

Study on the Antimicrobial Effects of Novel Thiazole Derivatives

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ABSTRACT

In this work, six 2-[(1/4-methylimidazol/triazol/tetrazol-2/3/5-yl)thio]-N-(4-substituted thiazol-2-yl) acetamide derivatives (**2a-f**) were synthesized. The antimicrobial activities of these substances were examined against some foodborne Gram positive and Gram negative test bacteria, *Candida albicans*,

Candida glabrata, *Candida krusei*, and *Candida parapsilosis* yeasts, and some *Aspergillus* and *Penicillium* filamentous fungi species. The substances were shown considerable antimicrobial effect to tested pathogenic microorganisms.

Keywords: antibacterial, antifungal, thiazole

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INTRODUCTION

Infectious diseases are one of the most important clinical problems in the worldwide. If microorganisms develop resistance to drugs that are used continuously, many diseases become difficult to control. Thanks to the progress in biosynthesis, it is possible to create libraries of molecules and some of them may have minor modifications that present special value if the molecule is to work as a drug. These new molecules may start to produce a different set of possibly valuable products that have biological activity. One of these molecules, thiazole is a five-membered heterocyclic ring which is important fragment of naturally existing molecules thiamine (vitamin B1), thiamine pyrophosphate (TPP, a coenzyme important in respiration in the Krebs cycle), epothilones, carboxylase, and the large family of macrocyclic thiopeptide antibiotics, thiostrepton and micrococcin P1. Because of its biological importance and easibility of ring synthesis, thiazole containing compounds have been widely studied (1-3). Their derivatives combined with various heterocyclics have been reported with a broad spectrum of biological and pharmacological activities such as anaesthetic (4, 5), antitubercular (6), antibacterial, antifungal (7-9), analgesic (10) anticancer (11) activity, and inhibition of acetylcholinesterase activity (12). Therefore much interest has been focused on biological activity of thiazole derivatives.

The purpose of this study was to synthesize of 2-[(1/4-methylimidazol/triazol/tetrazol-2/3/5-yl)thio]-N-(4-substituted thiazol-2-yl)acetamide derivatives (**2a-f**) by appropriate methods and screening for antimicrobial activity for some foodborne pathogenic bacteria, yeasts and filamentous fungi.

MATERIALS AND METHODS

Materials

General

All chemicals were purchased from Sigma-Aldrich Chemical Co (Sigma-Aldrich Corp., St. Louis, MO, USA) and Merck Chemicals (Merck KGaA, Darmstadt, Germany). All melting points (m.p.) were determined by MP90 digital melting point apparatus (Mettler Toledo, Ohio, USA) and were uncorrected. All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany). Spectroscopic data were recorded with the following instruments: IR, Shimadzu Affinity 1S spectrophotometer (Shimadzu, Tokyo, Japan); NMR, Agilent 300 MHz NMR spectrometer (Agilent technologies, California, USA), in DMSO- d_6 , using TMS as internal standard; M+1 peaks were determined by Shimadzu 8040 LC/MS/MS system (Shimadzu, Tokyo, Japan). Elemental analyses were performed on a Leco 932 CHNS analyzer (Leco, Michigan, USA).

General procedure for the synthesis of 2-[(1/4-methylimidazol/triazol/tetrazol-2/3/5-yl)thio]-N-(4-substituted thiazol-2-yl)acetamide derivatives (**2a-f**)

A mixture of 2-chloro-(4-substituted thiazol-2-yl)acetamide (**1a**, **1b**) (2 mmol) and the appropriate thiol derivative (2 mmol) and K_2CO_3 (2.4 mol) were stirred in 40 ml of acetone at room temperature for 5 h. Acetone was evaporated until dryness. The residue was washed with water and recrystallized from ethanol to obtain compounds **2a-f** (13).

2-[(1-Methyl-1H-imidazol-2-yl)thio]-N-(thiazol-2-yl)acetamide (**2a**)

Yield: 75 %. M.p. 159°C.

1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.59 (3H, s, $-CH_3$), 3.97 (2H, s, $-CH_2$), 6.94 (1H, s, Ar-H), 7.22 (1H, d, $J=3.54$ Hz, Ar-H), 7.25 (1H, s, Ar-H), 7.46 (1H, d, $J=3.54$ Hz, Ar-H), 12.47 (1H, s, -NH).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 33.44, 37.32, 114.17, 124.12, 129.05, 138.20, 139.70, 158.23, 167.30.

