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Antimicrobial properties of heterocyclic compounds against clinical mastitis isolates[1]

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ABSTRACT.- Acosta A.C., Cardoso M.V.O., Oliveira Filho G.B., Pinheiro Junior J.W., Leite A.C.L. & Mota R.A. 2021. **Antimicrobial properties of heterocyclic compounds against clinical mastitis isolates.** *Pesquisa Veterinária Brasileira 41:e06862, 2021.* Laboratório de Bacterioses dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: acabad80@gmail.com

Mastitis causes significant economic losses to the dairy cattle industry. The present study aimed to evaluate the antibacterial properties of 39 heterocyclic derivatives (1,3-thiazoles and 4-thiazolidinones) against clinical mastitis isolates from dairy cows. Milk samples were collected from cows with clinical mastitis and the bacterial species were identified by PCR. Antibacterial activity was assessed using the broth microdilution method. First, 39 heterocyclic compounds were tested against four bacterial isolates (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Escherichia coli*) randomly chosen from those recovered from the milk samples (Study 1). Subsequently, the compounds with the strongest antibacterial activity were tested against all the bacterial isolates recovered from the milk samples (Study 2). 1,3-thiazoles showed the strongest antibacterial activity, specially compounds 30 and 38, which also showed bactericidal properties according to their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values. *Corynebacterium* spp. and Enterobacteriaceae isolates were the most susceptible to compounds 30 and 38. Compounds 30 and 38 are promising targets for new antimicrobial agents.

INDEX TERMS: Heterocyclic compounds, clinical mastitis, thiazole derivatives, thiazolidinone derivatives, biological activity, antibacterial activity, MIC, MBC.

RESUMO.- [**Propriedades antimicrobianas de compostos heterocíclicos contra isolados de mastite clínica.**] A mastite causa significativas perdas econômicas à indústria leiteira bovina. O presente estudo teve como objetivo avaliar as propriedades antibacterianas de 39 derivados heterocíclicos (1,3-tiazóis e 4-tiazolidinonas) contra isolados clínicos de mastite em vacas leiteiras. Amostras de leite foram coletadas de vacas com mastite clínica e as espécies bacterianas isoladas foram identificadas por PCR. A atividade antibacteriana foi avaliada pelo método de microdiluição em caldo. Primeiramente, os 39 compostos heterocíclicos foram testados contra quatro isolados bacterianos (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* e *Escherichia coli*) escolhidos aleatoriamente dentre os recuperados das amostras de leite (Estudo 1). Posteriormente, compostos com atividade antibacteriana mais forte foram testados contra todos os isolados bacterianos recuperados das amostras de leite (Estudo 2). Os compostos 1,3-tiazóis apresentaram a maior atividade antibacteriana, principalmente os compostos 30 e 38, que também apresentaram propriedades bactericidas de acordo com seus valores de concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM). Os isolados *Corynebacterium* spp. e Enterobacteriaceae foram os mais suscetíveis aos compostos 30 e 38. Os compostos 30 e 38 mostraram-se promissores como novos agentes antimicrobianos.

TERMOS DE INDEXAÇÃO: Compostos heterocíclicos, mastite clínica, derivados de tiazóis, derivados de tiazolidinonas, atividade biológica, atividade antibacteriana, CIM, CBM.

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INTRODUCTION

Mastitis causes substantial economic losses to the dairy cattle industry worldwide (De Vliegher et al. 2012), due to reduced milk production, increased milk disposal and costs for therapy and replacement of animals (Seegers et al. 2003, Halasa et al. 2007). The aetiology of bovine mastitis involves several microbial agents and, in Brazil, *Staphylococcus* spp., *Corynebacterium* spp. and *Streptococcus* spp. are the most common mastitis causative pathogens (Acosta et al. 2016).

Antimicrobial therapy is an important tool for treating mastitis, but the overuse and misuse of antibiotics may lead to the selection of resistant strains, in particular when therapy is initiated before bacterial susceptibility testing (Thomas et al. 2015). Due to the increasing occurrence of antibiotic resistance, the development of new antimicrobial agents with novel mechanisms of action has become a hot topic (Qiu et al. 2001, Rafi et al. 2006, Qin et al. 2014).

The 1,3-thiazoles and 4-thiazolidinones nucleus are found in natural products and have been used to develop synthetic drugs and drug-like molecules with a variety of pharmacological properties. The biological activities of thiazole derivatives include: antibacterial (Abu-Melha et al. 2019, Tratrat et al. 2019), antifungal (Guo et al. 2019), antitubercular (Gundlewad & Patil 2018), antiviral (Güzeldemirci et al. 2018), antitumor (Shaik et al. 2017), anticonvulsant (Agarwal et al. 2018), anti-inflammatory (Sinha et al. 2018), antioxidant (Afifi et al. 2019), and antiparasitic (Aliança et al. 2017). Similarly, 4-thiazolidinones chemical properties and biological activities include anticancer (Gududuru et al. 2005), antiviral (Rawal et al. 2005), anti-inflammatory (Ottanà et al. 2005), analgesic (Ashour et al. 2016), antimicrobial (Liesen et al. 2010), antituberculosis (Babaoglu et al. 2003), antiparasitic (Moreira et al. 2012), among others.

Many researchers have used 1,3-thiazoles and 4-thiazolidinones to improve drug efficacy (Siddiqui et al. 2009, Ayati et al. 2015, Gomes et al. 2016). Therefore, the present study aimed to evaluate the antibacterial properties of 1,3-thiazoles and 4-thiazolidinones derivatives against clinical mastitis isolates from dairy cows.

