Study of Antibacterial Effect of Novel Thiazole, Imidazole and Tetrahydropyridine Derivatives against *Escherichia coli*

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ABSTRACT

***Background*:** *Escherichia coli* is one of the important pathogens in human with global importance. Because of the necessity for identification and the use of novel antibacterial compounds against *E. coli*, in this present study we focused on the antibacterial effects of synthesized thiazole, imidazole and tetrahydropyridine derivatives on *E. coli*.

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 (MIC).

Results: Assessing the antibacterial effect showed that only 6d derivative of thiazole had inhibitory effect on *E. coli* and the other thiazole, imidazole and tetrahydropyridine derivatives lacked any inhibitory result on this organism. The inhibitory effect of 6d derivative of thiazole was MIC=125 and growth inhibition zone diameter of 16±0.1.

Discussion: The antibacterial effect of thiazole, imidazole and tetrahydropyridine derivatives differs from each other and chemical linkages such as oxygen to thiazole ring in 6d derivative, could have reinforced this effect. The next step is determination of the toxicity and therapeutic effects in the laboratory animals.

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Introduction

Escherichia coli is the major reason for the incidence of travelers' diarrhea and children diarrheal. Due to the host variations, *E. coli* is being considered as a major public hygiene dilemma in developed and developing countries. Antibiotics usage is the cheapest and most popular way of controlling *E. coli* and their wide and sometimes unnecessary consumption whether in veterinary or medicine causes vast drug resistance in this pathogen which has led to increased mortality, health care costs and endangering the public hygiene. In recent years, researchers have recommended the use of novel antibacterial compounds to inhibit the drug-resistant strains of *E. coli* (1, 2).

Thiazoles have a crucial role in active biological compounds. The thiazole ring exists in B1 vitamin which is the important coenzyme of the carboxylase enzyme. Some of the thiazole derivatives are applicable as drugs in treatment of cancer, blood cholesterol, blood pressure and HIV virus infection (3). Also in-vitro high anti-oxidant potency, anti-inflammatory and inhibitory effects of thiazoles on parasites such as anopheles mosquito or trypanosoma or fungi (*Candida albicans*) have been observed (4-8). Scientists have proven the in-vitro potency of thiazole derivatives to inhibit the bacterial pathogens such as *S. aureus*, *E. coli*, *S. epidermidis*, *S. pyogenes*, *P. fluorescens* and *S. faecalis* (9). In addition, in the recent years, the imidazoline derivatives have also attracted researchers for studying inhibiting tumor cells, *Leishmania* parasite *Aspergillus* and *Fusarium* fungi (10-12). Studies have shown the antibacterial effect of imidazole derivatives on pathogens such as *E. faecalis*, *E. coli* and *S. aureus* (13).

Recently, the effect of tetrahydropyridine derivatives to inhibit tuberculosis and *Aspergillus niger* and *C. albicans* as well as preventing parkinson's disease and diabetes have also been documented (14-16). Antibacterial effect of tetrahydropyridine derivatives has been proven in-vitro on pathogens like vancomycin resistant *E. faecalis* and methicillin resistant *S. aureus* (17). The potent and broad-spectrum activity of thiazole, imidazole and tetrahydropyridine derivatives has generally caused that the antibacterial test to be amongst the first experiments carried out by the researchers after synthesis of such compounds. In this study, we have evaluated the antibacterial effects of novel thiazole, imidazole and tetrahydropyridine compounds, which have recently been synthesized in Iran, on *E. coli* organism.

Materials and Methods

Synthesizing compounds

Derivatives of thiazole 6a-d were synthesized in a three-step process and their chemical structures were confirmed by single crystal X-ray diffraction, 1H-NMR, 13C-NMR, IR spectrometry and elemental analysis. Afterwards, these derivatives were prepared as solution in DMSO solvent with concentration of 8000 µg/ml (3).

3a-g derivatives of imidazole and tetrahydropyridine were synthesized through a mono-step process from the malononitrile (10 mmol, 1.7 g) and 2a-g diaminoalkanes (10 mmol) and their chemical structure was confirmed by 1H-NMR, 13C-NMR, IR spectrometry and elemental analysis. Thereafter, these derivatives were prepared as solution in DMSO solvent with concentration of 8000 µg/ml (18).

