SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 3-(4'-HYDROXY-3'- METHYLPHENYL)-5-[(SUBSTITUTED) PHENYL]-4,5-DIHYDRO-1H-1-PYRAZOLYL-4-PYRIDYL METHANONE DERIVATIVES

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Abstract: A series of 3-(4'-hydroxy-3'-methylphenyl)-5-[(substituted) phenyl]-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives were synthesized by the reaction between isoniazid (INH) and various chalcones and were tested for their antimicrobial activity in vitro against Staphylococcus aureus 209p, Escherichia coli ESS 2231, Aspergillus fumigatus, Candida albicans, Candida albicans ATCC 10231, Candida krusei G03 and Candida glabrata H05. Among the synthesized compounds, all the compounds possess the significant antibacterial activity. Compounds Ia and Ib, i.e. 3-(4'-hydroxy-3'-methylphenyl)-5-4'-dimethylaminophenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridyl methanone and 3-(4'-hydroxy-3'-methylphenyl)-5-(2',6'-dichlorophenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone were found to be the most active agents against used bacterial and fungal strains with minimum inhibitory concentration of less than 0.5 µg/mL and were equally active as standard drugs Ofloxacin and Fluconazole.

Keywords: antibacterial, antifungal, pyrazoline

In the last decade, the frequency of fungal infections has increased in immunocompromised patients. Immunosuppression due to malignancy, immunosuppressive and cytotoxic therapies, human immuno deficiency virus infection, broad-spectrum antibacterial treatment and age, as well as procedures which cause breaks in skin and mucosal barriers places patients at risk for fungal infections. In bone marrow transplantation patients, aspergilloses are the most prevalent non-Candida fungal infections, causing 70% of such infectious disease (1). Newer azole derivatives active against experimental fungal infections have recently been reported. Their mechanism of action involves inhibition of ergosterol biosynthesis, the major sterol of the fungal membrane, by interacting with the cytochrome P450 of 14a-demethylase (4, 5). Nevertheless,azole resistance and emerging strains of Aspergillus flavus, naturally resistant toazole, have been reported which demonstrates the existing need for new azole derivatives or alternative antifungals. Pyrazoline derivatives are of considerable chemical and pharmacological importance as purine analogs (2–4).

Various compounds with related structures also possess antimicrobial (5), antitumor (6), antimycobacterial (7), and antileukemia (8) activities.

EXPERIMENTAL

The entire chemicals were supplied by E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene-ethyl formate-formic acid (5:4:1, v/v/v) and benzene-methanol (8:2, v/v), the spots were located under iodine vapors and UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). 1H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO-d6/CDCl3 and mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing voltage with a VG Prospec instrument and are presented as m/z.

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General method of synthesis of 1- (4'-hydroxy-3'-methyl phenyl) -3- [(substituted) phenyl] 2-propen-1-ones (C<sub>11</sub>Χ)  
1-(4'-Hydroxy-3'-methylphenyl)-3-[(substituted) phenyl] 2-propen-1-one derivatives were synthesized by condensing 4-hydroxy-3-methylacetophenone with appropriate aromatic aldehydes according to Claisen-Schmidt condensation.

1-(4'-Hydroxy-3'-methylphenyl)-3-(4''-methoxyphenyl)-2-propen-1-one (C<sub>11</sub>)  
IR: (KBr cm<sup>-1</sup>): 3200 (OH), 3042 (CH), 1686 (C=O).  
1H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 2.2 (3H, s, CH<sub>3</sub>), 3.9 (3H, s, OCH<sub>3</sub>), 6.8 - 6.9 (1H x 2, d J = 7.5 Hz, 8.5 Hz CH=CH), 7.2-7.9 (7H, s, aromatic), 9.2 (1H, s, OH).

1-(4'-Hydroxy-3'-methylphenyl)-3-(4''-chlorophenyl)-2-propen-1-one (C<sub>11</sub>)  
IR: (KBr cm<sup>-1</sup>): 3210 (OH), 3030 (CH), 1676 (C=O).  
1H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 2.2 (3H, s, CH<sub>3</sub>), 6.7 - 6.8 (1H x 2, d J = 8.34 Hz, 6.79 Hz CH=CH), 7.7 - 8.0 (7H, m, aromatic), 9.2 (1H, s, OH).

1-(4'-Hydroxy-3'-methylphenyl)-3-(4''-dimethylamino phenyl)-2-propen-1-one (C<sub>11</sub>)  
IR: (KBr cm<sup>-1</sup>): 3200 (OH), 3042 (CH), 1680 (C=O).  
1H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 2.2 (3H, s, CH<sub>3</sub>), 2.83 (6H, s, N (CH<sub>3</sub> x 2), 6.8 - 6.9 (1H x 2, d J = 7.61 Hz, 7.63 Hz CH=CH), 7.6-8.1 (7H, m, aromatic), 9.2 (1H, s, OH).