MATERIALS AND METHODS

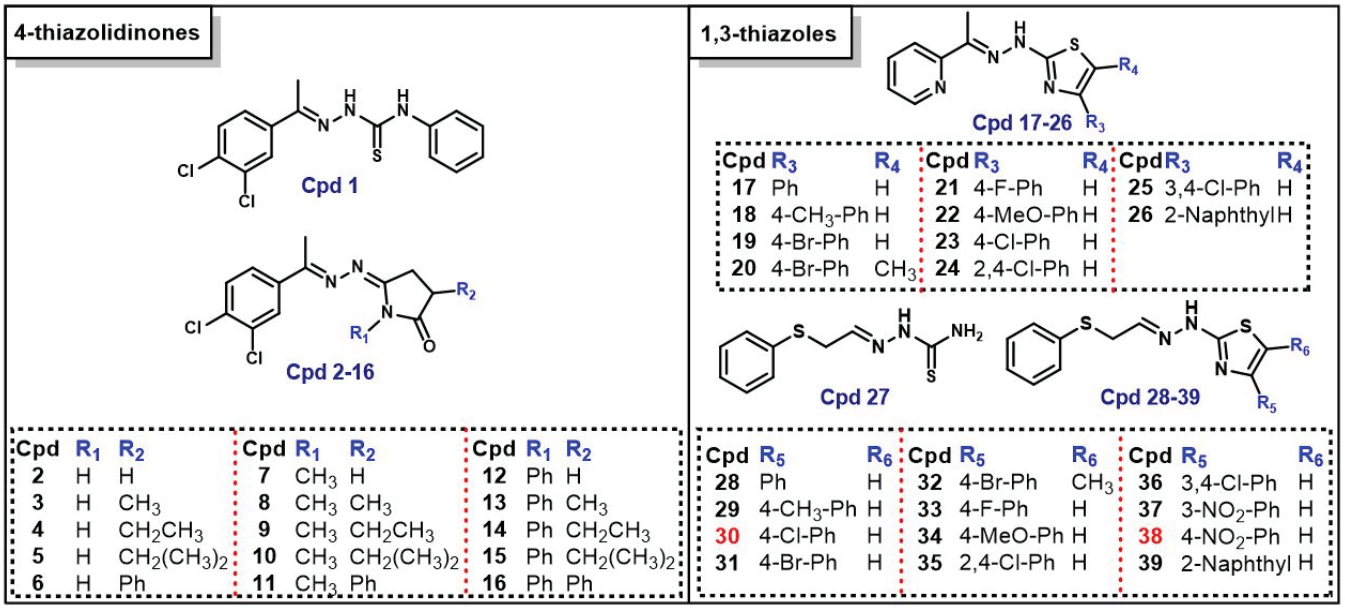
Ethical statement. The present study was approved by the Institutional Animal Care and Use Committee of the "Departamento de Medicina Veterinária" of the "Universidade Federal Rural de Pernambuco" (UFRPE), under the protocol number 079/2014-CEUA. All experimental procedures and the animal care were in strict accordance with the guidelines of the "Conselho Nacional de Controle de Experimentação Animal" (CONCEA; Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009).

Compounds. The 39 compounds used in the present study, 16 4-thiazolidinones (1-16) (Oliveira Filho et al. 2015) and 23 1,3-thiazols (17-39) (Cardoso et al. 2014), were obtained from the "Laboratório de Planejamento em Química Medicinal" of the "Universidade Federal de Pernambuco" (UFPE). The compounds' structures are presented in Figure 1.

Sample selection criteria. Milk samples were collected from 1000 cows from 24 bovine herds from the state of Pernambuco, Brazil as described by (Acosta et al. 2018). Only milk samples from cows with clinical signs of mastitis and that had not been treated with antibiotics for at least three weeks were used in the present study. The criteria for sample inclusion were milk flake, clots, pus or watery secretions and infected quarter showing clinical signs (swelling, heat or pain on palpation).

Milk samples were collected from individual quarters after disinfection of the ostium with 70% ethanol following the recommendations of the National Mastitis Council (1999), and transported to the laboratory under refrigeration (4-10°C).

Bacteriological culture. Primary cultures of milk samples were performed by plating 10µL of milk in 5% sheep blood agar and incubated aerobically at 37°C for 24 h to 72 h, considering growth bacterial time. Milk samples with three or more dissimilar colony types were considered contaminated (Hiitiö et al. 2015). Identification was based on standard phenotypic bacterial identification schemes that included colony and microscopic morphology, Gram stain, growth characteristics, catalase and coagulase activity (Hogan et al. 1999), and thermostable nuclease (TNase) (Rall et al. 2014). Gram negative bacteria were plated onto MacConkey agar (Merck) to



Cpd	R_1	R_2	Cpd	R_1	R_2	Cpd	R_1	R_2
2	H	H	7	CH_3	H	12	Ph	H
3	H	CH_3	8	CH_3	CH_3	13	Ph	CH_3
4	H	CH_2CH_3	9	CH_3	CH_2CH_3	14	Ph	CH_2CH_3
5	H	$CH_2(CH_3)_2$	10	CH_3	$CH_2(CH_3)_2$	15	Ph	$CH_2(CH_3)_2$
6	H	Ph	11	CH_3	Ph	16	Ph	Ph

Cpd	R_3	R_4	Cpd	R_3	R_4	Cpd	R_3	R_4
17	Ph	H	21	4-F-Ph	H	25	3,4-Cl-Ph	H
18	4-CH_3-Ph	H	22	4-MeO-Ph	H	26	2-Naphthyl	H
19	4-Br-Ph	H	23	4-Cl-Ph	H			
20	4-Br-Ph	CH_3	24	2,4-Cl-Ph	H			

Cpd	R_5	R_6	Cpd	R_5	R_6	Cpd	R_5	R_6
28	Ph	H	32	4-Br-Ph	CH_3	36	3,4-Cl-Ph	H
29	4-CH_3-Ph	H	33	4-F-Ph	H	37	3-NO_2-Ph	H
30	4-Cl-Ph	H	34	4-MeO-Ph	H	38	4-NO_2-Ph	H
31	4-Br-Ph	H	35	2,4-Cl-Ph	H	39	2-Naphthyl	H

Fig.1. Structures of the 4-thiazolidinones (1-16) (Oliveira Filho et al. 2015) and 1,3-thiazols (17-39) (Cardoso et al. 2014).

screen for *members* of *Enterobacterales order* (Saidani et al. 2018). All bacterial isolates were stored at -20°C in brain/heart infusion broth (Merck) supplemented with 50% glycerol.

DNA extraction and PCR assay. Bacterial species were identified by PCR. Colonies were freshly sub-cultured in brain/heart infusion broth (Merck) and incubated overnight at 37°C for 24 h. Subsequently, DNA was extracted using a commercial kit (Promega) following the manufacturer's instructions and stored at -20°C. All PCRs were performed in 0.2mL tubes with a final volume of 25µL using the protocol developed by Acosta et al. (2018). PCR primers and annealing temperature can be found in the Table 1. Simplex PCR method for species identification was principally used, and multiplex PCR assay for simultaneous detection of staphylococcal and streptococcal was performed using the protocol developed by Shome et al. (2011).

Antimicrobial susceptibility testing. Antibacterial activity was assessed using the broth microdilution method in 96-well microtiter plates (k12-096, KASVI, CH) as per Abu-Melha et al. (2019) and following the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2018) for the standard antibacterial drug tetracycline. Because broth microdilution method is costly, time-consuming and labor-intensive, the antimicrobials test was subdivided in two studies. First, were evaluated the antibacterial properties of all 39 heterocyclic compounds and tetracycline against four bacterial isolates randomly chosen from those recovered from the milk samples: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Escherichia coli* (Study 1). Then, the heterocyclic compounds with the strongest antibacterial activity were evaluated against all isolates recovered from the milk samples (Study 2).

