Antibacterial effect of thiazole derivatives on *Rhodoccocus equi*, *Brucella abortus*, and *Pasteurella multocida*

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Abstract:

BACKGROUND: *Rhodoccocus equi*, *Brucella abortus*, and *Pasteurella multocida* are important veterinary bacterial pathogens that in recent years have been resisted to current antibiotics, and this problem threats the livestock industry. To control this resistant microorganisms, use of new antibacterial compounds, such as thiazole derivatives, in veterinary is necessary. **OBJECTIVES:** In this study, antibacterial effects of thiazole derivatives on *Rhodoccocus equi*, *Brucella abortus*, and *Pasteurella multocida* were evaluated. **METHODS:** Synthesized thiazole derivatives were prepared in DMSO, then the disk diffusion method was used to measure growth inhibition zone diameter and the broth microdilution method was applied to determine the minimum inhibitory concentration (MIC). **RESULTS:** Results showed that thiazole derivatives had no significant inhibitory effects on *B. abrotus*, while they had inhibitory effects on *R. equi* and *P. multocida* with inhibition zone 12.7±0.4 -30.1±0.2 mm and MICs 32- 256 µg/ml. **CONCLUSIONS:** Results of this study indicate that thiazole derivatives have considerable inhibitory effects on *R. equi* and *P. multocida* as veterinary bacterial pathogens.

Introduction

Rhodoccocus equi, *Brucella abrotus*, and *Pasteurella multocida* are important veterinary bacterial pathogens that cause high mortality rates and economic losses in the livestock industry (Adesiyun et al., 2011; Bakavoli et al., 2009; Bakavoli et al; 2011). Development of bacterial resistant in these microorganisms to current antibiotics, such as ciprofloxacin, trimethoprim, and tetracycline have caused serious problems in veterinary in recent years and use of new antibacterial compounds is the best solution for this problem (Bondock et al., 2010; Bondock et al., 2013; Brvar et al., 2010). One of these novel antimicrobials is thiazole derivatives. These derivatives perceive to have multi-therapeutic effects and they have been utilized in treatment of cancer, blood fat, blood pressure, and HIV infection (Chementi et al., 2011). Strong antioxidant and anti-inflammatory effects of thiazoles have been proven (Cheng et al., 2013; Coleman et al., 2010).

In laboratory, thiazoles have shown the ability to kill anopheles insects (Helul et al., 2013). Thiazole derivatives have inhibitory effects on Trypanosoma and *Candida* spp. (Horohov et al., 2011; Jaishree et al., 2013). Thiazole derivatives can inhibit the growth of a variety of gram-positive and gram-negative bacteria, including *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*,

Streptococcus pyogenes, *Enterococcus faecalis* and *Pseudomonas fluorescens*. Strong and wide range of antibacterial properties of thiazole derivatives has generally made the antibacterial test to be among the initial experiments that is studied after synthesizing these agents in many countries (Juspin et al., 2010; Katsuda et al., 2013. Very few studies on thiazole antibacterial effects against these veterinary bacterial pathogens have been published. In the current study, the antibacterial effects of thiazole derivatives on *R. equi*, *P. multocida*, and *B. abortus* were assessed.

Materials and Methods

Preparation of thiazole derivatives: The number 6a-d of thiazole derivative was incorporated in a three-phase process and its chemical structure was verified with monocrystalline X-ray diffraction, HNMR, CNMR, IR, element decomposition, and spectrometry. Afterwards, this derivative was dissolved in the DMSO solvent with the concentration of 8192 µg/ml (7).

Synthesis of 2-[(E)-(benzo[d]thiazol-2(3H)-ylidene)(cyano)methyl]thiazoles (10a-c); the number 10a-c of thiazole derivative was incorporated in a three-phase process and its chemical structure was verified with monocrystalline X-ray diffraction, HNMR, CNMR, IR, element decomposition, and spectrometry. Afterwards, this derivative was dissolved in the DMSO solvent with the concentration of 8192 µg/ml (Khalil et al., 2009).

