INTRODUCTION
2-thioxoimidazolidin-4-one derivatives belong to heterocyclic compounds which have a wide range of biological and pharmacological properties such as antibacterial, anti fungal, anti thyroidal, antiviral, anti HIV, anti tuberculosis and anticonvulsant activities.

In the present work, some new 2-thioxoimidazolidin-4-one derivatives were synthesized from (E)-1-((1,3-diphenyl-1H-pyrazol-4-yl)methylene)thiosemicarbazide. The characterization of synthesized compounds was done by IR, NMR and mass spectral analysis. The antimicrobial activity of the synthesized compounds was done against some pathogenic Gram positive and Gram negative bacteria and fungi in DMSO. Among the screened compounds, RPI-10 containing hydroxyl group as substituent exhibited most potent antimicrobial activity.

EXPERIMENTAL SECTION
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 concentrated hydrochloric acid 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 3ml POCl₃ in ice cooled 15ml DMF) and the 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 overnight. The resulting product was filtered, washed and dried.

Synthesis of 3-((1,3-diphenyl-1H-pyrazol-4-yl)methyleneamino)-2-thioxoimidazolidin-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 thiosemicarbazide in methanol was refluxed for 1hr in presence of concentrated hydrochloric acid.
acid. 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 chloroacetate were dissolved in chloroform in presence of sodium acetate. The reaction mixture was refluxed for 8 hrs. The solid product was filtered and washed with hexane and water respectively to remove impurities. The crude product was recrystallized.

**MATERIAL AND METHODS**

The melting point of all the synthesized compounds was determined by Differential Scanning Calorimeter (Shimadzu-DSC-60). The characterization of all these compounds was done by IR, NMR and mass spectral data. The IR spectra were recorded on Shimadzu FT-IR-8400 instrument using KBr pellet method. ¹H NMR and was determined in DMSO solution on a Bruker Ac 400 MHz spectrometer. The Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique.

**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: *Pseudomonas aeruginosa* ATCC27853 (PA), *Escherichia coli* NCIM2931 (EC), *Klebsiella pneumoniae* NCIM2719 (KP), *Salmonella typhimurium* ATCC23564 (ST) and fungal strains were *Candida albicans* ATCC2091 (CA), *Cryptococcus neoformans* NCIM3542 (CN), *Candida glabrata* NCIM3448 (CG) and *Candida epilica* NCIM3367 (CE). The microorganisms studied are clinically important ones causing several infections and food spoilage.

*In vitro* antimicrobial activity of the 2-thioximidazolidin-4-one derivatives were studied against pathogenic microbial strains by the agar well diffusion method.

**RESULTS AND DISCUSSION**

In total 10 compounds were synthesized (RPI-1 to RPI-10). Analysis of their IR, NMR and Massspectral data confirmed their molecular structure. The spectral data of all the compounds are given below:

**RPI-1**: IR (KBr, cm⁻¹): -C=C(str.):1545, C=S(str.):1172, C=O(str.):1643, N-H(sym.):1597, C-H(str.):2773. ¹H-NMR (δ, ppm): 2.50(s,3H,CH₃), 3.91(s,2H,CH₂), 7.53(q,1H,CH), 7.55(t,1H,CH), 7.57(t,1H,Ar-CH), 8.41(s,1H,CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 421

**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): 2.40(s,3H,CH₃), 3.91(s,2H,CH₂), 7.33(d,2H,Ar-CH), 7.38(t,1H,CH), 7.76(d,2H,Ar-CH), 7.96(d,2H,Ar-CH), 8.41(s,1H,CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 375

**RPI-3**: IR (KBr, cm⁻¹): -C=C(str.):1489, C=S(str.):1157, C=O(str.):1643, N-H(sym.):1647, C-H(str.):2780. ¹H-NMR (δ, ppm): 2.50(s,3H,CH₃), 3.91(s,2H,CH₂), 7.53(q,1H,CH), 7.55(t,1H,CH), 7.57(t,1H,Ar-CH), 8.44(s,1H,CH hydrazide), 11.91(s,1H,SH). MS: (m/z) = 395

**RPI-4**: IR (KBr, cm⁻¹): -C=C(str.):1542, C=S(str.):1215, C=O(str.):1643, N-H(sym.):1599, C-H(str.):2780. ¹H-NMR (δ, ppm): 3.94(s,2H,CH₂), 6.94(m,2H,Ar-CH), 7.29(t,1H,CH), 7.27(t,1H,Ar-CH), 7.31(d,1H, Ar-CH), 7.34(t,2H, Ar-CH), 7.43(d,2H, Ar-CH), 8.18(s,1H,CH hydrazide), 8.86(s,1H, Ar-CH), 9.83(s,1H, -OH), 11.80(s,1H,SH). MS: (m/z) = 377

**RPI-5**: IR (KBr, cm⁻¹): -C=C(str.):1504, C=S(str.):1111, C=O(str.):1645, N-H(sym.):1597, C-H(str.):2752. ¹H-NMR (δ, ppm): 3.75(s,3H, OCH₃), 3.95(s,2H,CH₂), 7.18(t,1H, -NH), 7.20(d,1H, NH), 7.41(t,1H, Ar-CH), 7.42(d,2H, Ar-CH), 8.41(s,1H,CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 441