Sub-cultures were performed in 5% ovine blood agar plates incubated aerobically at 37°C for 24 h. Suspensions were prepared in 0.9% NaCl (w/v) and were adjusted to achieve turbidity equivalent to a 0.5 McFarland standard (approximately 1 to 2 × 10^8 colony-forming units (CFU)/mL). The final inoculum size in Mueller-Hinton broth (Merck, Germany) dilution was approximately 5 × 10^5 CFU/mL.

Stock solutions of all compounds tested were prepared in dimethyl sulfoxide (DMSO) at 12.8mg/mL (final concentration). Antimicrobial susceptibility was determined using concentrations derived from serial 2-fold dilutions indexed to the base 2. The final dilutions ranged from 0.625 to 320µg/mL for all 39 compounds. Control groups included: culture media only, culture media + microorganism, and culture media + microorganism + DMSO (Fig.2). Tetracycline in sterile distilled water (0.25 to 128µg/mL) was used as reference.

Table 1. Sequences and characteristics of the oligonucleotide primers

Organisms	Primer name	Primer sequence (5′-3′)	Annealing temperature (°C)	Expected size (bp)	Reference
Staphylococcus aureus	nuc_F	GGTTCTGAAGATCCAACAGTAT	61	296	Acosta et al. (2017)
	nuc_R	GCTAAGCCACGTCCATATTTA			
Staphylococcus hyicus	hy-F1	CATTATATGATTTGAACGTG	56	793	Sasaki et al. (2010)
	hy-R1	GAATCAATATCGTAAAGTTGC			
Staphylococcus simulans	SSMF	AGCTTCGTTTACTTCTTCGATTGT	60	472	Shome et al. (2011)
	SSMR	AAAAGCACAAGCTCACATTGAC			
Staphylococcus epidermidis	SERF	AAGAGCGTGGAGAAAAGTATCAAG	60	130	Shome et al. (2011)
	SERR	TCGATACCATCAAAAAGTTGG			
Staphylococcus chromogenes	SCHS1F	GCGTACCAGAAGATAAACAAACTC	60	222	Shome et al. (2011)
	SCHS1R	CATTATTTACAACGAGCCATGC			
Staphylococcus haemolyticus	SHS1F	CAAATTAAATTCTGCAGTTGAGG	60	214	Shome et al. (2011)
	SHS1R	AGAGCCCCATTGTTCTTTGA			
Staphylococcus xylosus	Xyl F	AACGCGCAACGTGATAAAATTAATG	55	539	Morot-Bizot et al. (2004)
	Xyl R	AACGCGCAACAGCAATTACG			
Staphylococcus pseudintermedius	pse-F2	TRGGCAGTAGGATTCGTTAA	56	926	Sasaki et al. (2010)
	pse-R5	CTTTTGTGCTYCMTTTTGG			
Streptococcus agalactiae	STAGF	GCTAATACCGCATAAGAGTAATTAAC	60	317	Shome et al. (2011)
	STAGR	GGTAGATTTTCCACTCCTACCAA			
Streptococcus dysgalactiae	STDGF	GGGAGTGGAAAATCCACCAT	60	572	Shome et al. (2011)
	STDGR	AAGGGAAAGCCTATCTCTAGACC			
Streptococcus uberis	Su-F	AATTGGCATTCGTCGCGGTA	53	239	Ashraf et al. (2017)
	Su-R	GCATCCCTTCAACCACTTCAA			
Streptococcus parauberis	Spa 301	GCG ACG TGG GAT CAA ATA CT	57	918	Riffon et al. (2001)
	Spa 1219	TAC CAT TAC CTC TAA AGG TA			
Corynebacterium bovis	C_bo_F	GGTGTGGGGATCTTCCACGAT	60	224	Kim et al. (2014)
	C_bo_R	ACCACCTGTGAACAAGCCCA			
Corynebacterium pseudotuberculosis	PLD-F	ATAAGCGTAAGCAGGGAGCA	58	203	Pacheco et al. (2007)
	PLD-R1	ATCAGCGGTGATTGTCTTCC			
Escherichia coli	ECPF	GGTAACGTTTCTACCGCAGAGTTG	60	468	Shome et al. (2011)
	ECPR	CAGGGTTGGTACACTGTCATTACG			
Klebsiella pneumoniae	SHV-F	GCTGGCGGTACACGCCAGCCCG	55	995	Fonseca et al. (2017)
	DeoR-R	AGAAGCATCCTGCTGTGCG			

Data analysis. Minimal inhibitory concentration (MIC) is the lowest concentration able to inhibit any microbial growth (turbidity). Minimal bactericidal concentration (MBC) is the lowest concentration of an antibiotic required to kill a microorganism. MBC is determined by plating-out samples (Mueller-Hinton agar, Merck, Germany) of completely inhibited dilution cultures and assessing growth (static) or no-growth (cidal) after incubation (Cos et al. 2006). The MIC_{50}, MIC_{90} (MICs at which at least 50% and 90% of the isolates in a test population are inhibited, respectively) (Thomas et al. 2015) and MBC values were determined for all bacterial strains and compounds used in the study. Optical densities were measured by spectrophotometry at 600nm and a growth curve was constructed after measurements at 0 h and 12 h.

RESULTS

Twenty-seven samples of milk were analyzed from cows with clinical mastitis. Three milk samples (11.11%) were contaminated with three or more environmental bacteria concomitantly and were excluded from the study. Of the 24 remaining samples, 11 (40.74%), 4 (14.81%), 3 (11.11%), 3 (11.11%), 2 (7.4%) and 1 (3.7%) were initially identified as *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., Enterobacteriaceae, *Bacillus* spp. and *Micrococcus* spp., respectively.

Of the 39 heterocyclic compounds tested against the four randomly chosen isolates, 15 (38.46 %), 10 (25.64%), 7 (17.94%) and 7 (17.94%) showed antibacterial activity against *Escherichia coli*, *Streptococcus agalactiae, Staphylococcus aureus* and *Corynebacterium bovis*, respectively (Table 2 and 3). 1,3-thiazoles (17-39), specially compounds 17, 30, 38 and 39 (Table 3), showed the strongest antibacterial activity. All four isolates were susceptible to tetracycline (Table 2).

