Design and synthesis of novel lactam/thiazole derivatives having five membered thiazolyl ring and their antimicrobial activity

Krishna SRIVASTAVA*, Ram Prakash TIWARI1, Raj Bahadur SINGH1, Abhishek SRIVASTAVA2, Deepa LAKHMANI1, Rishi Kumar VISHNOI3

1Faculty of Chemical Sciences, Shri Ramswaroop Memorial University (SRMU), Barabanki. 225 003, U.P. (India)
2Department of Chemistry, GLA University, Mathura, 281006, U.P. (India)
3Department of Chemistry University of Lucknow, Lucknow 226007 U.P. (India)

*Corresponding Author E-mail: rintu_srivastava@rediffmail.com (K.S.); Tel. +91-831-7004549

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ABSTRACT: In the present communication, we conceived a new synthetic approach for the preparation of novel thiazolyl-lactam/thiazole analogs. The reaction was started through cyclization of ketone with thiourea along with substituted aryl aldehydes to culminate imine derivatives which subsequently cyclized with thioglycolic acid/chloroacetyl chloride to produce final compounds. The structural elucidation of newly synthesized compounds was performed through elemental detections, FT-IR, 1HNMR, and Mass spectrometric techniques. Antimicrobial studies were performed for all synthesized molecules by serial dilution method by tackling the positive (K. pneumonia, S. aureus, B. subtills) and negative (P. aeruginosa and E. coli.) bacterial strains. The in vitro antimicrobial screening results show that the compounds 2e, 3c, and 3d containing o-hydroxy, p-chloro, and p-nitro substituent respectively exhibit exceptional activity against S. aureus while compounds 2d and 3f bearing p-nitro and o-chloro substituent respectively were deemed to be the most competent against B. subtills. Compounds 2d (p-nitro) and 2f (o-chloro) were found to be most potent against E. coli. In gram-negative bacterial strains, compounds 2c (p-chloro), 2g (4-OH-OCH3), 3b (p-hydroxy), and 3e (o-hydroxy) were extremely potent against P. aeruginosa while compound 2e containing o-hydroxy group shows excellent activity against E.coli.

KEYWORDS: Synthesis; Lactam; Thiazolyl ring; Antimicrobial activity; Thiourea.

1. INTRODUCTION

Infectious diseases are a challenge for chemists, who must concentrate their efforts on producing novel heterocyclic compounds so that fauna and flora can exist without fear of getting life-threatening infections [1]. Heterocyclic scaffolds are found in nature or can be built artificially. For decades, azetidinone, a heterocyclic scaffold having a nitrogen atom in the ring, has been studied as a pharmacophore in medicinal chemistry [2]. Changes in the four-membered -lactam nucleus's substituent have been demonstrated to have a substantial impact on conferring promising microbial activity [3]. B-lactam moiety exhibits outstanding antibacterial [4], antimicrobial [5], anti-inflammatory [6], anticonvulsant [7], and antitubercular effects [8]. They also inhibit enzymes and have a beneficial effect on the central nervous system. Antitubercular [9], anti-inflammatory [10], anti-tumor [11], anti-HIV [12], anti-parkinsonism [13], antidiabetic [14] and vasopressin antagonist characteristics have been discovered.

Thiazole derivatives have been discovered to be adaptable scaffolds with strong pharmacological properties that are frequently used in disease treatment. Thiazoles have different pharmacological properties against different diseases conditions [15-20]. These compounds also have an antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic intermediate called Schiff base. Imine or azomethine groups are be found in a variety of natural, natural-derived, and non-natural compounds. Schiff bases also exhibit fluorescence, photoluminescence, potentiometric titration, aggregation and anthelmintic activities [21-23]. As part of our ongoing effort to develop new heterocyclic compounds...
with distinct activity characteristics, we present this paper as a design and synthesis of thiadiazin-3-yl-2-one and thiazol-2-yl-azetidin-2-one/thiazolidine-4-one derivatives.

2. RESULTS AND DISCUSSION

Compounds 1a-h were synthesized by the reaction of cyclohexanone, substituted Aromatic aldehyde, and thiourea. The characteristic FTIR spectrum of the synthesized compounds exhibits the absorption bands of C-S, and C=N in the range of 899-886 cm\(^{-1}\), and 1662-1634 cm\(^{-1}\) respectively, while the absorption band corresponding to C=O str. has disappeared. The signal at ~980 cm\(^{-1}\) corresponds to the C=N stretching of the thiazole ring [24]. The singlet at δ 8.89-8.97 ppm is due to -N=CH-C- proton which is also following the proposed structure 1a-h [24]. The reaction of compounds 1a-h with thioglycolic acid results in cyclization to yield 2a-h. The FTIR spectra of synthesized compounds 2a-h show a new absorption band of C=O in the range of 1650-1698 cm\(^{-1}\) [25, 26]. In the \(^1\)H NMR spectrum of compounds 2a-h, the signal of the two protons (O=C-CH\(_2\)-S) appeared as a singlet at δ 2.81-2.94 ppm, the one proton singlet which was present at δ 8.89-8.97 ppm due to -N=CH-C- (1a-h) has been shifted to δ 3.32-3.45 ppm (due to –N-CH-S-). The IR and NMR data suggest the formation of 5 membered lactam ring in which the -CH\(_2\) and -CH groups are not attached directly [25, 26].