For $C_9H_{10}N_4OS_2$ calculated: 42.50 % C, 3.96 % H, 22.03 % N, found: 42.54 % C, 3.89 % H, 22.08 % N.

HRMS (m/z): $[M+H]^+$ calcd for $C_9H_{10}N_4OS_2$: 254.33; found 255.0359.

2-[(4-Methyl-4H-1,2,4-triazol-3-yl)thio]-N-(thiazol-2-yl)acetamide (**2b**)

Yield: 71 %. M.p. 212°C.

1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.59 (3H, s, $-CH_3$), 4.14 (2H, s, $-CH_2$), 7.23 (1H, d, $J=3.54$ Hz, Ar-H), 7.48 (1H, d, $J=3.54$ Hz, Ar-H), 8.56 (1H, s, Ar-H), 12.38 (1H, s, -NH).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 31.28, 36.61, 114.24, 138.23, 146.79, 148.83, 158.19, 166.59.

For $C_8H_9N_5OS_2$ calculated: 37.64 % C, 3.55 % H, 27.43 % N, found: 37.58 % C, 3.59 % H, 27.48 % N.

HRMS (m/z): $[M+H]^+$ calcd for $C_8H_9N_5OS_2$: 255.31; found 256.0319.

2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N-(thiazol-2-yl)acetamide (**2c**)

Yield: 73 %. M.p. 225°C.

1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.98 (3H, s, $-CH_3$), 4.36 (2H, s, $-CH_2$), 7.24 (1H, d, $J=3.54$ Hz, Ar-H), 7.49 (1H, d, $J=3.54$ Hz, Ar-H), 12.47 (1H, s, -NH).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 34.14, 36.62, 114.34, 138.24, 153.59, 158.17, 165.87.

For $C_7H_8N_6OS_2$ calculated: 32.80 % C, 3.15 % H, 32.79 % N, found: 32.88 % C, 3.17 % H, 32.82 % N.

HRMS (m/z): $[M+H]^+$ calcd for $C_7H_8N_6OS_2$: 256.30; found 257.0765.

2-[(1-Methyl-1H-imidazol-2-yl)thio]-N-(4-phenylthiazol-2-yl)acetamide (**2d**)

Yield: 69 %. M.p. 96°C.

1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.60 (3H, s, $-CH_3$), 3.99 (2H, s, $-CH_2$), 7.26-7.35 (2H, m, Ar-H), 7.39-7.45 (3H, m, Ar-H), 7.63 (1H, s, Ar-H), 7.89 (2H, d, $J=7.17$ Hz, Ar-H), 12.58 (1H, s, -NH).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 33.45, 37.38, 108.73, 124.15, 126.13, 128.30, 129.11, 129.21, 134.65, 139.58, 149.38, 158.15, 167.59.

For $\text{C}_{15}\text{H}_{14}\text{N}_4\text{OS}_2$ calculated: 54.53 % C, 4.27 % H, 16.96 % N, found: 54.57 % C, 4.29 % H, 16.92 % N.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{15}\text{H}_{14}\text{N}_4\text{OS}_2$: 330.42; found 331.0668.

2-[(4-Methyl-4H-1,2,4-triazol-3-yl)thio]-N-(4-phenylthiazol-2-yl)acetamide (2e) Yield: 67 %. M.p. 174°C.

^1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.60 (3H, s, $-\text{CH}_3$), 4.16 (2H, s, $-\text{CH}_2$), 7.32-7.46 (3H, m, Ar-H), 7.64 (2H, d, $J=7.17$ Hz, Ar-H), 8.56 (1H, s, Ar-H), 12.54 (1H, s, $-\text{NH}$).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 31.28, 36.65, 108.78, 126.13, 128.32, 129.22, 134.64, 146.82, 149.41, 158.11, 166.93.

For $\text{C}_{14}\text{H}_{13}\text{N}_5\text{OS}_2$ calculated: 50.74 % C, 3.95 % H, 21.13 % N, found: 50.77 % C, 3.92 % H, 21.18 % N.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{14}\text{H}_{13}\text{N}_5\text{OS}_2$: 331.41; found 332.0628.

2-[(1-Methyl-1H-tetrazol-5-yl)thio]-N-(4-phenylthiazol-2-yl)acetamide (2f)

Yield: 70 %. M.p. 193°C.

