Synthesis and antimycobacterial activity of novel heterocycles

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Abstract: In the present investigation 4-hydroxy-3-methylacetophenone on condensation with various aromatic aldehydes in methanolic KOH solution yielded the corresponding chalcones (C_I–C_XI). These chalcones were further reacted with hydrazine hydrate in ethanol which led to the formation of pyrazoline derivatives (H_I–H_XI). The newly synthesized heterocycles were characterized on the basis of their chemical properties and spectroscopic data. All newly synthesized compounds were evaluated for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv.

Keywords: pyrazoline, antimycobacterial, *Mycobacterium tuberculosis*.

INTRODUCTION

Tuberculosis (TB) is by far the most frequently encountered mycobacterial disease in the world. Although its incidence has diminished significantly in the industrially more developed countries, it remains a major public health problem in most developing nations. Tuberculosis is still the single largest infection having a high mortality rate and 0.1 to 0.3 percent of the population become infected each year in the developed countries. This year, 2 million people may develop the disease and 30 million may die worldwide (as per a WHO report). It is commonly known that *Mycobacterium tuberculosis* has developed resistance to the majority of the existing drugs. However, powerful new anti-TB drugs with new mechanisms of action have not been developed in the last forty years. In the developing countries, the annual infection rate is 20–50 times greater than in the developed countries and its high level shows little or no downward trend. It is expected that development of new effective anti-TB drugs will bring various outcomes viz: shortening the total duration of therapy, reducing the total expenditure and treatment of multiple drug resistant tuberculosis (MDR-TB) by single dosage regiment. In pursuit of achieving this goal, our research efforts are focused on the development of novel structural moieties having antimycobacterial properties. Chalcones have vari-

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ous biological activities such as cytotoxic, antimalarial, antioxidant, tyrosinase inhibitory, anti-inflammatory, cancer chemopreventive and antibacterial. Several pyrazolines are also known to have various biological activities, e.g., anti-bacterial, anti-inflammatory, hypoglycemic, anti-HIV and anti-tumor. Herein the synthesis and in vitro antimycobacterial activity of novel chalcone and pyrazoline derivatives are reported.

RESULTS AND DISCUSSION

Chemistry

The synthesis of chalcone and pyrazoline derivatives was performed following the steps shown in Scheme 1. In the initial step, chalcones \( (\text{C}_1-\text{C}_{11}) \) were synthesized by condensing 4-hydroxy-3-methylacetophenone with appropriate aromatic aldehydes in dilute methanolic potassium hydroxide solution at room temperature. The compounds \( (\text{H}_1-\text{H}_{11}) \) were synthesized by reacting the appropriate chalcone with hydrazine hydrate in ethanol. The purity of the compounds was controlled by TLC. Spectral data (IR and \( \text{H-NMR} \)) of all the newly synthesized compounds were in full agreement with the proposed structures.

![Scheme 1](image)

Biological screening

Microbiology

The in vitro activities of the synthesized compounds for tuberculosis inhibition against the Mycobacterium tuberculosis H37RV (ATCC27294) strain were performed using the micro plate almar blue assay (MABA) method. Compounds exhibiting fluorescence are tested in a BACTEC-460 radiometric system and/or broth micro dilution assay and the activities expressed as minimum inhibitory concentration (MIC, \( \mu g/ml \)) are summarized in Tables I and II. Compounds dem-
onstrating at least 90% inhibition were re-tested at lower concentrations by the broth micro dilution assay to determine the actual MIC, a value defined as the lowest concentration inhibiting ≈ 90% of the inoculum relative to the control.

**Antimycobacterial activity**

Twenty-two compounds were screened for their antimycobacterial activity against *Mycobacterium tuberculosis* H37RV using a BACTEC-460 radiometric system. Among the chalcones (C_I–C_XI) and pyrazolines (H_I–H_XI), compounds C_{III}, C_X and H_{III} produced the highest efficacy and exhibited > 90% inhibition at ≈ 6.25 μg/ml in the primary screen (Tables I and II). Compounds C_{II}, C_V, C_{VIII}, C_{IX}, H_{VII} and H_{XI} exhibited ≈ 90% inhibition against *Mycobacterium tuberculosis* at MIC > 6.25 μg/ml (Tables I and II). These antimycobacterial data clearly show that the presence of dimethylaminophenyl substituted chalcone and pyrazoline causes remarkable improvements in antitubercular activity.