Preparing bacterial suspension

In this research we used a standard strain such as *E. coli* (PTCC 1395) which was obtained from Iranian Research Organization for Science and Technology (IROST). Bacteria were cultured in Mueller-Hinton agar medium in 37 °C for 24 hours. Henceforth in sterile condition of Mueller-Hinton medium and in logarithmic growth phase, a concentration of

0.5 McFarland (1.5×10^8 CFU/ml) was obtained with spectrophotometer and standard McFarland tube number 0.5 from each bacterium was assigned as a stock solution (19).

Determining the minimum inhibitory concentration (MIC)

The MIC test was done three times in a sterile 96-well plate by broth microdilution. First 100 µl of Muller-Hinton broth medium was added to each well. Then 100 µl of thiazole, imidazole and tetrahydropyridine derivatives (in control groups, 100 µl of penicillin and gentamycin antibiotics with concentration of 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. 10 µl of bacterial suspension was added to each well. For negative control 100 µl of Muller-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 hour 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).

Determining the growth inhibition zone diameter

First, in Muller-Hinton agar medium the superficial bacterial culture was performed 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 hour incubation in 37 °C, the growth inhibition zone diameter was measured with coulisse. The results of growth inhibition zone diameter have been provided as average ± standard deviation and for the aim of analyzing data, the SPSS statistical software (version 22) was used (19).

Results

The results showed that imidazole and tetrahydropyridine compounds and 6a-c derivatives of thiazole don't have inhibitory effect on *E. coli* bacteria. Only the inhibitory effect of 6d derivative of thiazole was recorded on *E. coli* with halo diameter of 16 ± 0.1 mm and MIC of 125 µg/ml. In antibiogram test, the most susceptibility of *E. coli* was measured for Gentamycin and the least was recorded for Penicillin with MIC of 2 and 64 µg/ml, respectively. The results confirmed no inhibitory effect of DMSO on *E. coli* which was used as solvent for derivatives (Table 1).

Table 1. Growth inhibition zone diameter (mm) and MIC (µg/ml) of thiazole, imidazole tetrahydropyridine derivatives and antibiotics on *E. coli* (PTCC 1395).

Derivatives / Antibiotics	Growth inhibition zone	MIC
6a	-	-
6b	-	-
6c	-	-
6d	16 ± 0.1	125
3a	-	-
3b	-	-
3c	-	-
3d	-	-
3e	-	-
3f	-	-
3g	-	-
DMSO	-	-
Gentamycin	15 ± 0.1	2
Penicillin	17 ± 0.2	8

Discussion

In this study four tetrahydropyridine derivatives lacked any inhibitory effect on the tested *E. coli*. Evaluation of antibacterial effects of tetrahydropyridine derivatives on some bacterial pathogens by Prachayasittikul *et*

al. showed that amongst the tested bacteria such as *E. coli*, *A. hydrophila*, *P. aeruginosa*, *S. dysenteriae*, *S. typhi*, *M. catarrhalis*, *V. cholera*, *S. aureus*, *S. epidermidis*, *S. pyogenes* and *B. subtilis*, only one of the examined derivative had inhibitory effect on *M. catarrhalis* and *S. pyogenes*. In addition, this research indicated that tetrahydropyridine 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 *E. coli*, meanwhile some of imidazoline derivatives had the ability to inhibit gram negative bacteria like *Pseudomonas* and *E. coli* which could be due to the presence of compounds such as chlorine (21). One of the reasons for no effect of 3d-f derivatives is methyl nitroimidazole. The experiments have proven the potency of this substance to inhibit Enterobacteriaceae sp. like *P. vulgaris* and *P. mirabilis* and have shown that this derivative could damage bacteria and can lead to death by production of free radicals. Such effect was not seen in 3d-f derivatives (22).

