Synthesis and Antimicrobial Activity of new (Z)-2-((5-(4-Hydroxybenzylidene)-4-Oxo-4,5-Dihydrothiazol-2-Yl)Amino) Acid and its Derivatives

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ABSTRACT
A series of (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) acid and its derivatives were synthesized from appropriate 2-thioxothiazolidin-4-one using nucleophilic substitution and Knoevenagel condensation. All compounds have been synthesized, characterized and screened for their antimicrobial activity against gram positive bacteria and gram negative bacteria along with Ampicillin and Ciprofloxacin as standard drug. According to results obtained all compound shows good to moderate activity against all strain tested, compatible to Ampicillin but lower than Ciprofloxacin. Amongst these compounds (6c) shows very good and moderate activity as compare to standards drugs against Gram positive bacteria B. subtilus and S. aureus. Some of the most potent compounds, namely (6b, 6h and 6j) possessed selectively antimicrobial activity.

INTRODUCTION
Now a day the treatment of bacterial and fungal infections still remains a very challenging therapeutic problem because of emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens. Despite the many antibiotics and chemotherapeutics available, the emergence of old and newer antibiotic-resistant bacterial strains in the last two decades constitutes a substantial very needful for new classes of antimicrobial agents [1,2,3]. Compounds containing the 2-thioxothiazolidin-4-one (Rhodanine) and its derivatives have also been reported to exhibit a broad spectrum of biological activities, such as antibacterial [4,5], antidiabetic [6], antifungal [7,8], anticancer [9], antitubercular [10,11], anti-HIV [12-14], antiparasitic [15], hypnotic [16] and antiproliferative agents [17,18]. The rhodanine has been known for over 50 years, so there have been several attempts to design antimicrobial agents based on this heterocycle. There are various reports available on rhodanine derivatives as antimicrobial agents [19,20,21]. These reports suggested that a chain containing free carboxyl group at rhodanine nucleus was important to the observed levels of antimicrobial activity [20,23]. With this in mind, we initiated a program to synthesized rhodanine derivatives having amino acids chain as antimicrobial agent by preparing hybrid molecules having the similar features of reported potent antimicrobial agents (Figure 1).

In continuation of our work [24-26], on the synthesis of bioactive compounds, we have synthesized some rodanine analogues. In view of the facts mentioned above, rhodanine derivatives were synthesized (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) acid (6a-l). The compounds (3, 4, 6a-l) were characterized by different spectral analytical techniques and screened for their in vitro antimicrobial activities against pathogenic strains such as B. subtilis, S. aureus, E. coli, C. albicans, A. flavus, A. niger species. The synthesized compounds were characterized on the basis of IR, 1H NMR and Mass spectral data [4-7].
EXPERIMENTAL

Rhodanine (2-thioxothiazolidin-4-one), 4-hydroxybenzaldehyde, anhydrous sodium acetate, triethylamine, dichloromethane, iodomethane and various solvents were commercially available. The major chemicals were purchased from Sigma Aldrich and Avra labs. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates. Developed plates were examined with UV lamps (254 nm). IR spectra were recorded on a FT-IR (Bruker). Melting points were recorded on SRS Optimelt, melting point apparatus and are uncorrected. 1H NMR spectra (δ, ppm) were recorded in DMSO-d6 solutions on a Varian-Mercury 400 MHz spectrometer using tetramethylsilane as the internal reference. Chemical shifts are reported as δ ppm units. The following abbreviations are used; singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). 13C NMR spectra were recorded in DMSO-d6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer.

**Synthesis of (Z)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (3)**

In a 100 ml round bottom flask, equimolar amount of rhodanine (1 mmol), anhydrous sodium acetate (1 mmol) was added in glacial acetic acid (5 mL) and 4-hydroxybenzaldehyde was added to the reaction mixture. The mixture was stirred under reflux condition for 6 h. The progress of reaction was monitored by TLC (20% ethyl acetate: n-hexane). After completion of the reaction, the reaction mixture was poured into the ice-cold water. The precipitate was filtered, washed with water (3×10 mL), dried, and purified by recrystallization in ethanol as solvent to give 80% yield.