**RPI-6**: IR (KBr, cm⁻¹): C=C(str.):1446, C=S(str.):1215, C=O(str.):1651, N-H(sym.):1600, C-H(str.):2783. ¹H-NMR (δ, ppm): 2.50(s,3H,CH₃), 3.91(s,2H,CH₂), 7.53(q,1H,CH), 7.55(t,1H,CH), 7.57(t,1H,Ar-CH), 8.41(s,1H, CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 391

**RPI-7**: IR (KBr, cm⁻¹): -C=C(str.):1504, C=S(str.):1139, C=O(str.):1631, N-H(sym.):1601, 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,CH), 7.57(t,1H,Ar-CH), 8.41(s,1H,CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 379

**RPI-8**: IR (KBr, cm⁻¹): -C=C(str.):1504, C=S(str.):1101, C=O(str.):1653, N-H(sym.):1597, C-H(str.):2783. ¹H-NMR (δ, ppm): 2.50(s,3H,CH₃), 3.91(s,2H,CH₂), 7.53(q,1H,CH), 7.55(t,1H,CH), 7.57(t,1H,Ar-CH), 8.41(s,1H,CH hydrazide), 11.89(s,1H,SH). MS: (m/z) = 379
The antimicrobial activity was measured by the average diameter of the inhibition zones, expressed in mm. Fig. 5 shows antibacterial activity of synthesized 2-thioxoimidazolidin-4-one derivatives against Gram positive and Gram negative bacteria. It is evident from Fig. 5A that none of the synthesized compound showed activity against C. rurbum and L. monocytogenes but showed varied activity against B. cereus and S. aureus. B. cereus was inhibited by compound RPI-3, RPI-4, RPI-5, RPI-7 and RPI-10 while S. aureus was inhibited by RPI-1, RPI-2 and RPI-5. This differential inhibitory activity is because of different substitution. In all the studied compounds, central moiety is 2-thioxoimidazolidin-4-one but substituent groups are different (Table 1) and each group affected differently on different bacteria. RPI-6, RPI-8 and RPI-9 did not show activity against any of these bacteria. This suggests that -Br, -NO2, -OCH3 groups are not effective against these bacteria.

However, all the ten compounds showed better antibacterial activity towards Gram negative bacteria than Gram positive bacteria. Fig. 5B shows that P. aeruginosa was the most resistant Gram negative bacteria. It was not inhibited by any of the synthesized compound. Similar results were reported by Bhaluet al. for dihydropyranocchromenes. This is followed by S. typhimurium which was inhibited by only compounds RPI-8, RPI-9 and RPI-10. K. pneumonia was the most susceptible Gram negative bacteria. It was inhibited by all of the synthesized compounds except RPI-8 and RPI-9. E. coli was inhibited by RPI-2, RPI-3, RPI-6, RPI-8 and RPI-10. Similar results were reported by Cheng et al. for thiazole derivatives and Nasser et al. for 2-Thioxo-imidazolidin-4-one derivatives. This once again confirms our earlier conclusion that antibacterial activity depends on the molecular structure of the compounds and solvent and bacterial strain.

Further, antifungal activity of all the synthesized compounds were studied against four fungal strains but none of the compound exhibited any activity. This may be due to the fact that fungal cells are more complex organisms as compared to bacterial cell.

**CONCLUSION**

The synthesized compounds showed better activity towards Gram negative bacteria than Gram positive bacteria. RPI-10 containing hydroxyl group appears to be the most promising compound. However, the studied compounds are not effective against studied fungal strains.

**ACKNOWLEDGEMENT**

Authors are thankful to Heads of Chemistry and Biosciences Department for providing necessary facilities.
### Table 1: Physical properties of synthesized compounds

<table>
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<tr>
<th>Compound Code</th>
<th>Substitution</th>
<th>M.F.</th>
<th>M.W.</th>
<th>Yield (%)</th>
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<tr>
<td>RPI-1</td>
<td>-3,4-di-OCH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;19&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;S</td>
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<td>79</td>
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<td>RPI-2</td>
<td>-4-CH&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>84</td>
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<td>-4-Cl</td>
<td>C&lt;sub&gt;19&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;CIN&lt;sub&gt;5&lt;/sub&gt;OS</td>
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<td>80</td>
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<td>-2-OH</td>
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<td>85</td>
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<td>RPI-5</td>
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<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;N&lt;sub&gt;5&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S</td>
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<td>74</td>
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<tr>
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<td>377</td>
<td>80</td>
</tr>
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</table>

**Fig. 1:** Synthesis scheme of 2-thioxoimidazolidin-4-one derivatives

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**Step 1**

**Step 2**

RPI-1 to RPI-10  
R = different substitution
Fig. 2: IR spectra of compound RPI-4

Fig. 3: 1H NMR spectrum of compound RPI-4
Fig. 4: Mass spectrum of compound RPI-4

Fig. 5: Antibacterial activity of 2-thioximidazolidin-4-one derivatives in DMSO against Gram positive and negative bacteria

REFERENCES