**Table 1:** Mass fragmentation pattern of compounds 2a and 3a

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<th>Fragments of Compounds 2a</th>
<th>m/z value</th>
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<td><img src="image2.png" alt="Image" /></td>
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<table>
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The novel 3a-h was synthesized via cyclization of 1a-h with CH₂COCl₂. Based on analytical and spectral data (FTIR and ¹H NMR) the molecular structure of the novel synthesized series was characterized. The characteristic FTIR spectrum of the synthesized compounds exhibits new absorption bands of C=O, and C-Cl at ~1660 and 770 cm⁻¹ respectively [25, 26]. Proton NMR spectra suggest that the compounds 3a-h contain two new doublets at δ 3.11-3.21 and 4.17-4.31 ppm due to –C-CH-Cl and –N-CH- of lactam ring respectively, while a singlet of 1a-h at δ 8.89-8.97 ppm due to -N=CH- was absent in 3a-h [25, 26]. The mass fragmentation pattern of compounds 2a and 3a has been represented in table 1. The IR, NMR, and Mass fragmentation data suggest the formation of 4 membered lactam ring in which the –C-CH-Cl and -N=CH-C- groups are attached directly. All the compounds show an excellent agreement between calculated and experimentally obtained data.

The computed optical density value at 600 nm was used to assess the potency of the ten synthesized compounds. A routine antimicrobial susceptibility test (AST) was employed to evaluate the toxicity of the fabricated compounds towards gram-negative (P. aeruginosa, E. coli, K. pneumonia) (Figure 2 & 4) and gram-positive (B. subtilis, S. aureus) (Figure 1 & 3) bacteria. The MIC value of these compounds was found in the range of 12.5 - >100 µg/mL (Table 2). The relation between the physicochemical/biological activity of chemicals and their molecular structure is known as Structure-Activity Relationships (SAR). The thiazole derivative 2f having p-hydroxy substituent has shown good activity against S. aureus. Among all derivatives, compounds 2a, 2b, 2c, 2g, and 2h containing phenyl without substituent and p-hydroxy, p-chloro, o-chloro, and 3-OH, 4-OCH₃ group exhibit moderate antibacterial activity while compound 2d containing p-nitro group shown exceptional antibacterial against B. subtilis. Whereas 2c, 2d, and 2e exhibit exceptional activity against P. aeruginos and K. pneumonia while the rest were showing moderate activity towards Gram -ve bacteria.

Figure 1. Activity of compounds 2 a-h against Gram Positive Bacteria.
The lactam derivatives 3c, 3d bearing p-chlorophenyl, p-nitro, and 3f o-chlorophenyl substituent respectively in the main scaffold exhibit exceptionally good antibacterial activity towards B. subtilis/S. aureus. Compounds 3e and 3f having o-hydroxy and o-chlorophenyl show excellent and good activity against Gram -ve bacteria P. aeruginos. Whereas 3c, 3e, and 3h give good MIC values against K. pneumonia. The rest derivatives of this category show moderate activity against Gram -ve and Gram +ve bacteria. The structural activity relationship study concludes that the variation in substituent and its position plays an important role in the activity of the fabricated compounds against Gram - ve and Gram +ve bacteria. Scheme 1 represents the effect of various substituents on the activity of the synthesized compounds.
Figure 4. Activity of compounds 3, a-h against Gram Negative Bacteria.

Table 2: Antimicrobial activity: (MIC µg/mL) of compounds 2, a-h and 3, a-h

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R with benzene ring</th>
<th>MIC (µg/mL) against Gram +ve Bacteria</th>
<th>MIC (µg/mL) against Gram -ve Bacteria</th>
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</thead>
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<td></td>
<td></td>
<td>S. aureus</td>
<td>B. subtilis</td>
</tr>
<tr>
<td>2 a</td>
<td>phenyl</td>
<td>50</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2 b</td>
<td>p-OH-phenyl</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>2 c</td>
<td>p-Cl-phenyl</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>2 d</td>
<td>p-NO₂-phenyl</td>
<td>25</td>
<td>6.25</td>
</tr>
<tr>
<td>2 e</td>
<td>o-OH-phenyl</td>
<td>12.5</td>
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</tr>
<tr>
<td>2 f</td>
<td>o-Cl-phenyl</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>2 g</td>
<td>4-OH,3-OCH₃-phenyl</td>
<td>&gt;100</td>
<td>25</td>
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<tr>
<td>2 h</td>
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<td>50</td>
<td>50</td>
</tr>
<tr>
<td>3 a</td>
<td>phenyl</td>
<td>&gt;100</td>
<td>&gt;100</td>
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<tr>
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<td>p-OH-phenyl</td>
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<td>50</td>
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<td>p-Cl-phenyl</td>
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<td>3 h</td>
<td>3-OH,4-OCH₃-phenyl</td>
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<tr>
<td>Control</td>
<td>ciprofloxacin</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>
Scheme 1