1-(4'-Hydroxy-3'-methylphenyl)-3-furfuryl-2-propen-1-one (C<sub>11</sub>)  
IR: (KBr cm<sup>-1</sup>): 3200 (OH), 3040 (CH), 1680 (C=O).  
1H-NMR (DMSO-d<sub>6</sub>), δ (ppm): 2.2 (3H, s, CH<sub>3</sub>), 7.7-8.2 (3H, m, aromatic), 6.4-7.4 (3H, m, furan), 6.8 - 6.9 (1H x 2, d J = 3.0 Hz, 8.36 Hz, CH=CH), 9.2 (1H, s, OH).

Table 1. Physical constants of the newly synthesized 3-(4'-hydroxy-3'-methylphenyl)-5-[(substituted) phenyl]-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives.
Synthesis and antimicrobial activity of 3-(4’-hydroxy-3’-methylphenyl)-5-[(substituted) phenyl]-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives.

Table 2. Antibacterial and antifungal activity of novel 3-(4’-hydroxy-3’-methylphenyl)-5-[(substituted) phenyl]-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives.

<table>
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<th>Compounds (50 µg/mL)</th>
<th>Staphylococcus aureus 209p</th>
<th>Escherichia coli 2231</th>
<th>Aspergillus fumigatus</th>
<th>Candida albicans</th>
<th>Candida albicans ATCC 10231</th>
<th>Candida glabrata H05</th>
<th>Candida krusei G03</th>
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<td>+++</td>
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<td>+</td>
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</table>

Solvent controlf
- - - - - -

f +++++ = excellent activity; ++++ = very good activity; +++ = good activity; ++ = poor activity; + = very poor activity; - = non active
b Control: 0.5 µg/mL Ofloxacin and Fluconazole 0.5 µg/mL
c Solvent control 10% DMSO in methanol

IR: (KBr cm⁻¹): 3200 (OH), 3042 (CH), 1684 (C=O).

1H-NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 7.6-8.0 (7H, m, aromatic), 6.9 - 7.5 (1H × 2, d, J = 8.35 Hz, 3.63 Hz -CH=CH), 9.2 (1H, s, OH).

1-(4’-Hydroxy-3’-methylphenyl)-3-(2”’,6”’-dichlorophenyl)-2-propen-1-one (C₉)
IR: (KBr cm⁻¹): 3210 (OH), 3040 (CH), 1670 (C=O).

1H-NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 7.7-8.0 (6H, m, aromatic), 6.9 - 7.5 (1H × 2, d, J = 5.41 Hz, 15.68 Hz -CH=CH), 9.2 (1H, s, OH).

1-(4’-Hydroxy-3’-methylphenyl)-3-(3”’-nitrophenyl)-2-propen-1-one (C₃)
IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O).

1H-NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 7.7-8.2 (7H, m, aromatic), 6.9 - 7.5 (1H × 2, d, J = 5.46 Hz, 16.3 Hz -CH=CH), 9.2 (1H, s, OH).

General method of synthesis of 3-(4’-hydroxy-3’-methylphenyl)-5-[(substituted) phenyl]-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives (I₃X)
0.002 mol of isoniazid was added and the reaction mixture was refluxed for 12 h and cooled. An excess of solvent was removed under reduced pressure and the reaction mixture was cooled and poured onto crushed ice (20 g). The product so obtained was filtered, washed with water and recrystallized from methanol.

3-(4’-Hydroxy-3’-methylphenyl)-5-(4’-methoxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (I)

IR: (KBr cm⁻¹): 3210 (OH), 3034 (CH), 1682 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 1.4 (3H, s, CH₃), 2.3 (2H, s, CH₂), 3.7 (3H, s, OCH₃), 5.78 (1H, s, CH), 6.6-7.3 (7H, m, aromatic), 7.98-8.1 (4H, m, pyridine), 9.2 (1H, s, OH); EI-MS: m/z: 388 ([M+1]+); Analysis: (calcd.) found: C (71.30) 71.31, H (5.46) 5.41, N (10.85) 10.82

5-(4’-Chlorophenyl)-3-(4’-hydroxy-3’-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (II)

IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O), 780 (C-Cl); ¹H NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.3 (2H, s, CH₂), 4.1 (1H, s, CH), 5.78 (1H, s, CH), 6.6-7.5 (7H, m, aromatic), 7.9-8.9 (4H, m, pyridine), 11.49 (1H, s, OH) EI-MS: m/z: 392 ([M+1]+); Analysis: (calcd.) found: C (67.43) 67.51, H (4.63) 4.67, N (10.72) 10.72

5-(4’-Dimethylaminophenyl)-3-(4’-hydroxy-3’-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (III)

IR: (KBr cm⁻¹): 3230 (OH), 3020 (CH), 1676 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.1 (3H, s, CH₃), 2.6 (2H, s, CH₂), 2.9 (6H, s, N-(CH₃)₂), 4.1 (1H, s, CH), 6.5-7.5 (7H, m, aromatic), 7.6-8.6 (4H, m, pyridine), 11.2 (1H, s, OH); EI-MS: m/z: 400 ([M+1]+); Analysis: (calcd.) found: C (71.98) 71.96, H (6.04) 6.08, N (13.99) 13.93

