

RESEARCH ARTICLE

Thiazolidines; The Potential Antimicrobial Agents against Methicillin-Resistant Strains of *Staphylococcus aureus*

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Abstract: 2-Substituted 1,3-Thiazolidine-4-Carboxylic Acids (**1-11**) were synthesized and screened for their anti-methicillin-resistant *Staphylococcus aureus* (MRSA) potential. The synthesized compounds were evaluated for their antibacterial activities against four MRSA strains MRSA I, VI, VII and VIII with accession numbers KU662352, KR862285, KR862291 and KU662354 respectively by well diffusion method. In addition, antibacterial evaluations were also performed for gram positive strain *Bacillus subtilis* and gram-negative strains *klebsiella pneumoniae* and *pseudomonas aeruginosa* using same method. Most of the synthesized thiazolidine-4-carboxylic acid derivatives exhibited better antibacterial activities against studied bacterial strains. Amongst the synthesized compounds, **8-10** were found to possess significant activity (Zone in mm) against methicillin-resistant *Staphylococcus aureus* in addition to the other studied bacterial strains.

Keywords: antimicrobial, L-cystiene, bacterial strains, MRSA

Introduction

The use of antibacterial agents for treatment of infectious diseases is indispensable. Various natural, synthetic and semi-synthetic antimicrobial drugs have been discovered and used in the clinical practice but increasing use of antimicrobials has resulted in the emergence of resistant pathogens including Gram-positive bacteria methicillin-resistant *Staphylococcus aureus* (MRSA) [1]. MRSA infection is a problem causing challenging issues to the global health care [2]. The studies have shown that the MRSA cases have increased to several folds in

the past few years [3]. Emergence of resistance is an alarming issue globally. These resistant bacteria complicated microbial diseases making them hard to tackle as MRSA can easily develop resistance to commonly used antibiotics including macrolides, fluoroquinolones, lincosamides and aminoglycosides [4]. The *S. aureus* population's structure is primarily clonal and MLST (multi locus sequence typing) which results in ST and CC i-e sequence types and clonal complexes respectively. This system is used to provide framework for sketching the origin, evolution and spread of MRSA [5-6]. It

was basically a hospital-associated MRSA, also known as HA-MRSA (nosocomial pathogen), but in 1990s the infections were seen on the patients which were not sick previously, and also the infections then were not related to any health care settings. Then the pathogen was referred to as CA-MRSA (community-associated MRSA) [6]. MRSA infections that occurs in a community can also be instigated by livestock associated MRSA also known as LA-MRSA. This type of infection is different in genotype from HA-MRSA and CA-MRSA genomic traits [7]. The infections caused by MRSA imposes an increasing stress on health care budget, along with the increased mortality and morbidity rate [8]. In America alone the MRSA has killed many people and hospitalized ~20,000. These stats are on par with the number of combined deaths caused by AIDS, viral hepatitis and the tuberculosis [9].

In spite of significant developments in the antimicrobial therapy, the discovery and development of novel antimicrobial agents with increased activity towards resistant strains are highly desirable. In modern medicinal chemistry, a small molecule is used as a core scaffold for the purpose of designing new drugs depending on the pharmacological activity of the molecule. One of such promising frameworks are thiazolidine [10] pyrazoline and pyrazole nucleus [11]. 1,3 Thiazolidine is a significant platform to be connected with different biological potentials that include bactericidal [12], fungicidal [13], anti-inflammatory, histamine inhibition [14] and antihypertensive activities [15]. Various studies have also been reported showing antimicrobial potential of thiazolidines [12]. In present work, a series of thiazolidine 4- carboxylic acids (1-11) were synthesized and evaluated for their antibacterial potential against MRSA.