3. CONCLUSIONS

The present communication demonstrates the successful synthesis of novel series of lactam/thiazole derivatives having a five-membered thiazolyl ring. The spectral data of the synthesized compounds meet the standard values. The IR (absorbance band for C=O) and $^1$HNMR (signal of 2 H) data suggest the formation of a lactam ring. The in vitro antimicrobial screening results show that the compounds 2e, 3c, and 3d containing o-hydroxy, p-chloro, and p-nitro substituent respectively exhibit exceptional activity against S. aureus while compounds 2d and 3f bearing p-nitro and o-chloro substituent respectively were deemed to be the most competent against B. subtilis. Against E. coli compounds 2d (p-nitro) and 2f (o-chloro) were found to be most potent. In gram-negative bacterial strains, compounds 2c (p-chloro), 2g (4-OH-OCH$_3$), 3b (p-hydroxy), and 3e (o-hydroxy) were extremely potent against P. aeruginosa while compound 2e containing o-hydroxy group shows excellent activity against E.coli.

4. MATERIALS AND METHODS

4.1. Chemistry

Open capillaries methodology was used to access the melting point (m.p.) of all the newly synthesized compounds. The purity of synthesized compounds was checked by thin layer chromatographic plates (Merk, 60F-254) using I$_2$ vapor as a visualizer. All the new synthesized compounds were characterized by proton nuclear magnetic resonance spectroscopy using CDCl$_3$/DMSO as solvent. $^{300}$ MHz Bruker NMR spectrophotometer was used for the studies by taking TMS as the internal standard. The chemical shift ($\delta$) values are represented in ppm. For other analytical studies, Jasco FTIR-470 spectrophotometer and MS-JEOL SX102 Mass spectroscopy was used. KBr palates were used to record the IR spectra of synthesized compounds. NBA was used as a matrix and Xenon/Argon (10mA, 6Kv) was used as the FAB gas for the recording of mass spectra.

4.1.1 Synthesis of (E)substituted-N-benzylidene-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (1 a-h)

0.1 mole each of cyclohexanone, substituted aromatic aldehyde, and thiourea was taken in 25 ml dimethyl carbonate (solvent), the reaction mixture was refluxed at 95 °C temperature for 6 hrs. using NH$_4$OAc as a catalyst. The progress of reactions was monitored by thin-layer chromatography using a chloroform-methanol mixture as a developing solvent. After filtration the solid thus obtained was washed with ice-cold water, dried, and finally recrystallized with ethanol.

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(E)-N-benzyldiene-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (1a)

Yield 69 %; mp, 114 °C, Anal. Calcd. for C14H12N2S: C, 69.39; N, 11.56; S, 11.51; S, 13.26 %. IR νmax (KBr, cm⁻¹): 866 (C-S-C, str. thiazol ring), 3072 (Ar C-H), 1647 (C=N str.), 2918 (C-H, str. cyclohexane ring). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.38 (m, 8H, cyclohexane ring), 3.96 (d, 2H, cyclohexane ring), 7.79 (m, 4H, ArH). ¹³C NMR (75 MHz) (DMSO/CDCl₃): 20.7 ppm (d, 2H, cyclohexane ring).

4-((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2-imino)methyl)phenol (1b)

Yield 76 %; mp, 96-107 °C, Anal. Calcd. for C14H15ClN2S: C, 65.09; N, 6.19; S, 12.41, found: C, 65.01; N, 6.12; S, 12.35 %. IR νmax (KBr, cm⁻¹): 893 (C-S-C, str. thiazol ring), 3075 (Ar C-H), 1642 (C=N str.), 2923 (C-H, str. cyclohexane ring), 974 (C=N, str. thiazol ring), 3420 (p-OH-phenyl, str.). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.47 (m, 8H, cyclohexane ring), 8.92 (N=CH-C), 6.73-7.76 (m, 4H, ArH), 4.01 (d, 2H, cyclohexane ring), 4.21 (brs, 1H, s, exchangeable-OH).

N-(4-chlorobenzylidene)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (1c)

Yield 84 %; mp, 129-130 °C, Anal. Calcd. for C14H13ClN2S: C, 60.75; N, 10.12; S, 11.58, found: C, 60.70; N, 10.17; S, 11.52 %. IR νmax (KBr, cm⁻¹): 893 (C-S-C, str. thiazol ring), 3079 (Ar C-H), 1647 (C=N str.), 2932 (C-H, str. cyclohexane ring), 975 (C=N, str. thiazol ring), 770 (C-Cl str.). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.48 (m, 8H- cyclohexane ring), 8.95 (N=CH-C), 6.76-7.79 (m, 4H, ArH), 4.03 (d, 2H, cyclohexane ring).

