugopala KN, Krishnappa M, Nayak SK, et al. Synthesis and antimosquito properties of2,6-substituted benzo[d] thiazole and 2,4-substituted benzo[d]thiazole analogues against Anopheles arabiensis. Eur J Med Chem. 2013;65:295-303.
7. Zelisko N, Atamanyuk D, Vasylenko O, Grellier P, Lesyk R. Synthesis and antitrypanosomal activity of new 6,6,7-trisubstitutedthiopyrano[2,3-d][1,3]thiazoles. Bioorg Med Chem Lett. 2012;22:7071-4.
8. Chimenti F, Bizzarri B, Bolasco A, et al. Synthesis and biological evaluation ofnovel 2,4-disubstituted-1,3-thiazoles as anti-Candida spp. agents. Eur J Med Chem. 2011;46:378-82.
9. Ghasemi B, Najimi M, Jalaei J. Evaluation of antibacterial effects of benzothiazole derivatives on bacterial food pathogens. Iran J Med Microbiol. 2015;9:35-41. Persian

10. Brahmayya M, Venkateswararao B, Krishnarao D, et al. Synthesis and fungicidal activity of novel 5-aryl-4-methyl-3yl (imidazolidin-1yl methyl, 2-ylidene nitro imine) isoxazoles. J Pharm Res. 2013;7:516-9.

11. Robert JM, Sabourin C, Alvarez N, Robert-Piessard S, Le Baut G, Le Pape P. Synthesis and antileishmanial activity of new imidazolidin-2-one derivatives. Eur J Med Chem. 2003;38:711-8.

12. Wittine K, StipkovićBabić M, Makuc D, et al. Novel 1,2,4-triazole andimidazole derivatives of L-ascorbic and imino-ascorbic acid: synthesis, anti-HCV and antitumor activity evaluations. Bioorg Med Chem. 2012;20:3675-85.

13. Salhi L, Bouzroura-Aichouche S, Benmalek Y, et al. An efficient conversion of maleimide derivatives to 2-thioxo imidazolidinones. Org Commun. 2013;6:87-94.

14. Akhaja TN, Raval JP. Design, synthesis, in vitro evaluation of tetrahydropyrimidine–isatin hybrids as potential antibacterial, antifungal and anti-tubercular agents. Chinese Chem Lett. 2012;23:446-9.

15. Messer WS Jr, Rajeswaran WG, Cao Y, et al. Design and development of selective muscarinic agonists for the treatment of Alzheimer's disease: characterization of tetrahydropyrimidine derivatives and development of new approaches for improved affinity and selectivity for M1 receptors. Pharm Acta Helv. 2000;74:135-40.

16. Elumalai K, Ali M, Elumalai M, Eluri K, Srinivasan S. Novel isoniazid cyclocondensed 1,2,3,4-tetrahydropyrimidine derivatives for treating infectious disease: a synthesis and in vitro biological evaluation. J Acute Dis. 2013;2:316-21.

17. Hussein WM, Fatahala SS, Mohamed ZM, et al. Synthesis and kinetic testing of tetrahydropyrimidine-2-thione and pyrrolederivatives as inhibitors of the metallo-β-lactamase from Klebsiella pneumoniaand Pseudomonas aeruginosa. Chem Biol Drug Des. 2012;80:500-15.

18. Beyzaei H, Aryan R, Gomroki M. Synthesis of novel heterocyclic 2-(2-ylidene)malononitrile derivatives. Org Chem Indian J. 2015;11:3-10.

19. 19. Liaras K, Geronikaki A, Glamoclija J, Ciric A, Sokovic M. Thiazole-based chalcones as potent antimicrobial agents. Synthesis and biological evaluation. Bioorg Med Chem Lett. 2011;19:3135–3140.

20. Vishwakarma JN, Dutta MC, Chanda K, Das B, Laskar MA, Nongkhlaw RL. Synthesis and anti-bacterial activities of novel 5-isonicotinoyl-1,2,3,4-tetrahydropyrimidines and bis-(5-isonicotinoyl-1,2,3,4-tetrahydropyrimidines). Arkivoc. 2009;13:131-41.

21. Nasser AJA, Idhayadhulla A, Kumar RS, Selvin J. Synthesis and biological activities of new series of imidazolidin-2,4-dione derivatives. Asian J Chem. 2010;22:5853-8.

22. Shahid HA, Jahangir S, Yousuf S, Hanif M, Sherwani SK. Synthesis, crystal structure, structural characterization and in vitro antimicrobial activities of 1-methyl-4-nitro-1H-imidazole. Arabian J Chem. 2014;10:1016.

23. Bozdağ-Dündar O, Ozgen O, Menteşe A, et al. Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dionederivatives. Bioorg Med Chem. 2007;15:6012-7.

24. Zaky RR, Yousef TA. Spectral, magnetic, thermal, molecular modelling, ESR studies and antimicrobial activity of (*E*)-3-(2-(2-hydroxybenzylidene)hydrazinyl)-3-oxo-n(thiazole-2-yl) propanamide complexes. J Mol Struct. 2011;1002:76-85.

25. Brvar M, Perdih A, Oblak M, Masic LP, Solmajer T. In silico discovery of 2-amino-4-(2,4-dihydroxyphenyl)thiazoles as novel inhibitors of DNA gyrase B. Bioorg Med Chem Lett. 2010;20:958-62.

26. Lv PC, Wang KR, Yang Y, et al. Design, synthesis and biological evaluation of novel thiazole derivatives as potent FabH inhibitors. Bioorg Med Chem Lett. 2009;19:6750-4.

27. Cheng K, Xue JY, Zhu HL. Design, synthesis and antibacterial activity studies of thiazole derivatives as potent ecKAS III inhibitors. Bioorg Med Chem Lett. 2013;23:4235-8.

28. Shah NK, Shah NM, Patel MP, Patel RG. Synthesis, characterization and antimicrobial activity of some new biquinoline derivatives containing a thiazole moiety. Chinese Chem Lett. 2012;23:454-7.

29. Bondock S, Khalifa W, Fadda A. Synthesis and antimicrobial evaluation of some new thiazole,thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydronaphthalene-2-carboxaldehyde. Eur J Med Chem. 2007;42: 948-954.

30. Juspin T, Laget M, Terme T, Azas N, Vanelle P. TDAE-assisted synthesis of new imidazo[2,1-b]thiazole derivatives as anti-infectious agents. Eur J Med Chem. 2010;45:840-5.

31. Sarojini BK, Krishna BG, Darshanraj CG, Bharath BR, Manjunatha H. Synthesis, characterization, in vitro and molecular docking studies of new 2,5-dichlorothienyl substituted thiazole derivatives for antimicrobial properties. Eur J Med Chem. 2010;45:3490-6.

Corresponding Author:
Behzad Ghasemi, Doctor of Veterinary Medicine
Department of Pathobiology, Faculty of Veterinary, University of Zabol, Zabol, Iran.
Tel: +98 54 31232254
Fax: +98 54 31232250
E-mail: behzad.ghasemi99@gmail.com

Received July 2015
Accepted August 2015