Synthesis of several 2-(5-chloro-3-methyl-1-(pyridin-2-yl) pyrazolidin-4-yl)-3-substitutedphenylthiazolidin-4-ones as prospective antimicrobial agents

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ABSTRACT
Several 2-(5-chloro-3-methyl-1-(pyridin-2-yl) pyrazolidin-4-yl)-3-substitutedphenylthiazolidin-4-one (4a-f) have been synthesized by conventional synthesis methodology. The synthesized derivatives were characterized by IR, 1H-NMR, Mass and elemental analysis (C, H, N). Furthermore the synthesized 2-(5-chloro-3-methyl-1-(pyridin-2-yl) pyrazolidin-4-yl)-3-substitutedphenylthiazolidin-4-one (4a-f) were tested for antimicrobial activities. The compound 4c displayed significant biological activities among the all tested derivatives.

Keywords: Antibacterial, antifungal, thiazolidin-4-one, acute toxicity.

INTRODUCTION
Pyrazole symbolizes a class of simple aromatic ring organic compounds of the heterocyclic series which is a 5-membered ring skeleton composed of three carbon and two nitrogen atoms. Ludwig Knorr was the first who coined the term pyrazole in 1883. In 1959, the first natural pyrazole, 1-pyrazolyl-alanine, was isolated from seeds of watermelons [1-2]. A bulk of literature is available to show the biological versatility such as anti-inflammatory [3], antibacterial [4-5], anti-convulsant [6], anticancer [7-8], anti-depressant [9], anti-hyperglycemic [10], antiviral [11], antipyretic [12], antioxidant [13], antitubercular [14], fungicides [15], and analgesic activities [16]. These pyrazoles have also found applications in transition-metal chemistry as an analytical reagent [17]. On the other hand, thiazolidinone bearing derivatives have been known to exhibit a wide range of physiological and pharmacological activities [18-23]. Above observation encouraged us to design several pyrazole bearing thiazolidinones with the hope to show significant antibacterial and antifungal activity with lesser amount of toxicity.

RESULTS AND DISCUSSION
Chemistry
Ethyl acetoacetate and 2-hydrazinylpyridine undergo cyclo-condensation reaction to furnish 3-methyl-1-phenyl-1H-pyrazol-5 (4H)-one (1) which on chloroformylated yielded 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (2). Compound 2 on reaction with different 5-substituted anilines furnished 2-{[5-chloro-3-methyl-1-(pyridin-2-yl) pyrazolidin -4-yl] methylene} substituted anilines (3a-f). Cyclisation of compounds 3a-f was carried out by means of thioglycolic acid to produce 2-{[5-chloro-3-methyl-1-(pyridin-2-yl) pyrazolidin -4-yl] 3'-substitute dphenylthiazolidin -4- ones} (4a-f) (Scheme-1). Synthesised derivatives were assayed using the cup plate method for antimicrobial activity against selected pathogenic panel of microbes. The screening results were compared with standard ampicillin trihydrate and fluconazole respectively for antibacterial and antifungal testing. Furthermore the most potent congener was also tested for lethal dose. From the antimicrobial screening data of compounds 3 and 4a-f, it was found that conversion of compound 3a-f into thiazolidinone derivatives i.e. 4a-f, brought enhancing antibacterial and antifungal activity. Among the derivatives 4a-f, derivative 4c
and 4e showed remarkable potency against the used pathogens. On the other hand, remaining compounds exhibited mild to moderate activity. Results revealed that compound 4c was found the most potent one, showing broad spectrum inhibitory profile having lesser toxicity (Table-1).