N-(4-nitrobenzylidene)-4, 5, 6, 7-tetrahydrobenzo[d]thiazol-2-amine (1d)

Yield 63 %; mp, 122 °C, Anal. Calcd. for C14H11N3S: C, 58.52; N, 14.62; S, 11.16, found: C, 58.46; N, 14.54; S, 11.10 %. IR νmax (KBr, cm⁻¹): 892 (C-S-C, str. thiazol ring), 3074 (Ar C-H), 972 (C=N, str. thiazol ring), 2931 (C-H, str. cyclohexane ring), 1577 (sym str. N=O), 1298 (sym str. N=O). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.43 (m, 8H- cyclohexane ring), 8.91 (N=CH-C), 6.73-7.76 (m, 4H, ArH), 3.97 (d, 2H, cyclohexane ring).

2-((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2-imino)methyl)phenol (1e)

Yield 67 %; mp, 102-103 °C, Anal. Calcd. for C14H13ClN2S: C, 65.09; N, 10.84; S, 12.41, found: C, 65.01; N, 10.80; S, 12.36 %. IR νmax (KBr, cm⁻¹): 897 (C-S-C, str. thiazol ring), 3073 (Ar C-H), 1638 (C=N str.), 2921 (C-H, str. cyclohexane ring), 972 (C=N, str. thiazol ring), 3427 (p-OH-phenyl, str.). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.43 (m, 8H- cyclohexane ring), 8.91 (N=CH-C), 6.73-7.72 (m, 4H, ArH), 3.96 (d, 2H, cyclohexane ring), 4.19 (brs, 1H, s, exchangeable-OH).

N-(2-chlorobenzylidene)-4,5,6,7-tetrahydrobenzo[d]thiazol-2-amine (1f)

Yield 66 %; mp, 114-115 °C, Anal. Calcd. for C14H11ClN2S: C, 60.75; N, 10.12; S, 11.58, found: C, 60.70; N, 10.07; S, 11.50 %. IR νmax (KBr, cm⁻¹): 897 (C-S-C, str. thiazol ring), 3083 (Ar C-H), 1652 (C=N str.), 2938 (C-H, str. cyclohexane ring), 979 (C=N, str. thiazol ring), 781 (C-Cl str.). ¹HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.43 (m, 8H- cyclohexane ring), 8.92 (N=CH-C), 6.69-7.72 (m, 4H, ArH), 3.99 (d, 2H, cyclohexane ring).
(E)-2-methoxy-4-(((4,5,6,7-tetrahydrobenzo[d]thiazol-2-ylimino)methyl)phenol (1g)

Yield 70 %; mp, 106-107 °C. Anal. Calcd. for C_{15}H_{16}N_{2}O_{5}: C, 62.48; N, 9.71; S, 11.12. Found: C, 62.42; N, 9.64; S, 11.08 %. IR ν_{max} (KBr, cm⁻¹): 899 (C=S, thiazol ring), 3090 (Ar C-H, str.), 2944 (C-H, str. cyclohexane ring), 982 (C=N, str. thiazol ring), 3445 (3-OH-phenyl, str.).

1HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.24 (m, 8H, cyclohexane ring), 3.65 (d, 2H, cyclohexane ring), 6.63 (m, ArH), 3.87 (d, 2H, cyclohexane ring), 4.54 (brs, 1H), 3.36 (s, 3H, ArOCH₃).

2-methoxy-5-(((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)limino)methyl)phenol (1h)

Yield 78 %; mp, 125-126 °C. Anal. Calcd. for C_{15}H_{16}N_{2}O_{5}: C, 62.48; N, 9.71; S, 11.06. Found: C, 62.41; N, 9.65; S, 11.06 %. IR ν_{max} (KBr, cm⁻¹): 898 (C=S, thiazol ring), 3093 (Ar C-H, str.), 2945 (C-H, str. cyclohexane ring), 984 (C=N, str. thiazol ring), 3447 (3-OH-phenyl, str.), 1172 (4-OCH₃-phenyl, str.).

1HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.21 (m, 8H, cyclohexane ring), 3.37 (s, 3H, ArOCH₃), 6.68-7.72 (m, 5H, ArH), 3.32 (s, ArOCH₃), 7.66 (m, 4H, ArH), 3.35 (d, 2H, cyclohexane ring), 6.63-7.66 (m, 4H, ArH), 3.35 (s, -N-CHS-, 1H), 2.81 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 179, 141, 103, 78.