^1H -NMR (300 MHz, DMSO- d_6 , ppm) δ : 3.99 (3H, s, $-\text{CH}_3$), 4.39 (2H, s, $-\text{CH}_2$), 7.33-7.46 (3H, m, Ar-H), 7.65 (1H, s, Ar-H), 7.90 (2H, d, $J=7.14$ Hz, Ar-H), 12.65 (1H, s, $-\text{NH}$).

^{13}C -NMR (75 MHz, DMSO- d_6 , ppm) δ : 34.16, 36.63, 108.87, 126.14, 128.34, 129.23, 134.62, 149.45, 153.57, 158.06, 166.19.

For $\text{C}_{13}\text{H}_{12}\text{N}_6\text{OS}_2$ calculated: 46.97 % C, 3.64 % H, 25.28 % N, found: 50.01 % C, 3.67 % H, 22.32 % N.

HRMS (m/z): $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{13}\text{H}_{12}\text{N}_6\text{OS}_2$: 332.40; found 333.0577.

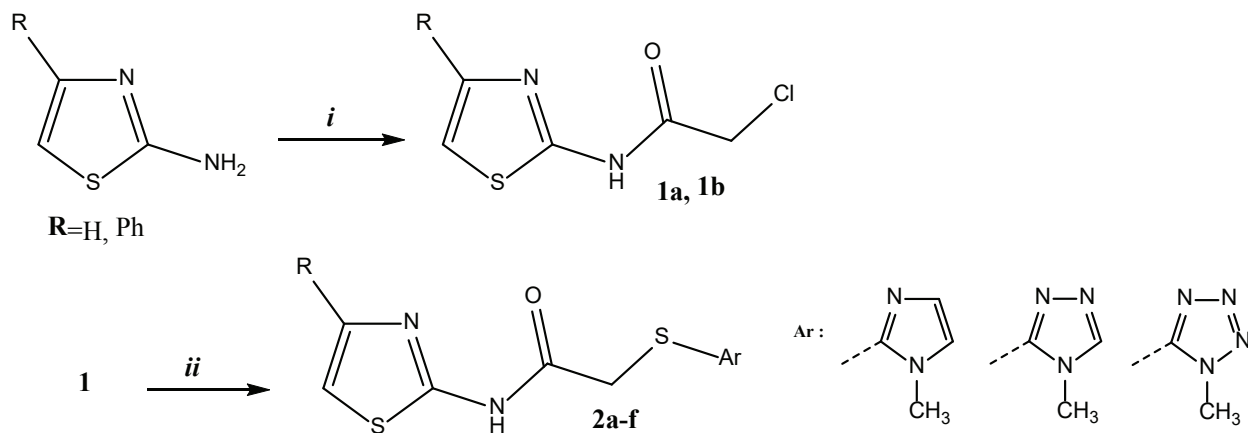
Antimicrobial activity

Microorganisms and media

12 bacteria, 4 yeasts, and 7 filamentous fungi were used as test organisms in this study.

Gram positive bacteria: *Bacillus cereus* ATCC 10876, *Bacillus subtilis* NRRL NRS-744, *Enterococcus faecalis* ATCC 51299, *Listeria monocytogenes* ATCC 19111 and *Staphylococcus aureus* ATCC 6538. Gram negative bacteria: *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* NRRL B-3567, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* NRRL B-123, *Salmonella typhimurium* ATCC 14028, and *Yersinia enterocolitica* Y53 bacteria. Yeasts: *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, and *Candida krusei* ATCC 6258. Filamentous fungi: *Aspergillus niger* ATCC 16404, *Aspergillus flavus* ATCC 9807, *Aspergillus parasiticus* NRRL 465, *Aspergillus fumigatus* NRRL 113, *Penicillium expansum* ATCC 24692, *Penicillium citrinum* ATCC 9849, *Penicillium chrysogenum* ATCC 10106.

Bacteria and yeasts were kept on nutrient agar and yeast extract agar plates at 4 °C, respectively. Fungal test cultures were subcultured on potato dextrose agar (PDA) for 5-7 d at 25 °C.