Due to their strong antimicrobial activity against *E. coli*, *S. agalactiae, S. aureus* and *C. bovis*, was decided to evaluate the MIC and MBC of compounds 30 and 38 against the 24 isolates recovered from the milk samples. Compounds 30 and 38 showed a MIC=MBC value or 2*MIC=MBC value against

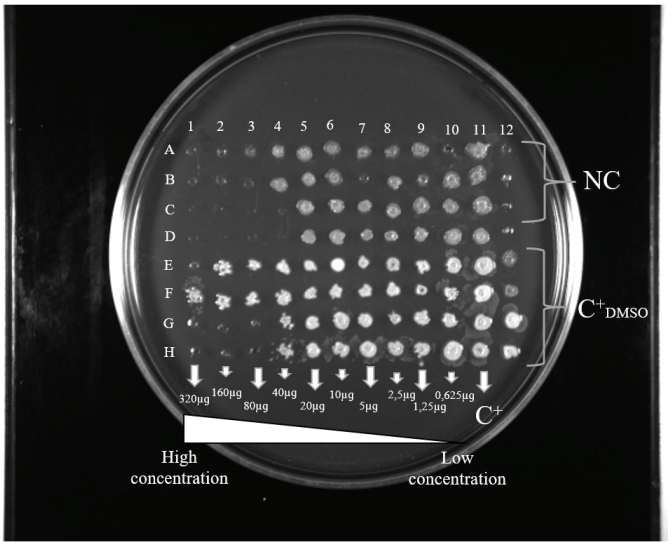


Fig.2. Minimal bactericidal concentration (MBC) of thiazoles compounds. NC (negative control), C^+ (positive growth control), C^+_{DMSO} (microorganism and solvent - DMSO). *Staphylococcus aureus* (A and B), *Streptococcus agalactiae* (C and D), *Corynebacterium bovis* (E and F) and *Escherichia coli* (G and H).

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 4-thiazolidinones derivatives compounds

Compounds	Clinical isolates																																							
	Staphylococcus aureus (n=1)												*Streptococcus agalactiae* (n=1)												*Corynebacterium bovis* (n=1)												*Escherichia coli* (n=1)					
	(µg/mL)												(µg/mL)												(µg/mL)												(µg/mL)					
	0.625	1.25	2.5	5	10	20	40	80	160	320	0.625	1.25	2.5	5	10	20	40	80	160	320	0.625	1.25	2.5	5	10	20	40	80	160	320	0.625	1.25	2.5	5	10	20	40	80	160	320
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tetracycline[a]	0.25	0.5	1	2	4	8	16	32	64	128	0.25	0.5	1	2	4	8	16	32	64	128	0.25	0.5	1	2	4	8	16	32	64	128	0.25	0.5	1	2	4	8	16	32	64	128

MIC value is indicated by vertical black line and MBC value is indicated by the negative symbol in the gray area; [a] Only the MIC values are shown for tetracycline.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 1,3-thiazoles derivatives compounds

Clinical isolates

Compounds	*Staphylococcus aureus* (n=1) (µg/mL) 0.625	1.25	2.5	5	10	20	40	80	160	320
17	+	+	+	+	+	+	+	+	-	-
18	+	+	+	+	+	+	+	+	+	-
19	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	-
21	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	-
31	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+
34	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+	+	+
38	+	+	+	+	+	+	+	+	-	-
39	+	+	+	+	+	+	+	+	+	-

Compounds	*Streptococcus agalactiae* (n=1) (µg/mL) 0.625	1.25	2.5	5	10	20	40	80	160	320
17	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	-
22	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	-
24	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	-
27	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	-
31	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+
34	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	+	+
36	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+	+	+
38	+	+	+	+	+	+	+	-	-	-
39	+	+	+	+	+	+	+	+	+	-

Compounds	*Corynebacterium bovis* (n=1) (µg/mL) 0.625	1.25	2.5	5	10	20	40	80	160	320
17	+	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	+	+
21	+	+	+	+	+	+	+	+	+	+
22	+	+	+	+	+	+	+	+	+	+
23	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	+
27	+	+	+	+	+	+	+	+	+	+
28	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	+	-
31	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+
34	+	+	+	+	+	+	+	+	+	+
35	+	+	+	+	+	+	+	+	-	-
36	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+	+	+
38	+	+	+	+	+	+	+	+	-	-
39	+	+	+	+	+	+	+	+	+	+

Compounds	*Escherichia coli* (n=1) (µg/mL) 0.625	1.25	2.5	5	10	20	40	80	160	320
17	+	+	+	+	+	+	+	-	-	-
18	+	+	+	+	+	+	+	-	-	-
19	+	+	+	+	+	+	+	+	+	-
20	+	+	+	+	+	+	+	-	-	-
21	+	+	+	+	+	+	-	-	-	-
22	+	+	+	+	+	+	+	+	+	-
23	+	+	+	+	+	+	+	+	+	+
24	+	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	+	+	+
26	+	+	+	+	+	+	+	+	+	-
27	+	+	+	+	+	+	+	+	+	-
28	+	+	+	+	+	+	+	-	-	-
29	+	+	+	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+	+	-	-
31	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
33	+	+	+	+	+	+	+	+	+	+
34	+	+	+	+	+	+	+	+	+	-
35	+	+	+	+	+	+	+	+	+	-
36	+	+	+	+	+	+	+	+	+	+
37	+	+	+	+	+	+	+	+	+	+
38	+	+	+	+	+	+	+	-	-	-
39	+	+	+	+	+	+	+	+	-	-

MIC value is indicated by vertical black line and MBC value is indicated by the negative symbol in the gray area.

most isolates (Table 4), and compound 38 displayed the most potent *in vitro* antibacterial activity against all isolates tested, with a MIC_{50} value of 80µg/mL, a MIC_{90} value of 160µg/mL and a MBC value of 40µg/mL (Table 4).

Corynebacterium spp. and Enterobacteriaceae isolates were the most susceptible to the antibacterial activity of compounds 30 and 38. Compound 30 showed MBC values of 40 and 80µg/mL against *Corynebacterium* spp. and Enterobacteriaceae, respectively, and MIC value of 40µg/mL for both isolates. Compound 38 showed MIC values of 10 and 40µg/mL against *Corynebacterium* spp. and Enterobacteriaceae, respectively, and MBC value of 40µg/mL against these two isolates (Table 4).

DISCUSSION

Due to their many biological activities, including antibacterial, 1,3-thiazoles and 4-thiazolidinones have attracted considerable attention over the years (Qin et al. 2014, Reddy et al. 2016, Abu-Melha et al. 2019, Tratrat et al. 2019). In the present study, two 1,3-thiazoles derivatives, compounds 30 and 38, showed significant antimicrobial activity (Table 4) with MIC_{50} values of 160µg/mL and 80µg/mL, respectively, which are similar to those previously observed for metronidazole-thiazole derivatives by Qin et al. (2014).