**Synthesis of (Z)-5-(4-hydroxybenzylidene)-2-(methylthio)thiazol-4(5H)-one (4)**

In a 100 ml round bottom flask, the compound (3) (1 mmol), triethylamine (1.5 mmol) was added in dichloromethane (5 mL) at room temperature. To the stirred reaction mixture with iodomethane (1.5 mmol) was added after 4 min and stirred for 2 h. at room temperature. The progress of reaction was monitored by TLC (10% chloroform: methanol). After completion of reaction the reaction mixture was concentrated in-vacuo. The residue was washed with water (3×15 mL) to afford the crude product. The crude product was recrystallized using ethanol as solvent to give yield in the range 82%.

**Synthesis of (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) amino) acid (6a-l)**

In a 50 ml round bottom flask, the compound (4) (1 mmol), amino acids (5a-l) (1.5 mmol) with a catalytic amounts of potassium carbonate (K2CO3) (1.5 mmol) were added in ethanol and water (1:1 v/v) and stirred for 30-55 min at room temperature. The progress of reaction was monitored by TLC (10% chloroform: methanol). After completion of reaction the reaction mixture was concentrated in-vacuo. The residue was washed with water (3×15 mL) to afford the crude product. The compounds (6a-l) were recrystallized from ethanol and isolated as yellow solid.
RESULTS AND DISCUSSION

The compound (3) was then subjected to a Knoevenagel condensation with the appropriate rhodanine, which had themselves been synthesized using the reported procedure [27,28], to provide new series of target compounds (6a-l). The structures of the desired compounds were confirmed by IR, 1H NMR, 13C NMR, mass spectral analysis. The (Z)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (3) was prepared in prominent good yields via a Knoevenagel condensation between the corresponding heterocyclic cores of rhodanine and 4-hydroxybenzaldehyde (Scheme 1).

![Scheme 1](image)

2-thioxothiazolidin-4-one based compounds were synthesized by reflux with sodium acetate which acts as a base and glacial acetic acid as catalysts. In theory of E and Z geometrical isomers around the exocyclic double bond (CH=C) are possible for 5-benzylidene derivatives (3). The IR spectrum of (Z)-5-(4-hydroxybenzylidene)-2-thioxothiazolidin-4-one (3), showed a strong absorption band at 1672 cm⁻¹ that is due to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z = 237 (M+H) corresponding to a molecular formula C₁₀H₇NO₂S₂. 1H NMR spectra of compounds (3) show only one signal for the methyne proton in the range δ 7.70 ppm, at lower field values than those expected for the E-isomers, which was strongly indicates that the compounds have the Z-configuration. The isolation of single crystals for some compounds permitted us to corroborate the postulated structures including their Z-configuration by single crystal X-ray diffraction analysis [29,30]. Compound (4) was synthesized (Scheme 1) from Compound (3) and the structures of the desired compounds were confirmed by spectral analysis. The IR spectrum of (Z)-5-(4-hydroxybenzylidene)-2-(methylthio)thiazol-4(5H)-one (4), showed a strong absorption band at 1694 cm⁻¹ that is due to a carbonyl group. The mass spectrum revealed a molecular ion peak at m/z = 252 (M+H) corresponding to a molecular formula C₁₁H₉NO₂S₂. 1H NMR spectra of compounds (4) show only one signal for the methyne proton in the range δ 7.70 ppm, sulfur attached methyl proton shows the singlet in the range δ 2.71 ppm. We synthesized series of novel (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl) amino) acid (6a-l) (Scheme 2) from compound (4), was displacement of a methyl sulfinyl group by amino acids from the C2 position of the thiazolone ring. The structures of the desired compounds (6a-l) were confirmed by IR, 1H NMR, 13C NMR, mass spectral analysis. The IR spectrum of (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) propanoic acid (6a), showed a strong absorption band at 1732 cm⁻¹ that is due to a carbonyl of carboxylic group and 3386 cm⁻¹ due to hydroxyl of carboxylic group. The mass spectrum revealed a molecular ion peak at m/z = 293.05 (M+H) corresponding to a molecular formula C₁₃H₁₂N₂O₄S. Their 1H NMR spectra revealed the signals of (6a) as a representative example, show one signal for the methyne proton in the range δ 7.70 ppm, phenyl ring proton shows the multiplet in the range δ 7.30–7.66 ppm, one of the methyl group proton shows doublet in the range of δ 1.25–1.42 ppm and adjacent to carboxylic acid proton shows quartet in the range of δ 4.51–4.85 ppm, carboxylic acid proton shows singlet in the range of δ 14.13 ppm (Table 1).

