

**Original Article****In Vitro Antimicrobial Activity of New Substituted Phenylthiazole Derivatives**Maryam Kouhkan<sup>\*1</sup>, Ali Souldozi<sup>2</sup>, Reza Talebi<sup>3</sup>

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**ABSTRACT**

**Background:** In this study, we evaluated antibacterial and antifungal activity of these derivatives against gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*), gram negative (*Proteus vulgaris*) bacteria and fungale (*Candida albicans*, *C. tropicalis* and *C. glabrata*).

**Methods:** The broth macro dilution and well agar diffusion methods were used for determination of inhibition zoom (IZ) and minimum inhibitory concentration (MIC) during preliminary evaluation of antimicrobial activity.

**Results:** The (MIC) values of tested compounds revealed that all compounds were active against *Staphylococcus aureus* and exhibited the same antibacterial activity in comparison to ceftizoxim (MIC=125µg/mL). The results of antifungal screening showed that all the compounds were potent antimicrobial activity against tested pathogenic fungi (MIC=250 to 1000 µg/ml). Compounds (6a) and (6b) showed the maximum activity with MIC value of 250 µg/ml against all tested fungi, but compound (6h) showed poor antifungal activity against all fungi. MIC values for title compounds were similar to MIC results for fluconazol.

**Conclusions:** All the compounds are potent antifungal and antibacterial activity against tested microorganism.

**Keywords:** Amino Thiazol, Antimicrobial Activity, Antifungal Activity, Minimum Inhibitory Concentration.

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**INTRODUCTION**

Overuse of antibiotics has made the number of resistant microorganisms rise during the past 30 years. Except a limited number of antibiotics, for example erythromycin and vancomycin, resistance was developed against majority of antibiotics only a few years after their introduction into clinical use [1]. The attempt to find such compounds with high antimicrobial activity in the treatment of drug resistant infections may be a good replacement to overcoming the problem of resistance in bacteria. The thiazole nucleus appears frequently in the structure of various natural products and biologically active compounds, like thiamine (vitamin-B) in some antibiotics drugs like penicillin and cephalosporin drugs [2].

Due to increasing of microbial resistance towards the existing antibiotics, new chemical compounds have been synthesized by thiazole with different aromatic substituted with a wide range of biological activities such as anticancer [3-5], antibacterial [6], antifungal [7], anti-inflammatory [8], antitubercular

[9], cardiotoxic [10] and antidegenerative activity on cartilage [11] etc. Already, we synthesised Alkyl 2-(Dialkylamino)-4 phenylthiazole-5-carboxylates derivatives (6a-4l) in good yields [12]. As synthesis and evaluation of antimicrobial activity is an important part of our research program [13, 14].

In this study, we evaluated antibacterial and antifungal activity of these derivatives against gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*), gram-negative (*Proteus vulgaris*) bacteria and fungal (*Candida albicans*, *C. tropicalis* and *C. glabrata*).

**MATERIAL AND METHODS****Compounds 6a-6l**

General Procedure: Benzoyl isothiocyanate 1 and secondary amine 2 in dry CH<sub>2</sub>Cl<sub>2</sub> was added drop wise a mixture of dialkyl acetylenedicarboxylates 4 in dry CH<sub>2</sub>Cl<sub>2</sub> at room temperature over 2 min. Then, after 0.5 h, SiO<sub>2</sub> powder (2g) was added and the solvent was evaporated. The dry materials were heated for 1 h at 90 °C and then placed on top of a

1. MSc of Applied Chemistry, Member of Young Researchers and Elite Club, Urmia Branch, Islamic Azad university, Urmia, Iran.

2. Department of Biology, Urmia Branch, Islamic Azad University, Urmia, Iran.

3. Department of Microbiology, Urmia Branch, Islamic Azad University, Urmia, Iran.

\*Corresponding Author: E-mail: kouhkanmaryam@gmail.com

column of SiO<sub>2</sub> (10 g). The column was washed with Accost/light petroleum ether. The solvent was then evaporate (product 6). The structures of

products 6 (Figure1) were confirmed by their IR and <sup>1</sup>H-NMR and <sup>13</sup>C-NMR [12].