**TABLE I. Physicochemical data and in vitro anti-mycobacterial screening of novel chalcones against Mycobacterium tuberculosis H37RV strain**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>M.p./°C</th>
<th>Yield/%</th>
<th>Mol. formula</th>
<th>% Inhibition</th>
<th>MIC μg/ml</th>
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</thead>
<tbody>
<tr>
<td>C_I</td>
<td>4-Methoxyphenyl-</td>
<td>190–192</td>
<td>75</td>
<td>C_{17}H_{16}O_{3}</td>
<td>72</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_{II}</td>
<td>4-Chlorophenyl-</td>
<td>156–158</td>
<td>77</td>
<td>C_{16}H_{13}O_{2}Cl</td>
<td>90</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_{III}</td>
<td>4-Dimethylaminophenyl-</td>
<td>126–128</td>
<td>67</td>
<td>C_{18}H_{19}O_{2}N</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td>C_{IV}</td>
<td>Phenyl-</td>
<td>113–115</td>
<td>90</td>
<td>C_{16}H_{14}O_{2}</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C_V</td>
<td>3,4-Dimethoxyphenyl-</td>
<td>156–158</td>
<td>85</td>
<td>C_{18}H_{18}O_{4}</td>
<td>92</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_{VI}</td>
<td>3,4,5-Trimethoxyphenyl-</td>
<td>144–146</td>
<td>65</td>
<td>C_{18}H_{20}O_{5}</td>
<td>77</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_{VII}</td>
<td>2-Furyl-</td>
<td>97–99</td>
<td>76</td>
<td>C_{14}H_{12}O_{3}</td>
<td>73</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_{VIII}</td>
<td>4-Fluorophenyl-</td>
<td>161–163</td>
<td>80</td>
<td>C_{18}H_{13}O_{4}F</td>
<td>94</td>
<td>&gt;6.25</td>
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<tr>
<td>C_{IX}</td>
<td>2-Chlorophenyl-</td>
<td>189–191</td>
<td>90</td>
<td>C_{16}H_{12}O_{2}Cl</td>
<td>88</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>C_X</td>
<td>2,6-Dichlorophenyl-</td>
<td>111–113</td>
<td>76</td>
<td>C_{16}H_{12}O_{2}Cl</td>
<td>90</td>
<td>6.25</td>
</tr>
<tr>
<td>C_{XI}</td>
<td>3-Nitrophenyl-</td>
<td>141–143</td>
<td>70</td>
<td>C_{16}H_{13}O_{2}N</td>
<td>65</td>
<td>&gt;6.25</td>
</tr>
</tbody>
</table>

Recrystallization: ethanol, acetic acid; ND = not done

**CONCLUSION**

To summarize, a new class of chalcone and pyrazoline derivatives, as a novel class of antitubercular agents, was synthesized. The newly synthesized novel heterocycles exhibited promising antitubercular activities against both drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis*. These results make
novel chalcone and pyrazoline derivatives interesting lead molecules for further synthetic and biological evaluation. It can be concluded that this class of compounds certainly holds great promise towards the pursuit to discover novel classes of antimycobacterial agents. Further studies to acquire more information concerning structure–activity relationships are in progress.

TABLE II. Physicochemical data and in vitro antimycobacterial screening of novel pyrazoline derivatives against Mycobacterium tuberculosis H37Rv strain

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>M.p./°C</th>
<th>Yield/%</th>
<th>Mol. formula</th>
<th>% Inhibition</th>
<th>MIC/μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>4-Methoxyphenyl-</td>
<td>147–149</td>
<td>75</td>
<td>C17H18N2O2</td>
<td>76</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HII</td>
<td>4-Chlorophenyl-</td>
<td>139–141</td>
<td>77</td>
<td>C18H15N2OCl</td>
<td>90</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HI</td>
<td>4-Dimethylaminophenyl-</td>
<td>160–162</td>
<td>67</td>
<td>C18H21N3O9</td>
<td>2.62</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HIV</td>
<td>Phenyl-</td>
<td>180–182</td>
<td>90</td>
<td>C16H16N2O</td>
<td>88</td>
<td>ND</td>
</tr>
<tr>
<td>H5</td>
<td>3,4-Dimethoxyphenyl-</td>
<td>119–121</td>
<td>85</td>
<td>C18H20N2O3</td>
<td>78</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HVI</td>
<td>3,4,5-Trimethoxyphenyl-</td>
<td>101–103</td>
<td>65</td>
<td>C19H22N2O4</td>
<td>86</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HIX</td>
<td>2-Furyl-</td>
<td>161–163</td>
<td>76</td>
<td>C14H14N2O2</td>
<td>90</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HX</td>
<td>4-Fluorophenyl-</td>
<td>142–144</td>
<td>80</td>
<td>C16H15N2OF</td>
<td>85</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HIX</td>
<td>2-Chlorophenyl-</td>
<td>140–142</td>
<td>90</td>
<td>C16H15N2OCl</td>
<td>83</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HX</td>
<td>2,6-Dichlorophenyl-</td>
<td>139–141</td>
<td>76</td>
<td>C16H14N2OCl</td>
<td>83</td>
<td>&gt;6.25</td>
</tr>
<tr>
<td>HX</td>
<td>3-Nitrophenyl-</td>
<td>149–151</td>
<td>70</td>
<td>C16H15N3O3</td>
<td>88</td>
<td>&gt;6.25</td>
</tr>
</tbody>
</table>