![Scheme 2](image)
The antibacterial activity of compounds was monitored by observing their Minimum Inhibitory Concentration (MIC, µg/mL) as previously mentioned. Compounds were active against the majority of bacterial and fungal strains tested to the narrow spectrum compounds, active only against only one specific bacteria; Bacillus subtilis (NCIM-2256) and three fungal strains; Candida albicans (NCIM-3471), Aspergillus flavus (NCIM-539) and Aspergillus niger (NCIM-1196). The antibacterial activity was used as solvent control for both antibacterial and antifungal testing (Table 2).

Antimicrobial activity

All the synthesized compounds (3,4 and 6a-l) were screened for their in-vitro antimicrobial activity against two gram positive bacteria; Bacillus subtilis (NCIM-2063) and Staphylococcus aureus (NCIM-2901), one gram negative bacteria; Escherichia coli (NCIM-2256) and three fungal strains; Candida albicans (NCIM-3471), Aspergillus flavus (NCIM-539) and Aspergillus niger (NCIM-1196). The antibacterial activity of compounds was monitored by observing their Minimum Inhibitory Concentration (MIC, µg/mL) as previously mentioned by broth dilution method with Ciprofloxacin and Ampicillin as control drugs. While the antifungal study was carried by standard agar dilution method were Fluconazole and Miconazole used as control drugs. Methanol was used as solvent control for both antibacterial and antifungal testing (Table 2).

Synthesized compounds of present series shows variety of antimicrobial activity, ranging from broad spectrum molecule active against the majority of bacterial and fungal strains tested to the narrow spectrum compounds, active only against only one strains. Amongst the series compounds (6b, 6g, 6h and 6j) are found to be most active molecules and they are specific towards

### Table 1: Physical and analytical data of thiazole derivatives (6a-l).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Substituent (R)</th>
<th>Time (min)</th>
<th>Mol. formula</th>
<th>Yielda (%)</th>
<th>M. P. (°C)</th>
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<tbody>
<tr>
<td>6a</td>
<td>-CH$_3$</td>
<td>35</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S</td>
<td>90</td>
<td>230-232</td>
</tr>
<tr>
<td>6b</td>
<td>-CH(CH$_3$)$_2$</td>
<td>35</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S</td>
<td>90</td>
<td>178-180</td>
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<tr>
<td>6c</td>
<td>-CH$_2$CH$_2$CH$_3$</td>
<td>40</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S</td>
<td>88</td>
<td>130-132</td>
</tr>
<tr>
<td>6d</td>
<td>-CH$_2$C$_6$H$_5$</td>
<td>30</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S</td>
<td>86</td>
<td>170-172</td>
</tr>
<tr>
<td>6e</td>
<td>-CH$_2$CH$_2$SCH$_3$</td>
<td>45</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S$_2$</td>
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<td>156-158</td>
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<td>6f</td>
<td>-CH$_2$CH$_2$(CH$_3$)$_2$</td>
<td>55</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S$_2$</td>
<td>92</td>
<td>229-231</td>
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<tr>
<td>6g</td>
<td>-CHOH</td>
<td>50</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S</td>
<td>92</td>
<td>185-187</td>
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<tr>
<td>6h</td>
<td>-CH$_2$SH</td>
<td>55</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S$_2$</td>
<td>90</td>
<td>169-171</td>
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<tr>
<td>6i</td>
<td>-CH$_2$COOH</td>
<td>50</td>
<td>C$<em>{16}$H$</em>{13}$N$_2$O$_5$S$_2$</td>
<td>85</td>
<td>155-157</td>
</tr>
</tbody>
</table>