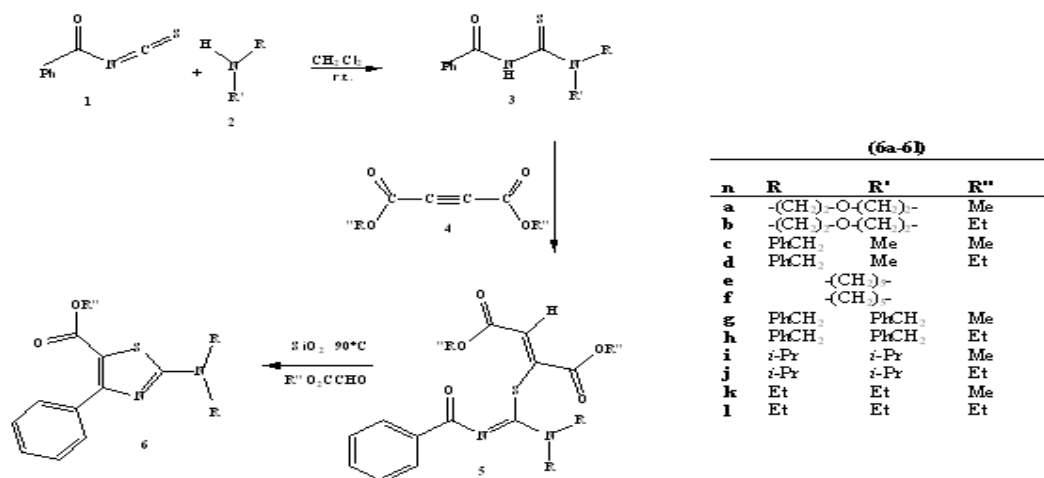


Figure 1. The synthesis scheme employed to obtain the target compounds.

## Antibacterial Activity

### Agar Diffusion Method-Individual Compounds Solutions

Antibacterial activity of synthesized compounds was tested against several gram positive and gram-negative bacteria including: *S. aureus* ATCC. 25923, *B. subtilis* PTCC.1254, *B. cereus* PTCC.1154 and *Proteus vulgaris* PTCC.0829. All microorganisms were obtained from Urmia University of Medical Sciences.

The standardization of each bacterial inoculum was done following the National Committee for Clinical Laboratory Standards (NCLS). Briefly, each bacterial strain inoculated into Mueller Hinton broth (MHB). Then it was incubated for 12 h at 35 °C to achieve the turbidity of 0.5 McFarland units. A standardized bacterial inoculum by concentrations of  $5 \times 10^5$  cfu/mL (forming units per millimeter) colony was used for antibacterial screening. The antibacterial activity of the prepared compounds was screened using the well diffusion method [15]. In short, each compound was dissolved in dimethyl sulfoxide (DMSO) and a solution with 1mg/ml concentration was prepared. Muller Hinton Agar (MHA, Merck, Germany) (200 ml) was melted over a boiling water bath then was stabilized at 45 °C and aseptically seeded with 100 µl inoculum, containing  $0.5 \times 10^6$  cells/ml of bacteria, transferred into a sterile Petri dish. Wells were made in agar using a sterile glass tube, 70 µl of compounds was transferred to each well, and 70 µl of DMSO was inoculated into another well as a

negative control. The antibacterial activity of compounds was determined by measuring the zones around each well against defined bacteria after incubation for 24 h. Cefprozime and ciprofloxacin used with the same method as standard antibacterial agents. Experiments performed at least three times and the moral values were selected.

### Broth Dilution Method

In the next step, antimicrobial activity of compounds was evaluated by broth dilution method [16]. The aim of this method is determining the lowest concentration of an antimicrobial product that inhibits the visible growth of the tested microorganism, which is considered by the lack of visible turbidity after incubation. Minimum Inhibitory Concentration (MIC) values are used in order to determining the susceptibility of the bacteria or fungus to antibiotics and new antimicrobial agents. In this study, sterile glass test tube containing Muller Hinton Broth was used. Ten of inoculum contained  $1.5 \times 10^6$  C.F.U/ml of tested microorganism was added to each test tube. Minimum Bactericidal Concentration (MBC) values were performed by sub culturing of the tested tubes on agar media that do not contain the antimicrobial agent. The MBC is defined by detecting the lowest concentration of compounds that decrease the viability of the bacterial inoculums by  $\geq 99.9$  [17]. Cefprozime and ciprofloxacin (Sigma, Aldrich, US) were used with the same method as standard antimicrobial agents.