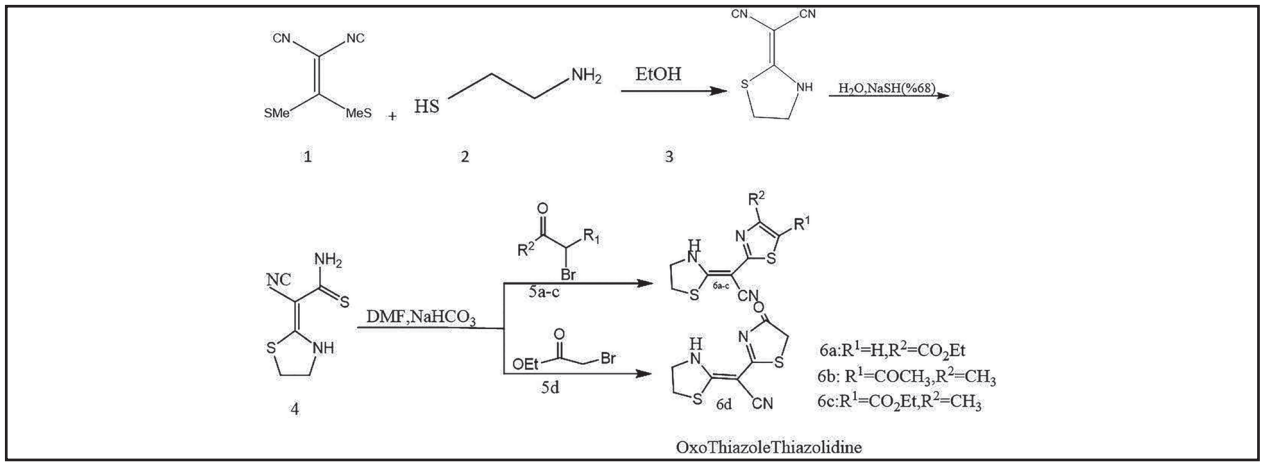
Bacterial suspensions: *Rhodococcus equi* ATCC 33701, *P. multocida* ATCC 12948, and *B. abortus* ATCC 23448 were provided by the Faculty of Veterinary Medicine, University of Shiraz. Each bacterium was cultured in Mueller-Hinton agar medium in 37°C for 24 hours. Henceforth, in sterile conditions of Mueller-Hinton medium and in logarithmic growth phase, a concentration of 0.5 McFarland (1.5 × 108 CFU/ml) was obtained with spectrophotometer and standard McFarland tube number 0.5 from each bacterium which is assigned as a stock solution (Kofteridis et al., 2009).

Minimum inhibition concentration (MIC): The MIC test was done in a sterile 96-well plates by broth micro dilution as CLSI standard. First, 100 µl of Muller-Hinton broth medium (Merck®, Germany) was added to each well. Then, 100 µl of thiazole derivatives (in control groups, 100 µl of penicillin and gentamycin antibiotics (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 the last well in each row. The result of incubation was read after 24 hours incubation in 37°C. The lucidity and turbidity in each well indicated lack or existence of bacterial growth, respectively. The last well that did not show any turbidity was reported as MIC (Kofteridis et al., 2009).

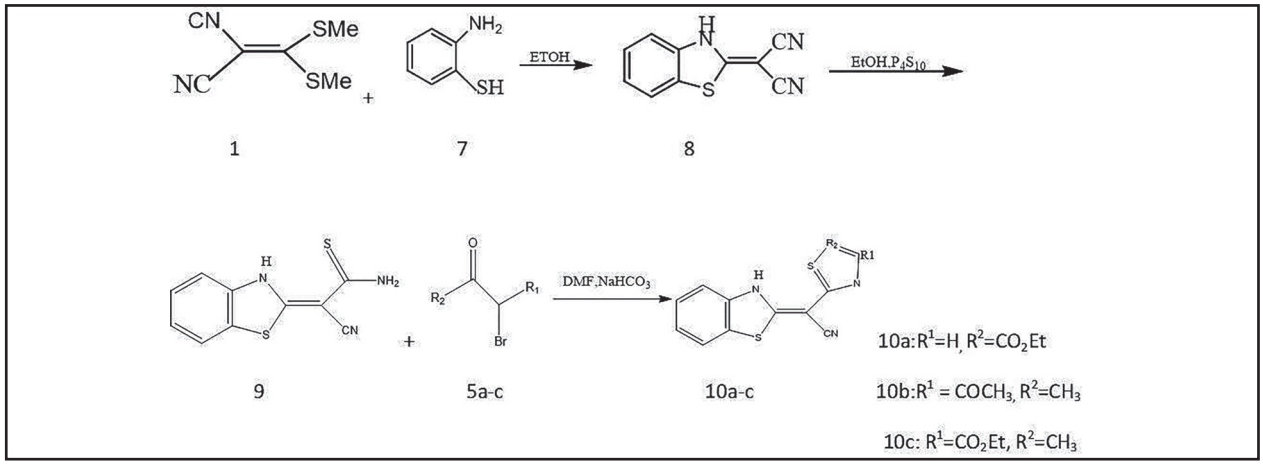
Inhibition zone diameter: First, in Muller-Hinton agar medium, the superficial bacterial culture was performed with a swab impregnated to bacterial suspension. 20 µl of obtained MIC thiazole derivatives and also antibiotics was shed on blank sterile disks. For negative control, the DMSO-impregnated disk was used. Then, after 24 hours incubation in 37°C, the growth inhibition zone diameter was measured with particular ruler. The results of growth inhibition zone diameter have been provided as average ± standard deviation and for the aim of analyzing the data, the SPSS statistical software (version 22) was used (Kofteridis et al., 2009).