Bacteriostatic agents inhibit the growth of bacterial cells but do not kill them, whereas bactericidal agents kill (French 2006). Agents that inhibit ribosome function and protein synthesis tend to be bacteriostatic, whereas those that disrupt the cell wall or membrane, or interfere with essential bacterial enzymes, are likely to be bactericidal (French 2006). Bactericidal agents are more advantageous because of the rapid elimination of bacteria and decreased possibility of resistance development or infection recurrence (Finberg et al. 2004).

In the present study, compounds 30 and 38 showed MIC_{90} values of 360µg/mL and 160µg/mL, respectively, and MBC values of 40µg/mL and 360µg/mL (Table 4), respectively, what demonstrates their bactericidal properties. Similarly,

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of compounds 30 and 38 against the 24 clinical isolates recovered

Clinical isolates	Compound 30[a] (µg/mL)										Compound 38[b] (µg/mL)									
	0.625	1.25	2.5	5	10	20	40	80	160	320	0.625	1.25	2.5	5	10	20	40	80	160	320
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
S. aureus	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-
S. aureus	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
Staphylococcus simulans	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
Staphylococcus xylosus	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
Staphylococcus spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Staphylococcus spp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Staphylococcus hyicus	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
Staphylococcus simulans	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
S. simulans	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
S. hyicus	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-
Streptococcus uberis	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-
Streptococcus agalactiae	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-
S. agalactiae	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-
Streptococcus spp.	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-	-
Corynebacterium bovis	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-
Corynebacterium spp.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
C. bovis	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
Escherichia coli	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	-	-
Enterobacteriaceae	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-
Enterobacteriaceae	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	-
Bacillus spp.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-
Bacillus spp.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-
Micrococcus spp.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-

[a] Compound 1,3-thiazole derivative R_5 (4-Cl-Ph), R_6 (H), [b] compound 1,3-thiazole derivative R_5 (4-NO_2-Ph), R_6 (H); MIC value is indicated by vertical black line and MBC value is indicated by the negative symbol in the gray area.

Reddy et al. (2016) has shown the bactericidal properties of thiazole derivatives against *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Proteus vulgaris* with MIC values ranging from 6.25 to 100µg/mL and MBC values ranging from 50 to 200µg/mL.

Since the literature is not conclusive regarding the bacteria species or clones responsible for the clinical or subclinical cases of bovine mastitis (Ronco et al. 2018, Cheng et al. 2019, Nüesch-Inderbinen et al. 2019), in the present study was evaluated the antimicrobial properties of the heterocyclic compound against isolates recovered from cows with clinical mastitis as an attempt to be more representative of a real life situation.

The identification of species level is an important task for veterinary diagnostic laboratories. In this study, the most frequently identified species in cows with clinical mastitis were *S. aureus* (n=3), *Staphylococcus simulans* (n=3), *Staphylococcus hyicus* (n=2), *Streptococcus agalactiae* (n=2) and *Corynebacterium bovis* (n=2) (Table 4). It is difficult to discriminate between species based on phenotypic differences because there is a lack of unique biochemical markers for species identification in special for coagulase-positive staphylococci (Sasaki et al. 2007). Molecular diagnostics by PCR have the ability to identify the organism with great sensitivity and specificity and can also distinguish between very closely related organisms. These molecular diagnostic methods have many advantages over the traditional bacteriology techniques in terms of low cost and accurate detection (Ashraf et al. 2017).

Because of the importance of antimicrobial therapies and antibiotic resistance for human and animal health (Ball 1999, Ball et al. 2002), the development of new antimicrobial agents (Qiu et al. 2001, Rafi et al. 2006, Qin et al. 2014) and studies that monitor the pathogens and their antibiotic resistance status (Saidani et al. 2018, Schabauer et al. 2018) are of extreme importance. Although the MIC and MBC results found in the present study do not reflect the actual antimicrobial susceptibility of the pathogens studied, the results should serve as a reference baseline for future studies and development of new antimicrobial agents.

CONCLUSION

The antibacterial compounds presenting a 1,3-thiazoles nucleus showed the strongest antibacterial activity. Two compounds, 30 and 38, showed bactericidal effects against several of the bacterial isolates evaluated (*Staphylococcus aureus* [n=3], *Staphylococcus simulans* [n=3], *Staphylococcus hyicus* [n=2], *Staphylococcus xylosus* [n=1], *Streptococcus agalactiae* [n=2], *Streptococcus uberis* [n=1], *Streptococcus* spp. [n=1], *Corynebacterium bovis* [n=2], *Corynebacterium* spp. [n=1], *Escherichia coli* [n=1], Enterobacteriaceae [n=2], *Bacillus* spp. [n=2] and *Micrococcus* spp. [n=1]), being promising targets for the development of new broad-spectrum antimicrobial agents.

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REFERENCES

Abu-Melha S., Edrees M.M., Salem H.H., Kheder N.A., Gomha S.M. & Abdelaziz M.R. 2019. Synthesis and biological evaluation of some novel thiazole-based heterocycles as potential anticancer and antimicrobial agents. Molecules 24(3):539. <https://dx.doi.org/10.3390/molecules24030539> <PMid:30717217>

Acosta A.C., Oliveira P.R.F., Albuquerque L., Silva I.F., Medeiros E.S., Costa M.M., Pinheiro Junior J.W. & Mota R.A. 2018. Frequency of *Staphylococcus aureus* virulence genes in milk of cows and goats with mastitis. Pesq. Vet. Bras. 38(11):2029-2036. <https://dx.doi.org/10.1590/1678-5150-PVB-5786>

Acosta A.C., Santos S.J., Albuquerque L., Soares K.D.A., Mota R.A. & Medeiros E.S. 2017. Frequência de genes codificadores de toxinas em *Staphylococcus aureus* isolados de leite de tanques expansão comunitários. Pesq. Vet. Bras. 37(7):691-696. <https://dx.doi.org/10.1590/S0100-736X2017000700007>

Acosta A.C., Silva L.B.G.d., Medeiros E.S., Pinheiro-Júnior J.W. & Mota R.A. 2016. Mastites em ruminantes no Brasil. Pesq. Vet. Bras. 36(7):565-573. <https://dx.doi.org/10.1590/S0100-736X2016000700001>

Afifi O.S., Shaaban O.G., Abd El Razik H.A., Shams El-Dine S.E.-D.A., Ashour F.A., El-Tombary A.A. & Abu-Serie M.M. 2019. Synthesis and biological evaluation of purine-pyrazole hybrids incorporating thiazole, thiazolidinone or rhodanine moiety as 15-LOX inhibitors endowed with anticancer and antioxidant potential. Bioorg. Chem. 87:821-837. <https://dx.doi.org/10.1016/j.bioorg.2019.03.076> <PMid:30999135>