Scheme 1. The synthesis protocol of the compounds. Reactants and conditions: *i*: Et_3N , ClCOCH_2Cl , THF, 0°C; *ii*: 2/3-Mercapto heterocyclics, K_2CO_3

Determination of antimicrobial activity and Minimum Inhibitory Concentration (MIC) values of the substances

Determination of antimicrobial activities and MIC values of the substances against to test microorganisms were used a serial dilution technique using 96-well microtitre plates, which has been described by the Clinical and Laboratory Standards Institute (14). For this aim, stock solutions of the substances were prepared in 20% dimethylsulfoxide (DMSO) and two fold dilution series from 4 mg/mL- 7.8125 µg/mL were prepared using sterile distilled water. 100 µl from each of the dilutions was added to 96-well microtiter plates. Bacterial and yeast cultures were standardized to 10⁸ CFU/mL using 0.5 McFarland standard solution. The fungal spore suspensions were prepared using sterile 0.1% Tween 80 and adjusted to about 10⁶ spores/mL. 100 µl of each microorganism suspension was then added into the wells and incubated at 30-35°C for 24-48 h in aerobic conditions. The culture medium alone and medium containing bacteria without test compounds were considered as two controls. Triplicate wells were applied for each concentration of the individual test materials. Chloramphenicol and ketoconazole were used as standard antibacterial and antifungal agent, respectively. MIC values were detected by using MTT method (15-17). A concentration of 5 mg/mL MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was prepared in PBS (pH 7.2). 20 µl of MTT solution was added to each single well and the 96-well micro-titer plates were incubated for 3 h at 30-35 °C to visually differentiate between the live and dead cells. A pink or red color in the microdilution well indicated bacterial growth; colorless wells were indicative of growth inhibition. The MIC was detected as the lowest substance concentration that completely inhibited microbial growth.

RESULTS AND DISCUSSION

Chemistry

Six new derivatives were synthesized with a simple, two-pot synthesis method as shown in Scheme 1. The structures of the compounds were confirmed by spectroscopic technics and elemental analysis results. In ¹H NMR spectra of the compounds, methyl protons were observed at 3.59-3.99 ppm whereas methylene protons were seen at about 3.97-4.39 ppm. The proton of nitrogen atom was detected at 12.38-12.65 ppm and the other protons were seen at

aromatic region and between 6.94-8.56 ppm as expected. In the ¹³C NMR spectra of the compounds, methyl, methylene and carbonyl carbons were resonated at about 33.50, 37.50 and 167.30 ppm, respectively. The other carbon atoms were resonated at about 108.23-158.23 ppm. In the mass spectra of compounds, [M+1] peaks were determined in agreement with their molecular formula. Elemental analysis results were determined within the theoretical values, ±0.4%.

Antimicrobial activity

All the synthesized compounds were screened for in vitro antimicrobial activity. The antimicrobial activity was evaluated against different bacterial, yeast and filamentous fungi strains. Results obtained in the present study relieved that all tested substances possess potential antimicrobial activity against all the test microorganisms. Results are summarized in Table 1. Among the bacteria *Bacillus subtilis*, *Enterobacter aerogenes*, and *Staphylococcus aureus* were the most resistant species to nearly all compound. In general evaluating, it was observed that all of the compounds had the activity in 0.25 and 1 mg/mL concentrations as MIC value. The lowest MIC values of the substances were found to be 0.125 mg/mL against only *Yersinia enterocolitica* with compounds **2e** and **2f**. There was no direct correlation between the effectiveness of the substances to tested microorganisms. In addition, the antifungal activity of the compounds was examined against four *Candida* species. Compound **2f** was found to be more effective against yeasts than the others with lower MIC values (Table 1). Otherwise, the MIC values obtained were similar for bacteria and yeast but higher for filamentous fungi. In antifungal activity evaluating for filamentous fungi, all the tested compounds inhibited the spore germination against tested fungi.

Among the synthesized compounds, **2a** most active compound for *Aspergillus parasiticus*, **2b** for *Penicillium citrinum*, **2c** for *Penicillium expansum*, **2d** for *Penicillium citrinum* and *Penicillium cyrisogenum*, **2e** for *Penicillium expansum*, **2f** for *Penicillium expansum* and *Penicillium citrinum* with 0.5 mg/mL MIC value when compared to others. According to these results, *Penicillium* species were more sensitive than *Aspergillus* species for the compounds. On the other hand, the compounds **2e** and **2f** (MIC 125 µg/mL) displayed half the potency of chloramphenicol to *Yersinia enterocolitica*. Other compounds had higher MIC values than standards.

Table 1. Minimum inhibitory concentration (MIC) in µg/mL of all substances and standard antibiotics in µg/mL obtained using the microdilution method.