4.1.2. Synthesis of 2-phenyl-3-((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl)-2)thiazolidin-4-one (2a-h)

The final derivatives were synthesized by refluxing 0.01 moles of compounds 1a-h with thioglycolic acid (0.01 mole) in DMF at 80 °C temperature for 8 hrs. using 0.01 gm of zinc chloride as a catalyst. The reaction mixture was then transferred into the ice-cold water and stirred vigorously. After one hour, the solid compound thus obtained was separated and washed with cold water. An analytically unblended sample was obtained via recrystallization with ethanol. Characterization data of the compounds thus synthesized are given as:

![Diagram](http://dx.doi.org/10.29228/jrp.308)

2-phenyl-3-((4, 5, 6, 7-tetrahydrobenzo[5,4-d]thiazolyl)-2)thiazolidin-4-one (2a)

Yield 70 %; mp, 167 °C. Anal. Calcd. for C_{16}H_{18}N_{2}O_{5}: C, 60.73; N, 8.85; S, 20.27. Found: C, 60.69; N, 8.80; S, 20 %. IR ν_{max} (KBr, cm⁻¹): 1650 (C=O, str. thiadiazolinedine ring), 1068 (CH₂-S-CH, str. thiadiazinyl ring), 3072 (Ar C-H, str.), 1558 (C=C, str.). 1HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.21 (m, 8H, cyclohexane ring), 3.63 (d, 2H, cyclohexane ring), 6.68-7.72 (m, 5H, ArH), 3.32 (s, -N-CHS-, 1H), 2.81 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 179, 141, 103, 78.

2-(4-hydroxyphenyl)-3-((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl)-2)thiazolidin-4-one (2b)

Yield 80 %; mp, 129-130 °C. Anal. Calcd. for C_{16}H_{18}N_{2}O_{5}: C, 57.81; N, 8.43; S, 19.29. Found: C, 57.87; N, 8.46; S, 19.22 %. IR ν_{max} (KBr, cm⁻¹): 1656 (C=O, str. thiadiazolinedine ring), 1069 (CH₂-S-CH, str. thiadiazinyl ring), 3074 (Ar C-H, str.), 1565 (C=C, str.), 3438 (p-OH-phenyl, str.). 1HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.24 (m, 8H, cyclohexane ring), 3.65 (d, 2H, cyclohexane ring), 6.63-7.66 (m, 4H, ArH), 3.35 (s, -N-CHS- 1H), 2.83 (s, 2H, O=CCH₂-S). 4.74 (brs, 1H, s, exchangeable-OH). Mass M⁺: 242, 194, 141, 103, 94.

2-(4-chlorophenyl)-3-((4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl)-2)thiazolidin-4-one (2c)

Yield 67 %; mp, 148 °C. Anal. Calcd. for C_{16}H_{18}ClN_{2}O_{5}: C, 54.77; N, 7.98; S, 18.28. Found: C, 54.72; N, 7.94; S, 18.21 %. IR ν_{max} (KBr, cm⁻¹): 1652 (C=O, str. thiadiazolinedine ring), 1069 (CH₂-S-CH, str. thiadiazinyl ring), 3076 (Ar C-H, str.), 1563 (C=C, str.), 760 (C-Cl, str.). 1HNMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.23 (m, 8H, cyclohexane ring), 3.64 (d, 2H, cyclohexane ring), 6.68-7.76 (m, 4H, ArH), 3.36 (s, -N-CHS-, 1H), 2.86 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 213, 141, 112, 103.

2-(4-nitrophenyl)-3-((4, 5, 6, 7-tetrahydrobenzo[5,4-d]thiazolyl)-2)thiazolidin-4-one (2d)
Yield 65 %; mp, 153 °C. Anal. Calcd. for C_{16}H_{18}N_{4}O_{3}S_{2}: C, 53.17; N, 11.63; S, 17.74, found: C, 53.11; N, 11.68; S, 17.77 %. IR ν_{max} (KBr, cm⁻¹): 1656 (C=O, str. thiazolidinone ring), 1055 (CH_{2}-S-CH, str. thiazadiazinyl ring), 3071 (Ar C-H, str.), 1561 (C=C, str.), 1720 (N=O, asym str.), 1292 (N=O, sym str.). ¹H NMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.21 (m, 8H, cyclohexane ring), 3.62 (d, 2H, cyclohexane ring), 6.68-7.76 (m, 4H, ArH), 3.36 (s, -N-CHS-, 1H), 2.81 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 224, 141, 123, 103.

2-(2-hydroxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)thiazolidin-4-one (2e)  

Yield 67 %; mp, 137.8 °C. Anal. Calcd. for C_{16}H_{18}N_{4}O_{3}S; C, 57.81; N, 8.43; S, 19.29, found: C, 57.86; N, 8.49; S, 19.21 %. IR ν_{max} (KBr, cm⁻¹): 1658 (C=O, str. thiazolidinone ring), 1071 (CH₂-S-CH, str. thiazadiazinyl ring), 3075 (Ar C-H str.), 1570 (C=C, str.), 3447 (o-OH-phenyl, str.). ¹H NMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.27 (m, 8H, cyclohexane ring), 3.69 (d, 2H, cyclohexane ring), 6.63-7.62 (m, 4H, ArH), 3.37 (s, -N-CHS-, 1H), 2.85 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 194, 141, 103, 94.