Agarwal S., Kalal P., Gandhi D. & Prajapat P. 2018. Thiazole containing Heterocycles with CNS activity. Curr. Drug Discov. Technol. 15(3):178-195. <https://dx.doi.org/10.2174/1570163814666170724170152> <PMid:28745208>

Aliança A.S.d.S., Oliveira A.R., Feitosa A.P.S., Ribeiro K.R.C., Castro M.C.A.B., Leite A.C.L., Alves L.C. & Brayner F.A. 2017. *In vitro* evaluation of cytotoxicity and leishmanicidal activity of phthalimido-thiazole derivatives. Eur. J. Pharm. Sci. 105:1-10. <https://dx.doi.org/10.1016/j.ejps.2017.05.005> <PMid:28478133>

Ashour H.M.A., El-Ashmawy I.M. & Bayad A.E. 2016. Synthesis and pharmacological evaluation of new pyrazolyl benzenesulfonamides linked to polysubstituted pyrazoles and thiazolidinones as anti-inflammatory and analgesic agents. Monatsh Chem. 147:605-618. <https://dx.doi.org/10.1007/s00706-015-1549-x>

Ashraf A., Imran M., Yaqub T., Tayyab M., Shehzad W. & Thomson P.C. 2017. A novel multiplex PCR assay for simultaneous detection of nine clinically significant bacterial pathogens associated with bovine mastitis. Mol. Cell. Probes 33:57-64. <https://dx.doi.org/10.1016/j.mcp.2017.03.004> <PMid:28336361>

Ayati A., Emami S., Asadipour A., Shafiee A. & Foroumadi A. 2015. Recent applications of 1,3-thiazole core structure in the identification of new lead compounds and drug discovery. Eur. J. Med. Chem. 97:699-718. <https://dx.doi.org/10.1016/j.ejmech.2015.04.015> <PMid:25934508>

Babaoglu K., Page M.A., Jones V.C., McNeil M.R., Dong C., Naismith J.H. & Lee R.E. 2003. Novel inhibitors of an emerging target in *Mycobacterium tuberculosis*; substituted thiazolidinones as inhibitors of dTDP-rhamnose synthesis. Bioorg. Med. Chem. 13(19):3227-3230. <https://dx.doi.org/10.1016/s0960-894x(03)00673-5> <PMid:12951098>

Ball P. 1999. Therapy for pneumococcal infection at the millennium: doubts and certainties. Am. J. Med. 107(1A):77S-85S. <https://dx.doi.org/10.1016/s0002-9343(99)00104-7> <PMid:10451013>

Ball P., Baquero F., Cars O., File T., Garau J., Klugman K., Low D., Rubinstein E. & Wise R. 2002. Antibiotic therapy of community respiratory tract infections: strategies for optimal outcomes and minimized resistance emergence. J. Antimicrob. Chemother. 49(1):31-40. <https://dx.doi.org/10.1093/jac/49.1.31> <PMid:11751764>

Cardoso M.V., Siqueira L.R.P., Silva E.B., Costa L.B., Hernandes M.Z., Rabello M.M., Ferreira R.S., Cruz L.F., Moreira D.R.M., Pereira V.R.A., Castro M.C.A.B., Bernhardt P.V. & Leite A.C.L. 2014. 2-Pyridyl thiazoles as novel anti-

Trypanosoma cruzi agents: structural design, synthesis and pharmacological evaluation. Eur. J. Med. Chem. 86:48-59. <https://dx.doi.org/10.1016/j.ejmech.2014.08.012> <PMid:25147146>

Cheng J., Qu W., Barkema H.W., Nobrega D.B., Gao J., Liu G., Buck J., Kastelic J.P., Sun H. & Han B. 2019. Antimicrobial resistance profiles of 5 common bovine mastitis pathogens in large Chinese dairy herds. J. Dairy Sci. 102(3):2416-2426. <https://dx.doi.org/10.3168/jds.2018-15135> <PMid:30639013>

CLSI 2018. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. CLSI document M07-A11, 11th ed., Vol. 38, Clinical and Laboratory Standards Institute, USA, p.112.

Cos P., Vlietinck A.J., Berghe D.V. & Maes L. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J. Heterocycl. Chem. 106(3):290-302. <https://dx.doi.org/10.1016/j.jep.2006.04.003> <PMid:16698208>

De Vliegher S., Fox L.K., Piepers S., McDougall S. & Barkema H.W. 2012. Invited review: mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. J. Dairy Sci. 95(3):1025-1040. <https://dx.doi.org/10.3168/jds.2010-4074> <PMid:22365187>

Finberg R.W., Moellering R.C., Tally F.P., Craig W.A., Pankey G.A., Dellinger E.P., West M.A., Joshi M., Linden P.K., Rolston K.V., Rotschafer J.C. & Rybak M.J. 2004. The importance of bactericidal drugs: future directions in infectious disease. Clin. Infect. Dis. 39(9):1314-1320. <https://dx.doi.org/10.1086/425009> <PMid:15494908>

Fonseca E.L., Ramos N.d.V., Andrade B.G.N., Morais L.L.C.S., Marin M.F.A. & Vicente A.C.P. 2017. A one-step multiplex PCR to identify *Klebsiella pneumoniae*, *Klebsiella variicola*, and *Klebsiella quasipneumoniae* in the clinical routine. Diagn. Microbiol. Infect. Dis. 87(4):315-317. <https://dx.doi.org/10.1016/j.diagmicrobio.2017.01.005> <PMid:28139276>

French G. 2006. Bactericidal agents in the treatment of MRSA infections - the potential role of daptomycin. J. Antimicrob. Chemother. 58(6):1107-1117. <https://dx.doi.org/10.1093/jac/dkl393> <PMid:17040922>

Gomes M.P.A.T., Barbosa M.O., Farias Santiago E., Cardoso M.V.O., Costa N.T.C., Hernandes M.Z., Moreira D.R.M., Silva A.C., Dos Santos T.A.R., Pereira V.R.A., Dos Santosd F.A.B., Pereira G.A.N., Ferreira R.S. & Leite A.C.L. 2016. New 1,3-thiazole derivatives and their biological and ultrastructural effects on *Trypanosoma cruzi*. Eur. J. Med. Chem. 121:387-398. <https://dx.doi.org/10.1016/j.ejmech.2016.05.050> <PMid:27295485>