Microorganisms	2a	2b	2c	2d	2e	2f	Standards
Bacteria							Chloramphenicol
<i>Bacillus cereus</i>	250	250	500	500	500	500	31.25
<i>Bacillus subtilis</i>	500	1000	1000	1000	1000	1000	31.25
<i>Escherichia coli</i>	250	250	250	500	500	500	62.5
<i>Enterococcus faecalis</i>	250	250	250	500	500	500	62.5
<i>Enterobacter aerogenes</i>	1000	500	1000	1000	1000	1000	31.25
<i>Klebsiella pneumoniae</i>	500	250	500	500	1000	1000	62.5
<i>Listeria monocytogenes</i>	250	250	250	500	500	500	125
<i>Pseudomonas aeruginosa</i>	500	250	500	500	500	500	125
<i>Proteus vulgaris</i>	500	250	500	500	500	500	125
<i>Salmonella thyphimurium</i>	500	500	500	500	500	500	125
<i>Staphylococcus aureus</i>	1000	1000	1000	1000	1000	500	125
<i>Yersinia enterocolitica</i>	250	250	250	500	125	125	62.5
Yeasts							Ketoconazole
<i>Candida albicans</i>	500	500	500	1000	1000	250	62.5
<i>Candida krusei</i>	500	500	500	1000	1000	250	62.5
<i>Candida parapsilosis</i>	500	500	1000	1000	500	250	62.5
<i>Candida glabrata</i>	500	1000	500	500	500	250	31.25
Filamentous fungi							Ketoconazole
<i>Aspergillus flavus</i>	1000	1000	1000	1000	1000	1000	31.25
<i>Aspergillus parasiticus</i>	500	1000	1000	1000	1000	1000	125
<i>Aspergillus niger</i>	1000	1000	1000	1000	1000	1000	62.5
<i>Aspergillus fumigatus</i>	1000	1000	1000	1000	1000	1000	125
<i>Penicillium expansum</i>	1000	1000	500	1000	500	500	125
<i>Penicillium citrinum</i>	1000	500	1000	500	1000	500	125
<i>Penicillium cyrisogenum</i>	1000	1000	1000	500	1000	1000	62.5

CONCLUSION

In conclusion, the objective of the present study was to synthesize and evaluate the antimicrobial activities of some new thiazole compounds that could be used as potent antimicrobial drugs. Six different compounds were synthesized newly using 2-aminothiazole derivatives as starting materials. It was observed that, the substances had bioactivity for the test bacteria in lower concentration than fungi. Although the MIC values obtained were higher than the standard antibiotics, it is an important result that the substances exhibited activity against for bacteria, yeast and filamentous fungi. Therefore all compounds were shown broad antimicrobial spectrum which have efficiency against pathogenic microorganisms associated with various human

diseases. However, our results clearly revealed that especially compounds **2e** and **2f** exhibited good antimicrobial activity to *Yersinia enterocolitica* bacterium. Also, compound **2b** exhibited half potency of standard with a MIC value of 125 µg/mL against *Pseudomonas aeruginosa* and *Proteus vulgaris*. In addition, bacteria *Listeria monocytogenes* and *Yersinia enterocolitica* were found as the most susceptible species for the tested compounds.

New antimicrobial drug development has global emphasis and needs to be supported and established so the resistance of microorganisms to antibiotics is a situation that is constantly increasing. Because of this reason, we have performed the synthesis and investigation of potential, antimicrobial agents, in this study.

Yeni Tiyazol Türevlerinin Sentezi ve Antimikrobiyal Aktivitelerinin Araştırılması**ÖZ**

Bu çalışmada 2-[(1/4-metilimidazol/triazol/tetrazol-2/3/5-il) tiyo]-N-(4-süstitüe tiyazol-2-il) asetamit türevleri (**2a-f**) sentezlenmiştir. Sentezlenen bu maddelerin bazı patojen Gram

negatif, Gram pozitif bakteriler, *Candida albicans*, *Candida glabrata*, *Candida krusei*, ve *Candida parapsilosis* mayaları ile bazı *Aspergillus* ve *Penicillium* filamentöz fungus türlerine karşı antimikrobiyal aktiviteleri araştırılmıştır. Sentezlenen maddelerin test mikroorganizmalarının tümüne karşı önemli bir antimikrobiyal aktivitesi olduğu gözlenmiştir.

Anahtar kelimeler: antibakteriyel, antifungal, tiyazol

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