2-(2-chlorophenyl)-3-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)thiazolidin-4-one (2f)  

Yield 68 %; mp, 121-122 °C. Anal. Calcd. for C_{16}H_{18}ClN_{4}O_{3}S; C, 54.77; H, 4.31; Cl, 10.10; N, 7.98; S, 18.28, found: C, 54.72; H, 4.38; Cl, 10.17; N, 7.98; S, 18.28 %. IR ν_{max} (KBr, cm⁻¹): 1698 (C=O, str. thiazolidinone ring), 1031 (CH₂-S-CH, str. thiazadiazinyl ring), 3187 (Ar C-H str.), 1553 (C=C, str.), 768 (C-Cl, str.). ¹H NMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.24 (m, 8H- cyclohexane ring), 3.65 (d, 2H, cyclohexane ring), 6.68-7.78 (m, 4H, ArH), 3.37 (s, -N-CHS-, 1H), 2.89 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 213, 141, 112, 103.

2-(4-hydroxy-3-methoxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)thiazolidin-4-one (2g)  

Yield 68 %; mp, 135.8 °C. Anal. Calcd. for C_{17}H_{18}N_{4}O_{4}S; C, 56.33; N, 7.73; S, 17.69, found: C, 56.37; N, 7.79; S, 17.63 %. IR ν_{max} (KBr, cm⁻¹): 1666 (C=O, str. thiazolidinone ring), 1078 (CH₂-S-CH, str. thiazadiazinyl ring), 3082 (Ar C-H str.), 1572 (C=C, str.), 3478 (4-OH-phenyl, str.), 1174 (3-OCH₃-phenyl, str.). ¹H NMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.24 (m, 8H- cyclohexane ring), 3.51 (d, 2H, cyclohexane ring), 6.63-7.71 (m, 3H, ArH), 3.43 (s, -N-CHS-, 1H), 2.92 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 195, 141, 124, 103.

2-(3-hydroxy-4-methoxyphenyl)-3-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)thiazolidin-4-one (2h)  

Yield 67 %; mp, 148.6 °C. Anal. Calcd. for C_{17}H_{18}N_{4}O_{4}S; C, 56.33; N, 7.73; S, 17.69, found: C, 56.39; N, 7.73; S, 17.63 %. IR ν_{max} (KBr, cm⁻¹): 1673 (C=O, str. thiazolidinone ring), 1077 (CH₂-S-CH, str. thiazadiazinyl ring), 3083 (Ar C-H str.), 1576 (C=C, str.), 3479 (3-OH-phenyl, str.), 1176 (4-OCH₃-phenyl, str.). ¹H NMR (300 MHz) (DMSO/CDCl₃) δ (ppm): 1.28 (m, 8H- cyclohexane ring), 3.58 (d, 2H, cyclohexane ring), 6.68-7.72 (m, 3H, ArH), 3.45 (s, -N-CHS-, 1H), 2.94 (s, 2H, O=CCH₂-S). Mass M⁺: 242, 195, 141, 124, 103.

4.1.3. Synthesis of 3-chloro-4-phenyl-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)azetidin-2-one (3-a-h)  

To synthesize 3a-h, 0.01 moles of 1a-h were taken in 30 ml of dioxane, thereafter 0.02 moles of CH₂COCl₂ and 0.02 moles of N(C₂H₅)₃ were added to the reaction mixture under stirring at 0 °C. Thereafter the reaction mixture was kept at 25 °C for 5 hrs. and subsequently refluxed at 90°C temperature for 12 hrs. After evaporation of the additional solvent, the final residue was poured into ice-cold water and ultimately recrystallized with ethanol. The analytical data of the prepared novel derivatives are given as:
3-chloro-4-phenyl-1-(4, 5, 6, 7-tetrahydrobenzo[5,4-d]thiazolyl-2)azetidin-2-one (3a)

Yield 78 %; mp, 119 °C, Anal. Calcd. for C_{16}H_{15}ClN_{2}O_{5}: C, 60.28; N, 8.79; S, 10.06; found: C, 60.38; N, 8.71; S, 10.11 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1619 (C=O, str.), 1412 (C-N, str.), 1564 (C=O, str.), 2912(C-H, str. cyclohexane ring), 1571 (C=C, str.), 874 (C-S, str. thiazolyl ring), 3066 (Ar C-H, str.), 966 (HC=N, str. thiazol ring), 768 (C-Cl, str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.31 (d, N-CH, lactam ring), 1.32 (m, 8H- C cyclohexane ring), 1.26 (2H, triplet, cyclohexane ring), 6.78-7.61 (m, 5H, ArH), 3.21 (d, C-CH-Cl, lactam ring). Mass M\(^+\): 244, 181, 141, 104, 78.