Recrystallization: ethanol, acetic acid; ND = not done

EXPERIMENTAL

Chemistry

Chemicals were supplied by E. Merck (Germany) and S. D. Fine Chemicals (India). Melting points were determined by the open tube capillary method and are uncorrected. The purity of the compounds was controlled by thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene–ethyl formate–formic acid (5:4:1) and benzene–methanol (8:2). The spots were located under iodine vapor or UV light. The spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). The 1H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as the internal standard in DMSO-d6/CDCl3.

General method for the synthesis of 1-(4-hydroxy-3-methylphenyl)-3-(substituted)phenyl-2-propen-1-ones (C1–XI). To a mixture of 4-hydroxy-3-methylacetophenone (0.005 mol) and the appropriate aromatic aldehyde (0.005 mol) in oxygen-free ethanol was added a solution of potassium hydroxide (0.005 mol) in oxygen-free distilled water with constant shaking of the reaction flask. The reaction mixture was stirred for a specified period on a magnetic stirrer and poured onto crushed ice. The solid mass which separated out was filtered, washed with water and crystallized from a suitable solvent to give the desired product.
s, CH₃), 7.7–8.2 (7H, m, aromatic), 3.9 (3H, s, OCH₃), 6.9–7.5 (1H+x2, dd, –CH=CH).
3-(4-Chlorophenyl)-1-(4-hydroxy-3-methylphenyl)-2-propen-1-one (C₉): IR: (KBr) cm⁻¹ 3210 (OH), 1682 (C=O), 3042 (CH). ¹H-NMR (DMSO-d₆ ppm): 9.2 (1H, s, OH), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 (1H+x2, dd, –CH=CH), 2.2 (3H, s, CH₃).
3-(4-Dimethylaminophenyl)-1-(4-hydroxy-3-methylphenyl)-2-propen-1-one (C₁₀): IR: (KBr) cm⁻¹ 3212 (OH), 1680 (C=O), 3032 (CH). ¹H-NMR (DMSO-d₆ ppm): 9.2 (1H, s, OH), 7.7–8.2 (7H, m, aromatic), 6.9–7.5 (1H+x2, dd, –CH=–CH), 2.9 (2H+x2, s, –N(CH₃)ₓ₂), 2.2 (3H, s, CH₃).

1-(4-Hydroxy-3-methylphenyl)-3-phenyl-2-pyrazoline (CIV): IR: (KBr) cm⁻¹ 3180 (OH), 1687 (C=O), 3040 (CH). ¹H-NMR (DMSO-d₆ ppm): 9.4 (1H, s, OH), 2.2 (3H, s, CH₃), 7.7–8.2 (8H, m, aromatic), 6.9–7.5 (1H+x2, dd, –CH=CH).
3-(3,4-Dimethoxyphenyl)-1-(4-hydroxy-3-methylphenyl)-2-propen-1-one (CV): IR: (KBr) cm⁻¹ 3200 (OH), 1680 (C=O), 3030 (CH). ¹H-NMR (DMSO-d₆ ppm): 9.2 (1H, s, OH), 2.2 (3H, s, CH₃), 7.7–8.2 (6H, m, aromatic), 3.9 (3H, s, OCH₃).