The only inhibitory effect in this research was related to 6d derivative of thiazole and no inhibitory effects were seen from 6a-c derivatives of thiazole. Study of the structure of this compound shows beside existence of thiazole ring, there are two major structures; 1) is the thiazolidine ring in the thiazolidine derivatives which has a broad-spectrum activities on bacteria such as *E. coli* and *S. aureus*. Here, we also have confirmed its inhibitory effect on *E. coli* with MIC of 6.25 - 25 µg/ml (23) and 2) the other major structure is oxygen linked to thiazole ring (oxothiazole) amongst 6a-d derivatives which is only present in 6d compound. The inhibitory power of oxothiazole-containing compounds has also been proven on *E. coli* which is in accordance with our results (24). Bondoc *et al*. have shown the inhibitory effect of thiazole derivatives on *E. coli* with MIC of 6.25 - 25 µg/ml and possibly due to existence of chlorine as trichlorophenyl, the inhibitory potency of these derivatives has been increased in comparison to 6d derivative 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 thiazoles can be used against quinolone-resistant bacteria or to have 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 in accordance to our study. Cheng *et al*. in 2013 showed the in-vitro potency of thiazole derivatives to inhibit *E. coli* bacteria by measuring MIC (27), Shah *et al*. (28) and Bondoc *et al*. (29) Juspin *et al*. (30), Sarojini *et al*. (31) have all reported the in-vitro activity of thiazole derivatives on *E. coli* by measuring of MIC.

Conclusion

By proving inhibitory potency of novel thiazole compound on standard strain of *E. coli*, the next step is to determine its effect on multiple drug resistant *E. coli* bacteria isolated from various places and patients.

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Conflict of interest

The authors report no conflicts of interest.

References

1. Jallat C, Darfeuille-Michaud A, Rich C, *et al*. Survey of clinical isolates of diarrhoeogenic *Escherichia coli*: diffusely

adhering *E. coli* strains with multiple adhesive factors. *Res Microbiol* 1994; **145**(8): 621–632.
2. Sahni G, Gopinath P, Jeevanandam P. A novel thermal decomposition approach to synthesize hydroxyapatite–silver nanocomposites and their antibacterial action against GFP-expressing antibiotic resistant *E. coli*. *Colloids Surf B* 2013; **103**: 441–447.
3. Bakavoli M, Beyzaei H, Rahimizadeh M, *et al*. Regioselective synthesis of new 2-(E)-cyano(thiazolidin-2-ylidene)thiazoles. *Molecules* 2009; **14**: 4849-4857.
4. Jaishree V, Ramdas N, Sachin J, *et al*. In-vitro antioxidant properties of new thiazole derivatives. *J Saudi Chem Soc* 2012; **16**(4): 371-376.
5. Helal MH, Salem MA, El-Gaby MS, *et al*. Synthesis and biological evaluation of some novel thiazole compounds as potential anti-inflammatory agents. *Eur J Med Chem* 2013; **65**: 517-526.
6. Venugopla KN, Krishnappa M, Nayak SK, *et al*. Synthesis and anti-mosquito properties of 2,6-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, *et al*. Synthesis and anti-trypanosoma activity of new 6,6,7-trisubstituted thiopyrano[2,3-d][1,3] thiazoles. *Bioorg Med Chem Lett* 2012; **22**(23): 7071-7074.
8. Chimenti F, Bizzarri B, Bolasco A, *et al*. Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles anti-Candida spp. agents. *Eur J Med Chem* 2011; **46**(1): 378-382.
9. Ghasemi B, Najimi M, Jalaei J. Evaluation of antibacterial effects of benzothiazole derivatives on bacterial food pathogens. *Iran J Med Microbiol* 2015; **9**(1): 35-41.
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**(6): 516-519.
11. Robert JM, Sabourin C, Alvarez N, *et al*. Synthesis and anti-leishmanial activity of new imidazolidin-2-one derivatives. *Eur J Med Chem* 2003; **38**(7-8): 711-718.
12. Wittine K, Stipković Babić M, Makuc D, *et al*. Novel 1,2,4-triazole and imidazole derivatives of L-ascorbic and imino-ascorbic acid: Synthesis, anti-HCV and antitumoractivity evaluations. *Bioorg Med Chem* 2012; **20**(11): 3675-3685.
13. Salhi L, Bouzroura-Aichouche S, Benmalek Y, *et al*. An efficient conversion of maleimide derivatives to 2-thioxo imidazolidinones. *Org Commun* 2013; **6**(2): 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**(4): 446-449.
15. Long S, Stefani FR, Biondi S, *et al*. N-Heteroarylmethyl-5-hydroxy-1,2,5,6-tetrahydropyridine-3-carboxylic acid a novel scaffold for the design of funcompetitive α-glucosidase inhibitors. *Bioorg Med Chem* 2013; **21**(18): 5811‑5822.
16. Martin HL, Mounsey RB, Sathe K, *et al*. A peroxisome proliferator-activated receptor-δ agonist provides neuroprotection in the1-methyl-4-pehnyl-1,2,3,6-tetrahydorpyridine model of Parkinson's disease. *Neuroscience* 2013; **240**: 191-203.
17. Aridoss G, Amirthaganesan S, Tae Jeong Y. Synthesis, crystal and antibacterial studies of diversely functionalized tetrahydropyridin-4-ol. *Bioorg Med Chem Lett* 2010; **20**(7): 2242‑2249.
18. Beyzaei H, Aryan R, Gomroki M. Synthesis of novel heterocyclic 2-(2-ylidene)malononitrile derivatives. *Org Chem Indian J* 2015; **11**(1): 10-13.
19. Karegoudar P, Karthikeyan MS, Prasad DJ, *et al*. Synthesis of some novel 2,4-disubstituted thiazoles as possible