Results

Thiazole derivatives showed no significant inhibitory effects on *B. abortus*; also 6a-c, 10a and 10c did not have inhibitory effects on any



Scheme 1. Synthesis of 2-(E)-cyano(thiazolidin-2-ylidene)thiazoles (6a-d) (derivative from reference No. 7).



Scheme2. Synthesis of 2-[(E)-(benzo[d]thiazol-2(3H)-ylidene)(cyano)methyl]thiazoles (10a-c) (Bakavoli et al., 2015).

bacterial tested. Just the two derivatives 6d and 10b showed inhibitory effects on *R. equi* and *P. multocida*. The maximum inhibitory effects on *R. equi* and *P. multocida* belonged to derivatives 6d and 10b with MIC of 64 and 32 µg/ml respectively. Ampicillin and neomycin had the maximum and minimum inhibitory effects on *R. equi*, respectively and penicillin and nalidixic acid had the maximum and minimum inhibitory effects on *P. multocida*, respectively. In antibiogram test, the most and the least susceptibility were recorded for *P. multocida* to penicillin with MIC of 0.5 µg/ml and for *B. abortus* to penicillin with MIC of 16 µg/ml (Tables 1 and 2).

Discussion

Thiazole derivatives are novel antibacterial compounds which promise good replacements for some antibacterial drugs. In the current study, inhibitory effects of eight thiazole derivatives have been assessed on three veterinary bacterial pathogens. Results show that the maximum inhibitory effect against *R. equi* belongs to derivative 6d. The structural study of this derivative shows that this compound includes thiazolidine ring as well as thiazole ring. This thiazolidine ring is similar to that of penicillin family of antibiotics. However, this derivative is expected to affect beta-lactamase producing bacteria due to the lack of a beta-lactam ring (Lv et al., 2009).

Thiazolidines are a novel class of antibacterial agents which include inhibitory effects on a broad-spectrum of gram-positive bacteria, such as streptococci and staphylococci. The inhibitory effect of thiazolidine derivatives on *S.*

Table 1. Bacterial growth inhibitory zone (mm) of the thiazole derivatives and antibiotics on studied bcteria. - absence of inhibition effect.

Derivatives/Drugs	*R. equi* ATCC 33701	*P. multocida* ATCC 12948	*B. abortus* ATCC 23448
6a-c	-	-	-
6d	25.6±0.1	26.3±0.0	-
10a	-	-	-
10b	12.7±0.4	30.1±0.2	-
10c	-	-	-
Gentamicin	25.4±0.3	21.2±0.0	16.3±0.2
Penicillin	27.2±0.5	30.5±0.3	22.1±0.1

Table 2. MICs (µg/ml) of thiazole derivatives and antibiotics on studied bcteria. - absence of inhibition effect.

Derivatives/ Drugs	*R. equi* ATCC 33701	*P. multocida* ATCC 12948	*B. abortus* ATCC 23448
6a-c	-	-	-
6d	64	64	-
10a	-	-	-
10b	256	32	-
10c	-	-	-
Gentamicin	1	8	2
Penicillin	2	0.5	16

faecalis and *S. aureus* has been proven (Majiduddin et al., 2002). Furthermore, the study of derivatives 6a-c has shown that only derivative 6d contains oxygen bonds with thiazole, resulting in the production of oxothiazole. Moreover, this derivative is the only compound within derivatives 6a-d that includes inhibitory effects on *R. equi* and *P. multocida*. Zaky and Yousef have shown that oxothiazole derivatives are able to inhibit the growth of *E. coli* (Patel et al., 2012).