Results and discussion

The synthesis of compounds (1-11) are outlined in Scheme 1. Compounds 1-11 were obtained from L-cysteine in reaction with different (het)aryl aldehydes in ethanol. These compounds were previously reported in the literature and have proven to be antioxidant [16], antimicrobial and enzyme inhibitory agents [17]. No studies have been carried out to explore biological potential of thiazolidine carboxylic acids against MRSA (Table 1). All compounds synthesized in current study have been evaluated against MRSA and encouraging results are attained. It is a well-known fact that 2-substituted-1,3-thiazolidine-4-carboxylic acids are obtained as mixture of (2*S*, 4*R*) and (2*R*, 4*R*) stereo isomers. All the obtained thiazolidines have (*R*)-amino acid chirality (C4-ring). During the process of thiazolidine ring formation the additional asymmetrical center appears at C2-atom. The structures of synthesized compounds were characterized by IR, ¹H-NMR, ¹³C-NMR and in comparison, with literature. All synthesized compounds were screened for antibacterial activities against methicillin resistant *staphylococcus aureus* strains and also for gram positive *Bacillus subtilis* and gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by well diffusion method. Zones of Inhibition are measured in mm, results for anti MRSA evaluation are shown in Figure 1 while for other bacterial strains are shown in Figure 2. It was found that many of the synthesized compounds showed activity against MRSA strains. Nitro substituted compounds 8-10 were found most effective for all four strains studied.

11		-	-	14	-
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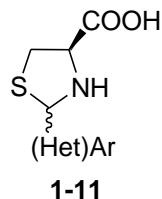


Table 1: Antimicrobial Activities of 1,3-Thiazolidine-4-Carboxylic Acids against MRSA strains (Zone of inhibition in mm)

Comp.	(Het)Ar	MRSA I	MRSA VI	MRSA VII	MRSA VIII
1		10	10	NP	NP
2		NP	NP	15	-
3		-	-	-	-
4		-	-	-	15
5		12	12	-	14
6		-	-	-	8
7		-	13	-	-
8		10	12	19	12
9		15	15	15	14
10		22	14	21	20

General trend observed in antimicrobial activity was $p\text{-NO}_2 > m\text{-NO}_2 > o\text{-NO}_2$ i.e., among nitro substituted compounds best results were obtained for 2-(4-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid (**10**). The antibacterial activity of compound **1-11** against the four strains is summarized in Table 1. Among other compounds better results were observed for 2-(4-chlorophenyl)-1,3-thiazolidine-4-carboxylic acid (**5**). According to the observed results for *B. subtilis*, *P. aeruginosa* and *K. pneumonia*, compound **10** showed results comparable to standard drug i.e., Ciproflaxacin (Table 2).

Table 2: Antimicrobial Activities of 1,3-Thiazolidine-4-Carboxylic Acids against *Bc*, *Ps*, *Kleb* strains (Zone of Inhibition in mm)

Comp.	(Het)Ar	Bc	Ps	Kleb
1		9	16	10
2		13	14	13
3		14	13	13
4		12	15	17
5		12	18	16
6		20	-	-
7		16	16	10
8		15	13	12
9		18	14	12
10		25	20	18

11		11	13	12
Ciprofloxacin		30	28	9

Bc = *Bacillus subtilis*; Ps = *Pseudomonas aeruginosa*; Kleb = *Klebsiella pneumoniae*

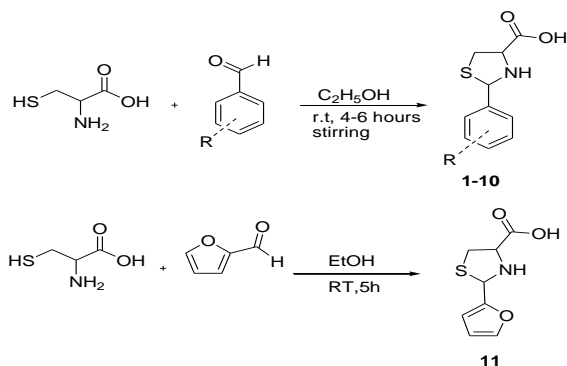
Experimental

Melting points are uncorrected. All chemicals are provided by sigma and used without further purification. Optical rotations of the formulated compounds were recorded on digital polarimeter model ADP410, code# 37-410 and serial # PX14052 by Bellingham Stanley Ltd. Bruker AM-400 and 500 MHz were used to record $^1\text{H-NMR}$ spectra and 100 MHz or 125 MHz to record $^{13}\text{C-NMR}$ spectra respectively, on the same instruments.

