SYNTHESIS, CHARACTERIZATION AND SCREENING OF 2-THIOXOIMIDAZOLIDIN-4-ONE AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

2-thioxoimidazolidin-4-one constitutes are an important group of heterocyclic compounds and have been shown to exhibit wide range of pharmacological activities such as anti-bacterial and anti-fungal. A series of 2-thioxoimidazolidin-4-one derivatives were synthesized and characterized by Mass, IR and ¹H NMR spectroscopy. All these synthesized compounds were tested for their in vitro antimicrobial activity against Gram positive, Gram negative bacteria and fungal strains in N,N-dimethylformamide(DMF). Among the screened compounds, RPI-3 showed most potent antimicrobial activity.

Keywords: 2-thioxoimidazolidin-4-one, antibacterial activity, Gram positive bacteria and Gram negative bacteria and fungi, N,N-dimethylformamide.

INTRODUCTION

Indiscriminate use of antibiotics has lead to multiple resistances by pathogenic microorganisms against these drugs. This has lead to a global threat of infectious diseases. Therefore, there is an urgent need to search for compounds which may have an entirely new mechanism because of their structural complexity or new ways to tackle these pathogenic bacteria. The most spectacular advances in medicinal chemistry were made when heterocyclic compounds played an important role in regulating biological activities. 2-thioxoimidazolidin-4-ones have a wide applications as intermediates and reagents as well as therapeutics, herbicides and fungicides. They are usually considered as useful intermediates in peptide synthesis and structure determination. In the present work, 2-thioxoimidazolidin-4-one derivatives were synthesized from (E)-1-(((1,3-diphenyl-1H-pyrazol-4-yl)methylene)mono derivatives were synthesized from (E)
thiosemicarbazide. 2-thioxoimidazolidin-4-one derivatives exhibit a wide range of biological and pharmacological properties such as anti-inflammatory and antinociceptive [1], antibacterial activity[2, 3], cytotoxicity [4].

In the present work, 2-thioxoimidazolidin-4-one derivatives were synthesized. Structures were confirmed by IR and NMR spectral analysis and antimicrobial activity was evaluated against some pathogenic microorganisms. Microorganism uses in the study are pathogenic. *Bacillus cereus* is a motile, aerobic or facultative anaerobic, spore-forming, Gram positive bacterium. It is also an opportunistic pathogen in immune compromised, critically ill, or otherwise debilitated patients, those with foreign bodies, and intravenous drug abusers [5]. *Escherichia coli* recognized as a common cause of both epidemic and sporadic diseases, notably bloody diarrhoea and hemo-lytic-uremic syndrome [6]. They are widely distributed in hospitals and are increasingly being isolated from community acquired infections [7]. *Pseudomonas aeruginosa* opportunistic, nosocomial pathogen of immune compromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections [8].

**Experimental section**

**Synthesis of 3-((1,3-diphenyl-1H-pyrazol-4-yl)methyleneamino)-2-thioxoimidazolidin-4-one**  
**Synthesis of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde**

*Synthesis of (E)-2-phenyl-1-(1-phenylethylidene)hydrazine:* To a methanolic solution of acetophenone (0.01M) and phenyl hydrazine (0.01M), small amount of con. HCl was added and solution was stirred at room temperature for about 10 to 15 minutes. The resulting solid was filtered, washed with cold methanol and crystallized.

*Vilsmeier-Haack Formylation:* The above synthesized product (E)-2-phenyl-1-(1-phenylethylidene) hydrazine was added in a mixture of Vilsmeier-Haack reagent(prepared by drop wise addition of 3 ml POCl₃ in ice cooled 15 ml DMF) and solution was refluxed for 1hr. The completion of reaction was confirmed by analytical thin layer chromatography (TLC) . The reaction mixture was poured into crushed ice and was kept for overnight. The resulting product was filtered, washed and dried.
Synthesis of 3-((1,3-diphenyl-1H-pyrazol-4-yl)methyleneamino)-2-thioximidazolidin-4-one

**Synthesis of (E)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)thiosemicarbazide:** Equimolar mixture of 1,3-diphenyl-1H-pyrazole-4-carbaldehyde and thio-semicarbazide in methanol was refluxed for 1hr in presence of conc.HCl. The resulting solid product was filtered, washed with cold methanol and dried.

**Synthesis of 3-((1,3-diphenyl-1H-pyrazol-4-yl)methyleneamino)-2-thioximidazolidin-4-one:** Equimolar amount of (E)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methylene) thiosemicarbazide and ethyl chloro acetate were dissolved in chloroform in presence of sodium acetate. The reaction mixture was refluxed for 8hrs. The solid product was filtered and washed with hexane and water respectively to remove impurities. The crude product was recrystallized.