3-chloro-4-(4-hydroxyphenyl)-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)azetidin-2-one (3b)

Yield 65 %; mp, 134-135 °C, Anal. Calcd. for C_{16}H_{15}ClN_{2}O_{5}: C, 57.40; N, 8.37; S, 9.58; found: C, 57.47; N, 8.32; S, 9.52 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1636 (C=O, str.), 1412 (C-N, str.), 1656 (C=O, str.), 2912 (C-H, str. cyclohexane ring), 1578 (C=C, str.), 875 (C=S, str. thiazolyl ring), 3066 (Ar C-H, str.), 974 (HC=N, str. thiazol ring), 769 (C-Cl, str.), 3476 (4-OH-phenyl, str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.27 (d, N-CH, lactam ring), 1.28 (m, 8H- C cyclohexane ring), 1.26 (2H, triplet, cyclohexane ring), 6.78-7.60 (m, 4H, ArH), 3.19 (d, C-CH-Cl, lactam ring), 4.37 (brs, 1H, s, exchangeable-OH). Mass M\(^+\): 244, 197, 141, 104, 94.

3-chloro-4-(4-nitrophenyl)-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2) azetidin-2-one (3c)

Yield 81 %; mp, 112-113 °C, Anal. Calcd. for C_{16}H_{15}ClN_{2}O_{5}: C, 54.40; N, 7.93; S, 9.08; found: C, 54.47; N, 7.99; S, 9.02 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1725 (C=O, str.), 1347 (C-N, str.), 1589(C=O, str.), 2977 (C-H, str. cyclohexane ring), 1576 (C=C, str.), 877 (C=S, str. thiazolyl ring), 3027 (Ar C-H, str.), 969 (HC=N, str. thiazol ring), 756 (C-Cl, str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.25 (d, N-CH, lactam ring), 1.29 (m, 8H- C cyclohexane ring), 1.22 (2H, triplet, cyclohexane ring), 6.78-7.62 (m, 4H, ArH), 3.17 (C-CH-Cl, lactam ring). Mass M\(^+\): 244, 215, 141, 112, 104.

3-chloro-4-(4-nitrophenyl)-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2) azetidin-2-one (3d)

Yield 67 %; mp, 136-137 °C, Anal. Calcd. for C_{16}H_{15}ClN_{2}O_{5}: C, 52.82; N, 11.49; S, 8.79; found: C, 52.87; N, 11.51; S, 8.81 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1641 (C=O, str.), 1418 (C-N, str.), 1662 (C=O, str.), 2927(C-H, str. cyclohexane ring), 1581 (C=C, str.), 889 (C-S, str. thiazolyl ring), 3086 (Ar C-H, str.), 962 (HC=N, str. thiazol ring), 777 (C-Cl, str.), 158 (N=O, asym str.), 1283 (N=O, sym str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.31 (d, N-CH, lactam ring), 1.27 (m, 8H- C cyclohexane ring), 1.29 (2H, triplet, cyclohexane ring), 6.78-7.68 (m, 4H, ArH), 3.20 (d, C-CH-Cl, lactam ring). Mass M\(^+\): 244, 215, 141, 112, 104.

3-chloro-4-(2-hydroxyphenyl)-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2) azetidin-2-one (3e)

Yield 68 %; mp, 155 °C, Anal. Calcd. for C_{16}H_{15}ClN_{2}O_{5}: C, 57.40; N, 8.37; S, 9.58; found: C, 57.47; N, 8.31; S, 9.51 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1640 (C=O, str.), 1415 (C-N, str.), 1657 (C=O, str.), 2915 (C-H, str. cyclohexane ring), 1582 (C=C, str.), 878 (C-S, str. thiazolyl ring), 3070 (Ar C-H, str.), 974 (HC=N, str. thiazol ring), 765 (C-Cl, str.), 3476 (OH-phenyl, str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.23 (d, N-CH, lactam ring), 1.29 (m, 8H- C cyclohexane ring), 1.28 (2H, triplet, cyclohexane ring), 6.78-7.65 (m, 4H, ArH), 3.16 (d, C-CH-Cl, lactam ring), 4.33 (brs, 1H, s, exchangeable-OH). Mass M\(^+\): 244, 226, 141, 123, 104.

3-chloro-4-(2-chlorophenyl)-1-(4, 5, 6,7-tetrahydrobenzo[5,4-d]thiazolyl-2) azetidin-2-one (3f)

Yield 79 %; mp, 122 °C, Anal. Calcd. for C_{16}H_{14}Cl_{2}N_{2}O_{5}: C, 54.40; N, 7.93; S, 9.08; found: C,54.48; N, 7.99; S, 9.02 %. IR v_{\text{max}} (KBr, cm\(^{-1}\)): 1632 (C=O, str.), 1415 (C-N, str.), 1662(C=O, str.), 2932(C-H, str. cyclohexane ring), 1576 (C=C, str.), 870 (C-S, str. thiazolyl ring), 3071 (Ar C-H, str.), 974 (HC=N, str. thiazol ring), 774 (C-Cl, str.). \(^1\)HNMR (300 MHz) (DMSO/CDCl\(_3\)) \(\delta\) (ppm): 4.26 (d, N-CH, lactam ring), 1.23 (m, 8H- C cyclohexane ring), 1.21 (2H, triplet, cyclohexane ring), 6.77-7.69 (m, 4H, ArH), 3.12 (d, C-CH-Cl, lactam ring). Mass M\(^+\): 244, 215, 141, 112, 104.