![Chemical structure](image)

**Scheme-1**

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>S. aureus (mm)</th>
<th>E. Coli</th>
<th>K. pneumoniae (mm)</th>
<th>P. vulgaris (mm)</th>
<th>A. fumigatus (mm)</th>
<th>C. glabrata (mm)</th>
<th>C. albicans (mm)</th>
<th>C. krusei (mm)</th>
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</thead>
<tbody>
<tr>
<td>4a</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>8</td>
<td>5</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>4d</td>
<td>12</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>4e</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4f</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Ampicillin trihydrate (std.)</td>
<td>16</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole (std.)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>15</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>DMF (control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

-: showing no activity

Table-1. Antibacterial and antifungal data for the synthesized compounds (4a-f).
Antimicrobial test

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial and fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Microorganisms employed antibacterial studies were Staphylococcus aureus, Escherichia coli, Klabsiella pneumoniae and Proteus vulgaris. Disk diffusion method [24-25] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an h. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The inhibition zone values of the tested compounds against the tested bacteria strains summarized in Table 1. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against Aspergillus fumigates (plant isolate), Candida glabrata, Candida albicans and Candida krusei in DMSO by the serial plate dilution method [26-27]. Fluconazole (antifungal) was used as reference drug. Sabouraud’s agar media were prepared by dissolving peptone (1 g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawnning. A loopful of particular fungal strain was dispensed to each screw-capped bottle and sterilized by dry heat for 1 hr. The resulting reaction mixture was refluxed for 2 h. On completion of the reaction, the reaction mixture was allowed to cool in ice bath to precipitate the solid. The solid filtered, dried and recrystallized from ethanol to give compound 1. Yield: 77%, Rf: 0.68. m.p. 127–129 °C. Anal. Caled. For C17H17N4O: C, 63.95; H, 5.70; N, 18.63%. Found: C, 63.89; H, 5.67; N, 18.54%. IR (KBr, cm-1): 3170 (NH), 3140 (C…H aromatic), 1760, 1688, 1682, 1565, 1277, 1237. 1H-NMR (DMSO-d6 ppm): 9.70 (s, 1H), 6.80-7.15 (m, 4H), 6.10 (brs, 1H), 5.33 (dd, 1H), 4.58 (d, 1H), 4.15 (d, 1H), 1.03 (s, 3H). MS (m/z): 225.07 (M)+.

Preparation of 3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one (1)
An ethanolic solution of the ethyl acetocetate (0.001 mol) and 2-hydrazinylpyridine (0.001 mol) was stirred at room temperature for 1 hr. The resulting reaction mixture was refluxed for 6 h. On completion of reaction, the reaction mixture was allowed to cool in ice bath to precipitate the solid. The solid filtered, dried and recrystallized from ethanol to give compound 1. Yield: 77%, Rf: 0.68, m.p. 127–129 °C. Anal. Caled. For C17H17N4O: C, 63.95; H, 5.70; N, 18.63%. Found: C, 63.89; H, 5.67; N, 18.54%. IR (KBr, cm-1): 3170 (NH), 3140 (C…H aromatic), 1760, 1688, 1682, 1565, 1277, 1237. 1H-NMR (DMSO-d6 ppm): 9.70 (s, 1H), 6.80-7.15 (m, 4H), 6.10 (brs, 1H), 5.33 (dd, 1H), 4.58 (d, 1H), 4.15 (d, 1H), 1.03 (s, 3H). MS (m/z): 225.07 (M)+.

Preparation of 5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (2)

Dimethylformamide solution of compound 1 (0.001 mol) cooled and followed by the dropwise addition of phosphorus oxychloride (0.002 mol). After completion of the addition the resulting mixture was refluxed for 2 h. On completion of the reaction, the reaction mixture was cooled, poured into crushed ice-water, filtered, dried and recrystallised from ethanol to furnish yellowish brown solid compound 2. Yield: 75%, m.p. 142–144 °C. Anal. Caled. For C10H7ClN2O: C, 53.22; H, 5.36; N, 18.62%. Found: C, 53.29; H, 5.37; N, 18.60%. IR (KBr, cm-1): 3162, 3146, 2964, 1688, 1620, 1565, 1277, 1237. 1H-NMR (DMSO-d6 ppm): 9.70 (s, 1H), 6.80-7.15 (m, 4H), 6.10 (brs, 1H), 5.33 (dd, 1H), 4.58 (d, 1H), 4.15 (d, 1H), 1.03 (s, 3H). MS (m/z): 225.07 (M)+.