Hydrazine hydrate (99 %) was added dropwise. The reaction mixture was heated under reflux for 7 h, then cooled and poured onto crushed ice. The so-obtained solid product was filtered and recrystallized from ethanol (H₁₋X).
3-(4-Hydroxy-3-methylphenyl)-5-(4-methoxyphenyl)-2-pyrazoline (HV): IR: (KBr) cm⁻¹ 3307 (OH), 1580 (C=O), 3042 (CH). ¹H-NMR (DMSO-d₆ ppm): 9.5 (1H, s, OH), 7.3–7.8 (8H, m, aromatic), 5.54 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH₃), 2.3 (2H, s, CH₂).
3-(4-Hydroxy-3-methylphenyl)-5-(3,4,5-trimethoxyphenyl)-2-pyrazoline (H_{VIII}). IR: (KBr cm$^{-1}$) 3307 (OH), 1596 (C=N), 1320 (C–N); 1H-NMR (DMSO-d$_6$ ppm): 9.5 (1H, s, OH), 7.3–7.8 (5H, m, aromatic), 5.48 (1H, s, NH), 4.24 (1H, s, CH), 3.6 (9H, s, OCH$_3$), 3.4 (3H, s, CH$_3$), 2.3 (2H, s, CH$_2$).

5-(2-Furyl)-3-(4-hydroxy-3-methylphenyl)-2-pyrazoline (HVII). IR: (KBr cm$^{-1}$) 3317 (OH), 1590 (C=N), 1320 (C–N); 1H-NMR (DMSO-d$_6$ ppm): 9.2 (1H, s, OH), 7.3–7.8 (3H, m, aromatic), 7.8–8.2 (3H, m, furan), 5.52 (1H, s, NH), 4.24 (1H, s, CH), 3.42 (3H, s, CH$_3$), 2.3 (2H, s, CH$_2$).

5-(4-Fluorophenyl)-3-(4-hydroxy-3-methylphenyl)-2-pyrazoline (HVIII). IR: (KBr cm$^{-1}$) 3312 (OH), 1590 (C–N), 700 (C–F); 1H-NMR (DMSO-d$_6$ ppm): 9.4 (1H, s, OH), 7.3–7.8 (7H, m, aromatic), 5.42 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH$_3$), 2.3 (2H, s, CH$_2$).

5-(2-Chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-2-pyrazoline (HIX). IR: (KBr, cm$^{-1}$) 3306 (OH), 1586 (C=N), 1320 (C–N), 774 (C–Cl); 1H-NMR (DMSO-d$_6$, ppm): 9.5 (1H, s, OH), 7.6–8.2 (7H, m, aromatic), 5.50 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH$_3$), 2.3 (2H, s, CH$_2$).

5-(2,6-Dichlorophenyl)-3-(4-hydroxy-3-methylphenyl)-2-pyrazoline (HX). IR: (KBr, cm$^{-1}$) 3317 (OH), 1594 (C–N), 770 (C–Cl); 1H-NMR (DMSO-d$_6$, ppm): 9.5 (1H, s, OH), 7.3–7.8 (6H, m, aromatic), 5.54 (1H, s, NH), 4.24 (1H, s, CH), 3.4 (3H, s, CH$_3$), 2.3 (2H, s, CH$_2$).

3-(4-Hydroxy-3-methylphenyl)-5-(3-nitrophenyl)-2-pyrazoline (HXI). IR: (KBr, cm$^{-1}$) 3307 (OH), 1590 (C–N), 700 (C–NO$_2$); 1H-NMR (DMSO-d$_6$, ppm): 9.4 (1H, s, OH), 7.8–8.4 (7H, m, aromatic), 5.56 (1H, s, NH), 4.20 (1H, s, CH), 3.2 (3H, s, CH$_3$), 2.7 (2H, s, CH$_2$).

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ИЗВОД

СИНТЕЗА И АНТИМИКОБАКТЕРИЈСКА АКТИВНОСТ НОВИХ ХЕТЕРОЦИКЛА

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Кондензацијом 4-хидрокси-3-метил-ацитофенона са различитим ароматичним алдехидима у метанолном раствору KOH добијени су одговарајући халкони (C$_1$–C$_{XI}$). Реакцијом ових халкона са хидразин-хидратом у станоу добијени су деривати пиразолина (H$_1$–H$_{XI}$). Нови синтезисани хетероциклли окажују барем унапредене својства и спектроскопским подацима. Испитивана је антимикобактеријска активност нових синтетизованих јединица према Mycobacterium tuberculosis H$37R_v$.


REFERENCES