Benzo[d]thiazole derivative 10b had a powerful inhibition on *P. multocida*. The important structure in this compound is benzothiazole. Antibacterial effect on gram-negtive bacteria, for example *Escherichia coli* and *Salmonella typhi*, were shown from derivatives which have benzothiazole in their structure (Shirharsha 2006). MICs of thiazole derivatives have demonstrated the ability of these compounds to significantly inhibit the growth of *Pseudomonas aeruginosa*, *S. aureus*, and *B. subtilis*, compared to penicillin G and kanamycin.

Results have also shown that these derivatives have higher inhibition effects with MICs of 12.5-100 µg/ml. Derivatives within the current study possibly include excited Cl and Br in thiazole ring and, therefore, show intensified inhibitory effects (Venugolpa et al., 2013). In a study by Zaky and Yousef (2011), MIC and inhibition zone of thiazole derivatives on *S. aureus* and *P. aeruginosa* were assessed and showed high antibacterial effects, compared to gentamicin as control (Zaky and Yousef, 2011). Furthermore, high inhibitory effects of thiazole derivatives on bacterial pathogens, such as *Bacillus thuringiensis* and *E. coli*, as well as *S. aureus*, *S. pyogenes*, *Proteus vulgaris* and *Klebsiella pneumonia*, have been studied using growth inhibition zone (Zelisko et al., 2013; Zhang et al., 2013).

In recent studies, inhibition of DNA or enzyme has been proposed as the influential mechanism of thiazole derivatives to inhibit bacteria. The inhibition of one enzyme, ecKA-SIII (or FabH) that is essential for synthesis of fatty acids in gram-negative and gram-positive bacteria, and DNA Gyrase, that is needed to replicate DNA, has been studied. Noting that Quinolone family antibiotics and thiazole derivatives could inhibit subunit A and subunit B of DNA Gyrase enzyme, respectively, has increasingly promised the inhibition of Quinolone-resistant bacteria by thiazole derivatives (Zitouni et al., 2003).

Conclusion: Few studies have been published on antibacterial effects of thiazole derivatives against veterinary bacterial pathogens. In this study, antibacterial effect of thiazole derivatives was proved against *R. equi*, *P. multocida*, and clinical use of these compounds needs in vivo studies for detection therapeutic and toxicity effects of them.

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مطالعه اثر ضد باکتریایی مشتقات تیازول روی رودو کوکوس \کوئی، پاستورلا مولتی سید\و بروسلا \بورتوس

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چکیده

زمینه مطالعه: رودوکوکوس \کوئی، پاستورلا مولتی سید\و بروسلا \بورتوس از مهمترین باکتری‌های پاتوژن دامپزشکی محسوب شده که در سال‌های اخیر مقاومت این باکتری‌ها به آنتی بیوتیک‌های رایج باعث نگرانی‌هایی در صنعت دامپروری شده است. برای کنترل این باکتری‌ها، استفاده از ترکیبات ضد باکتریایی جدیدی چون مشتقات تیازول در دامپزشکی ضروری است. **هدف:** در این مطالعه به بررسی اثر ضد باکتریایی مشتقات جدید تیازول بر روی سه باکتری رودوکوکوس \کوئی، پاستورلا مولتی سید\و بروسلا \بورتوس پرداختیم. **روش کار:** پس از حل کردن مشتقات در DMSO، برای بررسی مقایسه اثر ضد باکتریایی، از روش انتشار در دیسک برای اندازه‌گیری قطر هاله مهار رشد و از روش براس میکرودایلوشن برای تعیین حداقل غلظت بازدارندگی رشد (MIC) استفاده شد. **نتایج:** اثر مهاری از هیچ کدام از مشتقات روی بروسلا \بورتوس مشاهده نشد اما قطر هاله مهار رشد ۰/۴mm±۱۲/۷تا ۰/۲±۳۱/۲ و MIC ۳۲ تا ۲۵۶ µg/ml برای اثر مهاری مشتقات روی رودوکوکوس \کوئی و پاستورلا مولتی سید\ ثبت گردید. **نتیجه‌گیری نهایی:** نتایج این تحقیق قدرت مهاری مشتقات تیازول را بر روی دو باکتری مهم دامپزشکی (رودوکوکوس \کوئی و پاستورلا مولتی سید\) اثبات می‌کند.

واژه های کلیدی: اثر ضد باکتریایی، بروسلا \بورتوس، پاستورلا مولتی سید\، رودوکوکوس \کوئی، مشتقات تیازول

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