3-chloro-4-(4-hydroxy-3-methoxyphenyl)-1-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)azetidin-2-one (3g)

http://dx.doi.org/10.29228/jrp.308
Yield 66 %; mp, 142-143 °C, Anal. Calcd. for C17H17CIN2O5S: C, 55.96; N, 7.68; S, 8.79, found: C, 55.91; N, 7.62; S, 8.74 %.

IR νmax (KBr, cm−1): 1634 (C=N, str.), 1415 (C-N, str.) , 1662 (C=O, str.), 2939 (C-H, str. cyclohexane ring), 1577 (C=C, str.), 874 (C-S, str. thiazolyl ring), 3075 (Ar C-H, str.), 978 (HC=N, str. thiazol ring), 779 (C-Cl, str.), 3468 (4-OH-phenyl, str.), 1179 (3-OCH3-phenyl, str.). 1H NMR (300 MHz) (DMSO/CDCl3) δ (ppm): 4.24 (d, N-H, lactam ring), 1.25 (m, 8H- C cyclohexane ring), 1.24 (2H, triplet, cyclohexane ring), 6.73-7.68 (m, 4H, ArH), 3.16 (d, C-CH3-Cl, lactam ring), 4.44 (brs, 1H, s, exchangeable-OH), 3.36 (s, 3H, ArOCH3). Mass M+: 244, 227, 141, 124, 104.

3-chloro-4-(3-hydroxy-4-methoxyphenyl)-1-(4,5,6,7-tetrahydrobenzo[5,4-d]thiazolyl-2)azetidin-2-one (3 h)

Yield 61 %; mp, 105-106 °C, Anal. Calcd. for C17H17CIN2O5S: C, 55.96; N, 7.68; S, 8.79, found: C, 55.98; N, 7.61; S, 8.71 %. IR νmax (KBr, cm−1): 1640 (C=N, str.), 1415(C-N, str.) , 1666 (C=O, str.), 2941 (C-H, str. cyclohexane ring), 1579 (C=C, str.), 878 (C-S, str. thiazolyl ring), 3082 (Ar C-H, str.), 980 (HC=N, str. thiazol ring), 779 (C-Cl, str.), 3471 (3-OH-phenyl, str.), 1183(4-OCH3-phenyl, str.). 1H NMR (300 MHz) (DMSO/CDCl3) δ (ppm): 4.17 (d, N-H, lactam ring), 1.20 (m, 8H- C cyclohexane ring), 1.19 (2H, triplet, cyclohexane ring), 6.78-7.69 (m, 4H, ArH), 3.11 (d, C-CH3-Cl, lactam ring), 4.30 (brs, 1H, s, exchangeable-OH), 3.31(s, 3H, ArOCH3). Mass M+: 244, 227, 141, 124, 104.

4.2. In vitro anti-microbial susceptibility test (AST)

A routine antimicrobial susceptibility test (AST) was employed to evaluate the toxicity of the fabricated compounds toward the gram-negative (P. aeruginosa, E. coli, K. pneumonia) and gram-positive (B. subtilis, S. aureus) bacteria. The pure isolates of the test microbial species were obtained from the Department of Microbiology KGMU Lucknow. A reported Mueller-Hinton serial dilution methodology was used to confirm the identity of the working strains by gram staining and colony morphology [27, 28].

The variable concentrations of the synthesized compound (2, a-h and 3, a-h) were used to grow the test microbial species in numerous identical sets of LB media. Growth was estimated, by recording the absorbance value after 24 hours at 600 nm for all the test microbial species. Minimum inhibitory concentration (MIC) values of the synthesized compounds against the pathogenic gram-negative and gram-positive bacteria were obtained from the plot of optical density versus compound concentrations. Bacterial growth was done by incubating LB media (10 ml) containing P. aeruginosa, E. coli, B. subtilis, K. pneumonia, and Staphylococcus aureus, for 8 hrs at 37°C in a conical tube. 100 µl of the above bacterial suspension with 10 ml of LB media was then transferred to each conical tube having test compounds of varying concentration (1200, 600, 300, 150, 75, 37.5, 18.75, 9.38, 4.69, 2.34, 1.17, 0.59, and 0.30 µg ml−1). The control experiment was performed, in a conical tube containing ciprofloxacin and LB media (10 ml), by adding 100 µl of the bacterial suspension. Bacterial growth for every conical tube was checked after 24 hrs by computing the absorbance value at 600 nm. The plot of compound concentration and absorbance value was used to obtain the MIC (corresponding to the drop in optical density) of the particular compound.

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**REFERENCES**