Gududuru V., Hurh E., Dalton J.T. & Miller D.D. 2005. Discovery of 2-arylthiazolidine-4-carboxylic acid amides as a new class of cytotoxic agents for prostate cancer. J. Med. Chem. 48(7):2584-2588. <https://dx.doi.org/10.1021/jm049208b> <PMid:15801848>

Gundlewad G.B. & Patil B.R. 2018. Synthesis and evaluation of some novel 2-amino-4-aryl thiazoles for antitubercular activity. J. Heterocycl. Chem. 55(3):769-774. <https://dx.doi.org/10.1002/jhet.3098>

Guo X., Zhao B., Fan Z., Yang D.-y., Zhang N., Wu Q., Yu B., Zhou S., Kalinina T. & Belskaya N.P. 2019. Discovery of novel thiazole carboxamides as antifungal succinate dehydrogenase inhibitors. J. Agricult. Food. Chem. 67(6):1647-1655. <https://dx.doi.org/10.1021/acs.jafc.8b06935> <PMid:30669828>

Güzeldemirci N.U., Pehlivan E. & Naesens L. 2018. Synthesis and antiviral activity evaluation of new 4-thiazolidinones bearing an imidazo [2, 1-b] thiazole moiety. Marmara Pharm. J. 22(2)237-248. <https://dx.doi.org/10.12991/MPJ.2018.61>

Halasa T., Huijps K., Østerås O. & Hogeveen H. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet. Q. 29(1):18-31. <https://dx.doi.org/10.1080/01652176.2007.9695224> <PMid:17471788>

Hiitiö H., Riva R., Autio T., Pohjanvirta T., Holopainen J., Pyörälä S. & Pelkonen S. 2015. Performance of a real-time PCR assay in routine bovine mastitis diagnostics compared with in-depth conventional culture. J. Dairy Res. 82(2):200-208. <https://dx.doi.org/10.1017/S0022029915000084> <PMid:25704849>

Hogan J., Gonzalez R., Harmon R., Nickerson S., Oliver S., Pankey J. & Smith K.L. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI, p.6-10.

Kim T.-H., Kim D.-S., Han J.-H., Chang S.-N., Kim K.-S., Seok S.-H., Kim D.-J., Park J.-H. & Park J.-H. 2014. Detection of *Corynebacterium bovis* infection in athymic nude mice from a research animal facility in Korea. J. Vet. Sci. 15(4):583-586. <https://dx.doi.org/10.4142/jvs.2014.15.4.583> <PMid:24962412>

Liesen A.P., Aquino T.M., Carvalho C.S., Lima V.T., Araújo J.M., Lima J.G., Faria A.R., Melo E.J.T., Alves A.J., Alves E.W., Alves A.Q. & Góes A.J.S. 2010. Synthesis and evaluation of anti-*Toxoplasma gondii* and antimicrobial activities of thiosemicarbazides, 4-thiazolidinones and 1,3,4-thiadiazoles. Eur. J. Med. Chem. 45(9):3685-3691. <https://dx.doi.org/10.1016/j.ejmech.2010.05.017> <PMid:20541294>

Moreira D.R.M., Costa S.P.M., Hernandes M.Z., Rabello M.M., Oliveira Filho G.B., Melo C.M.L., Rocha L.F., Simone C.A., Ferreira R.S., Fradico J.R.B., Meira C.S., Guimarães E.T., Srivastava R.M., Pereira V.R.A., Soares M.B.P. & Leite A.C.L. 2012. Structural investigation of anti-*Trypanosoma cruzi* 2-iminothiazolidin-4-ones allows the identification of agents with efficacy in infected mice. J. Med. Chem. 55(24):10918-10936. <https://dx.doi.org/10.1021/jm301518v> <PMid:23167554>

Morot-Bizot S.C., Talon R. & Leroy S. 2004. Development of a multiplex PCR for the identification of *Staphylococcus* genus and four staphylococcal species isolated from food. J. Appl. Microbiol. 97(5):1087-1094. <https://dx.doi.org/10.1111/j.1365-2672.2004.02399.x> <PMid:15479426>

National Mastitis Council 1999. Laboratory and field handbook on bovine mastitis. National Mastitis Council, Madison.

Nüesch-Inderbinen M., Käppeli N., Morach M., Eicher C., Corti S. & Stephan R. 2019. Molecular types, virulence profiles and antimicrobial resistance of *Escherichia coli* causing bovine mastitis. Vet. Rec. Open. 6(1):e000369. <https://dx.doi.org/10.1136/vetreco-2019-000369> <PMid:31897302>

Oliveira Filho G.B., Cardoso M.V.O., Espíndola J.W.P., Ferreira L.F.G.R., Simone C.A., Ferreira R.S., Coelho P.L., Meira C.S., Moreira D.R.M., Soares M.B.P. & Lima Leite A.C. 2015. Structural design, synthesis and pharmacological evaluation of 4-thiazolidinones against *Trypanosoma cruzi*. Bioorg. Med. Chem. 23(23):7478-7486. <https://dx.doi.org/10.1016/j.bmc.2015.10.048> <PMid:26549870>

Ottanà R., Maccari R., Barreca M.L., Bruno G., Rotondo A., Rossi A., Chiricosta G., Di Paola R., Sautebin L., Cuzzocrea S. & Vigorita M.G. 2005. 5-Arylidene-2-imino-4-thiazolidinones: design and synthesis of novel anti-inflammatory agents. Bioorg. Med. Chem. 13(13):4243-4252. <https://dx.doi.org/10.1016/j.bmc.2005.04.058> <PMid:15905093>

Pacheco L.G.C., Pena R.R., Castro T.L.P., Dorella F.A., Bahia R.C., Carminati R., Frota M.N.L., Oliveira S.C., Meyer R., Alves F.S.F., Miyoshi A. & Azevedo V. 2007. Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. J. Clin. Microbiol. 56(Pt 4):480-486. <https://dx.doi.org/10.1099/jmm.0.46997-0> <PMid:17374887>

Qin Y.-J., Wang P.-F., Makawana J.A., Wang Z.-C., Wang Z.-N., Yan G., Jiang A.-Q. & Zhu H.-L. 2014. Design, synthesis and biological evaluation of metronidazole-thiazole derivatives as antibacterial inhibitors. Bioorg. Med. Chem. 24(22):5279-5283. <https://dx.doi.org/10.1016/j.bmcl.2014.09.054> <PMid:25318998>

Qiu X., Janson C.A., Smith W.W., Head M., Lonsdale J. & Konstantinidis A.K. 2001. Refined structures of β-ketoacyl-acyl carrier protein synthase III. J. Mol. Biol. 307(1):341-356. <https://dx.doi.org/10.1006/jmbi.2000.4457>