**Scheme**

The melting point of all the synthesized compounds was determined by open capillary method and was uncorrected. The characterization of all these compounds was done by IR, NMR and mass spectral data. The IR spectra was recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. The Mass spectra was recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. $^1$H NMR was determined in DMSO solution on a Bruker Ac 400 MHz spectrometer.
Antimicrobial activity

Microorganisms tested

The studied microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. The microorganisms were maintained at 4°C. The Gram positive bacteria studied were Staphylococcus aureus ATCC29737 (SA), Corynebacterium rubrum ATCC14898 (CR), Listeria monocytogenes ATCC19112 (LM), Bacillus cereus ATCC11778 (BC); Gram negative bacteria were Pseudomonas aeruginosa ATCC27853 (PA), Escherichia coli NCIM2931 (EC), Klebsiella pneumoniae NCIM2719 (KP), Salmonella typhimurium ATCC23564 (ST) and Fungi were Candida albicans ATCC2091 (CA), Cryptococcus neoformans NCIM3542 (CN), Candida glabrata NCIM3448 (CG), Candida epicola NCIM3367 (CE). The organisms were maintained on nutrient agar and MGYP medium (Hi Media, India) for bacteria and fungi respectively, at 4°C and sub-cultured before use. The microorganisms studied are clinically important ones causing several infections and food spoilage.

Agar well diffusion method

In vitro antimicrobial activity of the 2-thioxoimidazolidin-4-one derivatives were studied against pathogenic microbial strains by the agar well diffusion method [9].

RESULTS AND DISCUSSION

Table 1: The physical constants of all the synthesized compounds

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Substitution R</th>
<th>M.F.</th>
<th>M.W.</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPI-1</td>
<td>-3,4-di-OCH₃</td>
<td>C₂₁H₁₉N₅O₃S</td>
<td>421</td>
<td>79</td>
</tr>
<tr>
<td>RPI-2</td>
<td>-4-CH₃</td>
<td>C₂₀H₁₇N₅OS</td>
<td>375</td>
<td>84</td>
</tr>
<tr>
<td>RPI-3</td>
<td>-4-Cl</td>
<td>C₁₉H₁₄ClN₅OS</td>
<td>395</td>
<td>80</td>
</tr>
<tr>
<td>RPI-4</td>
<td>-2-OH</td>
<td>C₁₉H₁₅N₅O₂S</td>
<td>377</td>
<td>85</td>
</tr>
<tr>
<td>RPI-5</td>
<td>-2-OCH₃</td>
<td>C₂₀H₁₇N₅O₂S</td>
<td>391</td>
<td>74</td>
</tr>
<tr>
<td>RPI-6</td>
<td>-4-Br</td>
<td>C₁₉H₁₄BrN₅OS</td>
<td>441</td>
<td>76</td>
</tr>
<tr>
<td>RPI-7</td>
<td>-4-F</td>
<td>C₁₉H₁₄FN₅OS</td>
<td>379</td>
<td>82</td>
</tr>
<tr>
<td>RPI-8</td>
<td>-3-NO₂</td>
<td>C₁₉H₁₄N₆O₃S</td>
<td>406</td>
<td>74</td>
</tr>
<tr>
<td>RPI-9</td>
<td>-4-OCH₃</td>
<td>C₂₀H₁₇N₅O₂S</td>
<td>391</td>
<td>82</td>
</tr>
<tr>
<td>RPI-10</td>
<td>-4-OH</td>
<td>C₁₉H₁₅N₅O₂S</td>
<td>377</td>
<td>80</td>
</tr>
</tbody>
</table>

In total, 10 compounds were synthesized (RPI-1 to RPI-10). The physical constants of all the synthesized compounds are given in Table 1. The IR and NMR spectral data confirmed their molecular structure. Data of RPI-1 compound is given below:
RPI-2:IR (KBr, cm⁻¹): -C=C(str.): 1545, -C=S(str.): 1172, -C=O(str.): 1643, -N-H(sym.): 1597, -C-H(str.): 2785.

¹H-NMR (δ, ppm): 2.50 (s, 3H, CH₃), 3.91 (s, 2H, CH₂), 7.53 (q, 1H, CH), 7.55 (t, 1H, NH), 7.57 (t, 1H, Ar-CH), 8.41 (s, 1H, CH hydrazide), 11.89 (s, 1H, SH).

Fig 1: Antibacterial activity of 2-thioximidazolidin-4-one derivatives in DMF against Gram positive bacteria(A) and gram negative bacteria(B).

Antimicrobial assay
Antimicrobial potentiality of 2-thioximidazolidin-4-one derivatives was investigated against some pathogenic microbial strains. All the ten compounds showed better antibacterial activity towards Gram positive bacteria than Gram negative bacteria. None of the compounds showed activity against any of the fungal strains. RPI 8 showed best activity.
against Gram positive bacteria though highest activity was shown by RPI-6 against *B. cereus* (14 mm). Similar results were also reported by Baluja and Chanda (2012) with some Schiff bases [10]. *B. cereus* and *L. monocytogenes* were the most susceptible and resistant bacteria respectively (Fig. 1a). None of the compounds showed activity against Gram negative bacteria *E. coil* and *P. aeroginosa*. *K. pneumonia* and *S. typhimurium* were inhibited by only 4 and 3 synthesized compounds (Fig. 1b). This differential activity of the compounds is because of 2 reasons viz. the central moiety of all the compounds is 2-thioxoimidazolin-4-one with different side chains (Table 1). RPI-3 showed best antibacterial activity i.e. chloride group appears to be most promising. The synthesized compounds showed better activity towards Gram positive bacteria than Gram negative bacteria. Similar results were reported by Bhalu et al. (2014) for dihydropyrano[c]chromenes derivatives [11].

The reason for the differences in activity of Gram positive and negative bacteria might be due to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative bacteria have multilayered cell wall [12] The antibacterial activity depends on structure (molecular) of the compound, solvent used and strain (bacterial) used for investigation [13].

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**REFERENCES**