Synthesis of 2-aryl substituted thiazolidine-4-carboxylic acids:

Compounds 1-11 were synthesized following procedure reported by Gududuru [18]. A mixture of L-cysteine (2 g, 16.5 mmol) and appropriate (het)arylaldehyde (16.5 mmol) in EtOH (30 ml) was stirred at room temperature for 2-5 h. Solid formed was separated by filtration and washed with diethyl ether, dried to get compounds 1-11 (yields 60-90%). All synthesized compounds were epimeric ($2R,4R/2S,4R$) mixtures.

Scheme 1



Scheme 1: Synthesis of 2-aryl-1,3-thiazolidine-4-carboxylic acids

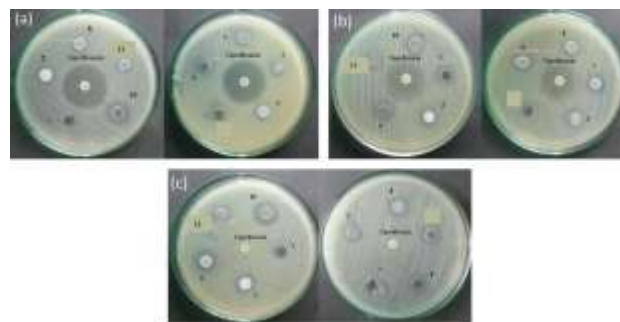


Figure 1: Antimicrobial evaluation of Thiazolidines against (a) *Bacillus subtilis* (Bc) b) *Pseudomonas aeruginosa* (Ps) (c) *Klebsiella pneumoniae* (Kleb) Cp: Ciprofloxacin

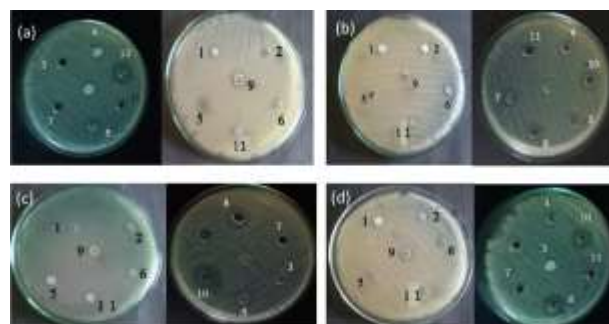


Figure 2: Antimicrobial Evaluation against MRSA strains a) MRSA-I (KU662352) b) MRSA-VI (KR862285) c) MRSA-VII (KR862291) d) MRSA-VIII (KU662354)

Method for antibacterial bioassay

Bacterial Strains Preparation:

The bacterial strains used in this bioassay were MRSA I, MRSA VI, MRSA VII and MRSA VIII *B. subtilis*, *P. aeruginosa* and *K. pneumoniae*. All strains were provided by Dr. Imran Sajid, Department of MMG, University of the Punjab University. The bacterial strains were refreshed from Glycerol stock by using Nutrient broth (Sigma). 50ml of sterilized nutrient broth in 100ml conical flask was used to introduce 100 ul of each bacterial strain. These strains were grown on shaking incubator for 24 hours. For this bioassay, bacterial strains were cultured on Muller Hinton agar medium (Sigma) [19].

Assay for antibacterial activity:

All the Petri plates were autoclaved and 10-15ml Muller Hinton agar medium was poured in each plate. After all the plates had set at room temperature, sterilized cotton swabs were used to inoculate cell suspension of each bacterial strain (10⁸ cfu/ml) over agar medium surface. In each plate, 5 or 6 wells of 8mm were scooped by using sterilized cork borer and micropipette with sterilized tips were utilized to add solutions of the test compounds (80 mmol) into the wells. DMSO was used as negative control and 48 hours of incubation period was provided to these plates at 37°C. After this period, zone of inhibition was recorded in mm against each bacterial strain (Figure 1 & 2)

Conclusion

2-Substituted 1,3-Thiazolidine-4-Carboxylic Acids were synthesized and evaluated for their antibacterial activities against MRSA as well as for gram positive and gram-negative strains. Amongst the synthesized compounds, nitro substituted compounds exhibited best results where 2-(4-nitrophenyl)-1,3-thiazolidine-4-carboxylic acid (10) was found to possess significant activity (Zone in mm) against methicillin-resistant *Staphylococcus aureus* in addition to the other studied bacterial strains.

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