antimicrobial agents. *Eur J Med Chem* 2008; **43**(2): 261-267.
20. Prachayasittikul, S, Pingaew, R, Worachartcheewan, A, *et al*. A new sulfoxide analog of 1,2,3,6- tetrahyarophenylpyridine and antimicrobial activity. *Excli Journal* 2010; **9**: 102-107.
21. Jamal abdul Nasser A, Idhayadhulla A, Surendra Kumar R, *et al*. Synthesis and biological activities of new series of imidazolidin-2,4-dione derivatives. *Asian J Chem* 2010; **22**(8): 5853-5858.
22. Shahid HA, Jahangir S, Yousuf S, *et al*. Synthesis, crystal structure, structural characterization and in vitro antimicrobial activities of 1-methyl-4-nitro-1H-imidazole. *Arabian J Chem* 2014; Doi: 10.1016/j.arabjc.2014.11.001 [In Press].
23. Bozdağ-Dündar O, Ozgen O, Menteşe A, *et al*. Synthesis and antimicrobial activity of some new thiazolyl thiazolidine-2,4-dione derivatives. *Bioorg Med Chem* 2007; **15**(18): 6012–6017.
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**(1-3): 76–85.
25. Brvar M, Perdih A, Oblak M, *et al*. In silico discovery of 2-amino-4-(2,4-dihydroxyphenyl) thiazoles as novel inhibitors of DNA gyrase B. *Bioorg Med Chem Lett* 2010; **20**(3): 958–962.
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**(23): 6750–6754.
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**(14): 4235–4238.
28. Shah NK, Shah NM, Patel MP, *et al*. Synthesis, characterization and antimicrobial activity of some new biquinoline derivatives containing a thiazole moiety. *Chinese Chem Lett* 2012; **23**(4): 454–457.
29. Bondock S, Khalifa W, Fadda AA. 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**(7): 948-954.
30. Juspin T, Laget M, Terme T, *et al*. TDAE-assisted synthesis of new imidazo[2,1-b] thiazole derivatives as anti-infectious agents. *Eur J Med Chem* 2010; **45**(2): 840–845.
31. Sarojini BK, Krishna BG, Darshanraj CG, *et al*. Synthesis, characterization, in vitro and molecular docking studies of new 2,5-dichloro thienyl substituted thiazole derivatives for antimicrobial properties. *Eur J Med Chem* 2010; **45**(8): 3490-6.