### Antifungal Activity

The in vitro antifungal activity of the Alkyl 2-(Dialkylamino)-4 phenylthiazole-5-carboxylates derivatives (6a-6l) were evaluated against standardized clinically important fungi, including: *Candida tropicalis*, *C. albicans* ATCC 10239 and *C. glabrata*. These microorganisms were chosen because they cause opportunistic local and systemic infections in immune compromised individuals [18]. The antifungal assay was performed by the agar well diffusion and broth macrodilution methods [19, 20]. In brief, fungal suspension containing  $1.5 \times 10^6$  cell/mL of yeast was swabbed and spread on Sabouraud dextrose agar (SDA, Merck, Germany). A well was cut at the center of each inoculated SDA using a sterile cork borer of 6 mm diameter and 70  $\mu$ g/mL of phenylthiazole derivatives dissolved in DMSO were introduced into the wells. The plates were incubated for 48 h at a temperature of  $28 \pm 2$  °C and then growth and inhibition diameter zones (in mm) were recorded. Fluconazol used with the same method as standard agent.

In the next step, the MICs were determined by broth dilution technique. The Sabouraud dextrose broth (SDB, Merck, Germany) tubes containing test compounds were serially diluted. The 48 h grown cultures of fungi was inoculated in each tube. The tubes were incubated at room temperature for 48 h. The lowest concentration required to inhibit the growth of fungi was regarded as MIC. To get the minimum fungicidal concentration (MFC), a loopful was taken from the MIC tubes and streaked on SDA plates. The growth was observed after

incubation at 37 °C at 24 h. The lowest concentration, which showed no growth, was recorded as MFC [21, 22].

### RESULTS

Herein, we have evaluated antibacterial and antifungal activity of some of previously prepared alkyl 2-(Dialkylamino)-4 phenylthiazole-5-carboxylates derivatives (6a-4l) (Scheme 1).

#### Determination of MIC

The MIC and inhibition zones (IZ) of synthesized compounds against tested bacteria (6a-6l) are shown in Table 1. All tested compounds (6a-6l) inhibit the growth of *S. aureus* and *Proteus mirabilis* but compounds 6a and 6c were more active than the others. The growth of *B. subtilis* was also inhibited by the compounds 6a, 6b, 6c, 6j and 6l at the range of 250-500  $\mu$ g/ mL. In addition, the thiazols 6a 6b and 6h were active against *B. cereus*. Some of the compounds showed the same antibacterial activity with *ceftizoxim* however, they showed poor activity compared with *ciprofloxacin*. All compounds were active against *S. aureus* and *P. mirabilis*. The lowest activity of compounds (6a-6l) was observed against *B. cereus* and *B. subtilis* (MIC = 500-1000 $\mu$ g/ml). The MBC of compounds was the same or three fold higher than the corresponding MIC results.

#### Antifungal Activity

The antifungal activity of new substituted phenylthiazole derivatives (6a-6l) against three kinds of pathogenic fungi was investigated and summarized in Table 2.

**Table1.** Minimum inhibitory concentrations ( $\mu$ g/ mL) and inhibition zones (mm) of synthesized compounds against tested bacteria.

Compounds	MIC(IZ)			
	<i>Staph. Aureus</i>	<i>Bacillus Subtilis</i>	<i>Bacillus Cereus</i>	<i>Proteus mirabilis</i>
6a	250 (14)	250(17)	250 (14)	500 (13)
6b	500 (12)	500 (17)	1000 (14)	500 (12)
6c	125 (14)	500 (17)	1000 (12 $\geq$ )	500 (13)
6d	500 (12)	1000 (17)	1000 (12 $\geq$ )	500 (12)
6e	250 (12)	1000 (15)	1000 (12 $\geq$ )	500 (13)
6f	500 (12)	1000 (12 $\geq$ )	1000 (12 $\geq$ )	500 (12)
6j	500 (12 $\geq$ )	1000 (12 $\geq$ )	1000 (12 $\geq$ )	1000 (12 $\geq$ )
6h	250 (14)	1000 (12 $\geq$ )	1000 (14)	1000 (13)
6i	250 (14)	1000 (15)	1000 (12 $\geq$ )	1000 (12 $\geq$ )
6j	500 (12 $\geq$ )	500 (15)	1000 (12 $\geq$ )	500 (12 $\geq$ )
6l	500 (15)	500 (13)	1000 (12 $\geq$ )	500 (13)
Ceftizoxim	125 (15)	62/5 ( 12 $\geq$ )	500 (12 $\geq$ )	500 (14)
Ciprofloxacin	0/07 (14)	0/7 (7)	0/75 (14)	1/5 (14)