General preparation of 2-[5-chloro-3-methyl-(pyridin-2-yl)pyrazolin-4-yl]methylene]substituted anilines (3a-f)

An ethanolic mixture of compound 1 (0.001 mol) and substituted anilines (0.001 mol) in the presence of few drops of glacial acid was refluxed for 5-7 hr. On completion of the reaction, reaction mixture cooled, poured onto crushed ice-water, stirred, filtered, dried, recrystallized from suitable solvents to yield compounds 3a-f.

Measurement

The melting points of the compounds were determined in open glass capillaries with the help of thermonic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyser. The results were found within the ±0.4% of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and 1H-NMR spectra on Bruker DPX 200 using TMS as internal standard.

Synthesis

Preparation of 3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one (1)
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General preparation of 2-[5-chloro-3-methyl-(pyridin-2-yl)pyrazolin-4-yl]methylene]substituted anilines (3a-f)

An ethanolic mixture of compound 2 (0.001 mol) and substituted anilines (0.001 mol) in the presence of few drops of glacial acid was refluxed for 5-7 hr. On completion of the reaction, reaction mixture cooled, poured onto crushed ice-water, stirred, filtered, dried, recrystallized from suitable solvents to yield compounds 3a-f.
In the present study, our attention was focused on the synthesis and antimicrobial evaluation of a series of 2-[[5-chloro-3-methyl-1-(pyridin-2-yl)pyrazolidin-4-yl]-3-substitutedphenyl-thiazolidin-4-ones 4a-f. A mixture of compound 3a-f (0.001 mol) in N,N'-dimethyl formamide and thioglycolic acid (0.001 mol) in presence of anhydrous zinc chloride was refluxed for 2-4 h. After completion of reaction (monitored by TLC), excess of solvent was distilled and cooled residual mass diluted with ice-water. The solid filtered, washed with water, dried and recrystallised by appropriate solvents to yield desired compounds 4a-f.

**CONCLUSION**

In the present study, our attention was focused on the synthesis and antimicrobial evaluation of a series of 2-[[5-chloro-3-methyl-1-(pyridin-2-yl)pyrazolidin-4-yl]-3-substitutedphenyl-thiazolidin-4-ones 4a-f. Yield: 67%, m.p. 139–141 °C. Anal. Calcd. For C_{18}H_{20}N_{5}ClSO: C, 57.67; H, 5.11; N, 14.94%. Found: C, 57.56; H, 5.16; N, 14.88%. IR (KBr, cm⁻¹): 3150, 3122, 2960, 1672, 1608, 1554, 1268, 1216, 667. ¹H-NMR (DMSO-d₆ /ppm): 6.70-7.35 (m, 9H), 5.89 (brs, 1H), 4.35-4.80 (m 3H), 3.60 (s, 2H), 2.73 (s, 1H, CH), 1.00 (s, 3H). MS (m/z): 374.89 (M⁺).

2-[[5-chloro-3-methyl-1-(pyridin-2-yl)pyrazolidin-4-yl]-3-substitutedphenyl-thiazolidin-4-ones 4a.

**General preparation of 2-[[5-chloro-3-methyl-1-(pyridine-2-yl)pyrazolidin-4-yl]-3-substitutedphenyl-thiazolidin-4-ones 4a-f**

A mixture of compound 3a-f (0.001 mol) in N,N'-dimethyl formamide and thioglycolic acid (0.001 mol) in presence of anhydrous zinc chloride was refluxed for 2-4 h. After completion of reaction (monitored by TLC), excess of solvent was distilled and cooled residual mass diluted with ice-water. The solid filtered, washed with water, dried and recrystallised by appropriate solvents to yield desired compounds 4a-f.
stututedphenylthiazolidin-4-ones (4a-f). Based on the resulting biological evaluation data, compound 4c possessing 3-aminophenyl substitution; exhibited the most potent antimicrobial activity with lesser toxicity among all the synthesized thiazolidin-4-one derivatives of the series.

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