a - Reaction condition (6a-l): Compound (4) (1 mmol), amino acids (5a-l) (1.5 mmol), K$_2$CO$_3$ (1.5 mmol), 4 ml ethanol and 4 mL water at room temperature

b - Isolated yields

### Table 2: Antimicrobial minimal inhibitory concentrations (MIC, µg/mL) of synthesized compounds (3, 4, 6a-l).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC Values (µg/mL)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. subtilis</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
</tr>
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<td>6a</td>
<td>85</td>
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<td>6b</td>
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<td>6c</td>
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<td>6g</td>
<td>80</td>
</tr>
<tr>
<td>6h</td>
<td>5.5</td>
</tr>
<tr>
<td>6i</td>
<td>60</td>
</tr>
<tr>
<td>6j</td>
<td>90</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>12.5</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
</tr>
<tr>
<td>Miconazole</td>
<td>-</td>
</tr>
</tbody>
</table>

aValues are the average of three readings
Our results clearly revealed that the substituted thiazolone exhibited good antimicrobial activity of amino acids attached at C2 position on thiazolone moiety, which are very effective for the enhanced antimicrobial activities. Some new (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) acid with the hope of discovering new structures directly attributed with the structural variations. The important highlights of structure-activity relationship are as follows:

**Structure activity relationship (SAR)**

The results of the antimicrobial screening demonstrated some definite and interesting facts about the structural-activity relationship (SAR) of synthesized thiazolone moiety. In majority of cases, dependence of activity profile on structural modifications of the molecule is clear and fascinating. Due to different types of amino acid and variation in activity profile of molecules are also directly attributed with the structural variations. The important highlights of structure-activity relationship are as follows:

**Effect of alkane chain:** In the present study it is clear that the activity profile of molecule is strongly affected by the branching pattern and chain length of alkane chain. Attachment of methyl group at C2 position on the thiazolone moiety (6a) makes molecule inactive against bacterial and fungal strains may be due to its small size and electron donating effects.

**Effect of alkane chain with hydroxyl group:** Compounds (6g and 6l) containing alkane chain with hydroxyl groups with deferent position. Substitution by hydroxymethyl (6g) at C2 position on the thiazolone moiety make the molecule selective active against gram positive bacteria, S. aureus, while substitution by 1-hydroxyethyl (6l), however, make the molecule inactive.

**Effect of heterocyclic ring:** Substitution by phenylmethyl (6d) at thiazolone moiety gives the inactive molecule towards S. aureus, B. subtilis, A. niger and C. albicans, not good as standard drugs. While 4-hydroxyphenyl (6k) substitution make the molecule inactive. This observation clearly shows that only phenyl ring gives inactive molecule and addition of hydroxyl group at position 4 causes lose in this activity.

**Effect of heterocyclic ring:** The compounds (6j) containing methyl-imidazole ring at thiazolone moiety makes the molecule specifically active towards Gram positive bacteria B. subtilis and S. aureus.

**Effect of sulfur containing group:** The compound (6e) with terminal methylthio group is inactive to all strain, but compound (6h) with terminal mercapto group shows specificity active towards Gram positive bacteria B. subtilis and S. aureus.

**CONCLUSION**

In conclusion, the objective of the our present study was to synthesize and investigated the potent antimicrobial activities of some new (Z)-2-((5-(4-hydroxybenzylidene)-4-oxo-4,5-dihydrothiazol-2-yl)amino) acid with the hope of discovering new structures that could be used as potent antimicrobial agents. Our aim has been verified by the synthesis of thiazolone moiety, different types of amino acids attached at C2 position on thiazolone moiety, which are very effective for the enhanced antimicrobial activities. Our results clearly revealed that the substituted thiazolone exhibited good antimicrobial activity.

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**REFERENCE**