IZ= Mean inhibition zone of derivatives in 1mg/ml concentration.

**Table 2.** Minimum inhibitory concentrations ( $\mu\text{g}/\text{mL}$ ) and inhibition zones (mm) of synthesized compounds against tested bacteria.

N	MIC(IZ)		
	<i>Candida albicans</i>	<i>Candida glabrata</i>	<i>Candida tropicali</i>
6a	250(16)	250(13)	250(12)
6b	250(16)	250(13)	250(12)
6c	500(15)	500(13)	250(13)
6d	250(14)	250(13)	500(12)
6e	250(16)	250(14)	500(13)
6f	250(17)	250(14)	500(13)
6h	500(15)	500(13)	500(13)
6i	250(15)	500(13)	250(13)
6j	500(15)	250(13)	500(13)
6k	250(15)	500(12)	500(13)
6l	500(14)	500(12)	250(12)
Floconazol	250(14)	250(14)	250(14)

IZ= Mean inhibition zone of derivatives in 1mg/ml concentration.

Antifungal potential of synthesised compounds (6a-6d) were evaluated according to their zone of inhibition against three pathogens as well as the MIC and MFC values were determined in the range of 250 to 500  $\mu\text{g}/\text{ml}$ . All compounds showed potent fungicidal activities against *C. tropicalis*, *C. albicans* and *C. glabrata*. The zones inhibition diameters and the MIC values indicate that all synthesized compounds (6a-6l) showed moderate to good inhibitory activity against human pathogenic fungi. Compounds 6a and 6b showed the maximum activity with MIC value of 250  $\mu\text{g}/\text{ml}$  against tested fungi. MFC values for title compounds were similar to MIC.

## DISCUSSION

Antibiotic resistance is the main crisis in treatment of infectious diseases caused by bacteria. As a result, there is global threat about the increasing numbers of antibiotic resistant bacteria isolated from human, animal, food, water and soil samples. One way to deal with this crisis is finding new antimicrobial compounds to replace current antibiotics. Thiazole derivatives are novel antibacterial compounds, which are good replacements for some antibacterial drugs [23].

In the current study, inhibitory effects of 12thiazole derivatives were assessed on bacterial and fungal pathogens. *S. aureus* is multi-drug resistant bacterial pathogens. It is the most common and dangerous causes of hospital infections, so there is a constant need to develop new antimicrobial agents to combat these infections. The result showed the maximum inhibitory activity of our derivatives was against *S. aureus*. They were mostly active against gram-positive bacteria than gram-negative cases.

From the screening data, the aminothiazols having morfolin and phenyl methyl substituent respectively 6a and 6c, showed better activity against bacteria strain. The results show that the compounds 6a and 6b, respectively, methyl 2-(Morpholin)-4-phenylthiazole and ethyl 2-(Morpholin)-4-phenylthiazole with morpholin ring at the C-2 position on the linker of 1,3-thiazole were a potent antifungal compound with good MICs. Minimal bactericidal concentrations of these compounds were significantly higher than *Ciprofloxacin* but they were equally as active as the *Floconazol* and *Ceftizoxim* so this derivative is expected to affect beta-lactamase producing bacteria due to the lack of a betalactam ring [24].

## CONCLUSION

Our phenylthiazole derivatives showed a narrow spectrum of antibacterial activity. Quite interesting results were found in antifungal assays. Our results show that morpholin-4-phenylthiazole derivatives will be helpful structure for possible development of new antifungal drugs. Furthermore the easy workup, high yield, and short reaction times makes the method a useful addition for preparing modern pharmaceutical synthetics.

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