Rafi S.B., Cui G., Song K., Cheng X., Tonge P.J. & Simmerling C. 2006. Insight through molecular mechanics Poisson-Boltzmann surface area calculations into the binding affinity of triclosan and three analogues for FabI, the *E. coli* enoyl reductase. J. Med. Chem. 49(15):4574-4580. <https://dx.doi.org/10.1021/jm060222t> <PMid:16854062>

Rall V.L.M., Miranda E.S., Castilho I.G., Camargo C.H., Langoni H., Guimarães F.F., Araújo Júnior J.P. & Fernandes Júnior A. 2014. Diversity of *Staphylococcus* species and prevalence of enterotoxin genes isolated from milk of healthy cows and cows with subclinical mastitis. J. Dairy Sci. 97(2):829-837. <https://dx.doi.org/10.3168/jds.2013-7226> <PMid:24359821>

Rawal R.K., Prabhakar Y.S., Katti S.B. & De Clercq E. 2005. 2-(Aryl)-3-furan-2-ylmethyl-thiazolidin-4-ones as selective HIV-RT Inhibitors. Bioorg. Med. Chem. 13(24):6771-6776. <https://dx.doi.org/10.1016/j.bmc.2005.07.063> <PMid:16198576>

Reddy G.M., Garcia J.R., Reddy V.H., Andrade A.M., Camilo Jr A., Ribeiro R.A.P. & Lazaro S.R. 2016. Synthesis, antimicrobial activity and advances in structure-activity relationships (SARs) of novel tri-substituted thiazole derivatives. Eur. J. Med. Chem. 123:508-513. <https://dx.doi.org/10.1016/j.ejmech.2016.07.062> <PMid:27494167>

Riffon R., Sayasith K., Khalil H., Dubreuil P., Drolet M. & Lagacé J. 2001. Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J. Clin. Microbiol. 39(7):2584-2589. <https://dx.doi.org/10.1128/JCM.39.7.2584-2589.2001> <PMid:11427573>

Ronco T., Klaas I.C., Stegger M., Svennesen L., Astrup L.B., Farre M. & Pedersen K. 2018. Genomic investigation of *Staphylococcus aureus* isolates from bulk tank milk and dairy cows with clinical mastitis. Vet. Microbiol. 215:35-42. <https://dx.doi.org/10.1016/j.vetmic.2018.01.003> <PMid:29426404>

Saidani M., Messadi L., Soudani A., Daaloul-Jedidi M., Châtre P., Chehida F.B., Mamlouk A., Mahjoub W., Madec J.-Y. & Haenni M. 2018. Epidemiology, antimicrobial resistance, and extended-spectrum beta-lactamase-producing Enterobacteriaceae in clinical bovine mastitis in Tunisia. Microb. Drug. Resist. 24(8):1242-1248. <https://dx.doi.org/10.1089/mdr.2018.0049> <PMid:29757079>

Sasaki T., Kikuchi K., Tanaka Y., Takahashi N., Kamata S. & Hiramatsu K. 2007. Reclassification of phenotypically identified Staphylococcus intermedius strains. J. Clin. Microbiol. 45(9):2770-2778. <https://dx.doi.org/10.1128/JCM.00360-07> <PMid:17596353>

Sasaki T., Tsubakishita S., Tanaka Y., Sakusabe A., Ohtsuka M., Hirotaki S., Kawakami T., Fukata T. & Hiramatsu K. 2010. Multiplex-PCR method for species identification of coagulase-positive staphylococci. J. Clin. Microbiol. 48(3):765-769. <https://dx.doi.org/10.1128/JCM.01232-09> <PMid:20053855>

Schabauer A., Pinior B., Gruber C.-M., Firth C.L., Käsbohrer A., Wagner M., Rychli K. & Obritzhauser W. 2018. The relationship between clinical signs and microbiological species, spa type, and antimicrobial resistance in bovine mastitis cases in Austria. Vet. Microbiol. 227:52-60. <https://dx.doi.org/10.1016/j.vetmic.2018.10.024> <PMid:30473352>

Seegers H., Fourichon C. & Beaudeau F. 2003. Production effects related to mastitis and mastitis economics in dairy cattle herds. Vet. Res. 34(5):475-491. <https://dx.doi.org/10.1051/vetres:2003027> <PMid:14556691>

Shaik S.P., Nayak V.L., Sultana F., Rao A.V.S., Shaik A.B., Babu K.S. & Kamal A. 2017. Design and synthesis of imidazo[2,1-b]thiazole linked triazole conjugates: microtubule-destabilizing agents. Eur. J. Med. Chem. 126:36-51. <https://dx.doi.org/10.1016/j.ejmech.2016.09.060>

Shome B.R., Das Mitra S., Bhuvana M., Krithiga N., Velu D., Shome R., Isloor S., Barbuddhe S.B. & Rahman H. 2011. Multiplex PCR assay for species identification of bovine mastitis pathogens. J. Appl. Microbiol. 111(6):1349-1356. <https://dx.doi.org/10.1111/j.1365-2672.2011.05169.x> <PMid:21972842>

Siddiqui N., Arshad M.F., Ahsan W. & Alam M.S. 2009. Thiazoles: a valuable insight into the recent advances and biological activities. Int. J. Pharm. Sci. Drug Res. 1(3):136-143.

Sinha S., Doble M. & Manju S. 2018. Design, synthesis and identification of novel substituted 2-amino thiazole analogues as potential anti-inflammatory agents targeting 5-lipoxygenase. Eur. J. Med. Chem. 158:34-50. <https://dx.doi.org/10.1016/j.ejmech.2018.08.098> <PMid:30199704>

Thomas V., Jong A., Moyaert H., Simjee S., El Garch F., Morrissey I., Marion H. & Vallé M. 2015. Antimicrobial susceptibility monitoring of mastitis pathogens isolated from acute cases of clinical mastitis in dairy cows across Europe: VetPath results. Int. J. Antimicrob. Agents 46(1):13-20. <https://dx.doi.org/10.1016/j.ijantimicag.2015.03.013> <PMid:26003836>

Tratrat C., Haroun M., Xenikakis I., Liaras K., Tsolaki E., Eleftheriou P., Petrou A., Aldhubiab B., Attimarad M., Venugopala K., Harsha S., Elsewedy H.S., Geronikaki A. & Soković M. 2019. Design, synthesis, evaluation of antimicrobial activity and docking studies of thiazole-based chalcones. Curr. Top. Med. Chem. 19(5):356-375. <https://dx.doi.org/10.2174/1568026619666190129121933> <PMid:30706816>