3-(4’-Hydroxy-3’-methylphenyl)-5-phenyl-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (IV)

IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.1 (3H, s, CH₃), 2.5 (2H, s, CH₂), 4.1 (1H, s, CH), 5.78 (1H, s, CH), 6.5-7.8 (8H, m, aromatic), 7.9-8.9 (4H, m, pyridine), 11.49 (1H, s, OH); EI-MS: m/z: 357 ([M+1]+); Analysis: (calcd.) found: C (73.53) 73.50, H (5.36) 5.41, N (11.76) 11.72

5-(4’-Hydroxy-3’-methylphenyl)-5-(4’-methoxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (V)

IR: (KBr cm⁻¹): 3220 (OH), 3044 (CH), 1686 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.4 (2H, s, CH₂), 3.8 (6H, s, 2 × OCH₃), 4.1 (1H, s, CH), 6.7-7.3 (6H, m, aromatic), 7.6-7.9 (4H, m, pyridine), 11.90 (1H, s, OH); EI-MS: m/z: 418 ([M+1]+); Analysis: (calcd.) found: C (69.05) 69.08, H (5.55) 5.41, N (10.07) 10.02

3-(4’-Hydroxy-3’-methylphenyl)-5-(3’’,4’’,5’’-trimethoxyphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (VI)

IR: (KBr cm⁻¹): 3210 (OH), 3040 (CH), 1680 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.4 (2H, s, CH₂), 3.8 (9H, s, 3 × OCH₃), 4.1 (1H, s, CH), 6.7-7.5 (5H, m, aromatic), 7.6-7.9 (4H, m, pyridine), 11.84 (1H, s, OH); EI-MS: m/z: 448 ([M+1]+); Analysis: (calcd.) found: C (67.10) 67.51, H (5.63) 5.61, N (9.39) 9.36

5-(2’-Furyl)-3-(4’-hydroxy-3’-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (VII)

IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.0 (3H, s, CH₃), 2.7 (2H, s, CH₂), 5.8 (1H, s, CH), 6.3-6.9 (3H, m, aromatic), 7.4-7.7 (3H, m, furan) 8.7-9.0 (4H, m, pyridine), 10.38 (1H, s, OH); EI-MS: m/z: 348 ([M+1]+); Analysis: (calcd.) found: C (69.15) 69.21, H (4.93) 4.86, N (12.10) 12.12

5-(4’-Fluorophenyl)-3-(4’-hydroxy-3’-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (VIII)

IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.9 (2H, s, CH₂), 4.1 (1H, s, CH), 6.5-7.3 (7H, m, aromatic), 7.9-8.9 (4H, m, pyridine), 11.49 (1H, s, OH); EI-MS: m/z: 376 ([M+1]+); Analysis: (calcd.) found: C (70.39) 70.41, H (4.83) 4.81, N (11.19) 11.17

5-(2’-Chlorophenyl)-3-(4’-hydroxy-3’-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (IX)

IR: (KBr cm⁻¹): 3200 (OH), 3040 (CH), 1680 (C=O); ¹H NMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.8 (1H, s, CH), 5.78 (1H, s, CH), 6.7-7.3 (6H, m, aromatic), 7.5-8.2 (4H, m, pyridine), 8.8 (1H, s, OH); EI-MS: m/z: 392 ([M+1]+); Analysis: (calcd.) found: C (67.43) 67.51, H (4.63) 4.61, N (11.9) 11.92
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EI-MS: m/z: 427 ([M+1]+); Analysis: (calcd.) found: C (61.98) 61.96, H (4.02) 4.08, N (9.86) 9.84

3-(4'-Hydroxy-3'-methylphenyl)-5-(3'-nitrophenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (IXI)
IR: (KBr cm⁻¹): 32740 (OH), 3042 (CH), 1678 (C=O); ¹HñNMR (DMSO-d₆), δ (ppm): 2.2 (3H, s, CH₃), 2.5 (2H, s, CH₂), 4.4 (1H, s, CH), 6.5-7.3 (6H, m, aromatic), 7.9-8.4 (4H, m, pyridine), 10.49 (1H, s, OH); EI-MS: m/z: 402 ([M+1]+); Analysis: (calcd.) found: C (65.67) 65.69, H (4.51) 4.53, N (13.92) 13.82

Biological evaluation

Anti-microbial screening
The anti-microbial test was performed by disk diffusion method. All the synthesized compounds were screened for their in vitro anti-microbial activity against Staphylococcus aureus 209p Escherichia coli 2231ESS 2231, Aspergillus Fumigatus, Candida albicans ATCC 10231, Candida krusei 403, Candida glabrata H05. Whatman No. 2 filter paper disks were impregnated with different compounds 50 µg/mL/disk). Agar plates were surface inoculated uniformly from the broth culture and the tested microorganism. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for 7 days for fungal strain. Control studies were done with Ofloxacin and Fluconazole (0.5 mg/mL) using 10% DMSO in methanol.

RESULTS AND DISCUSSION

Chemistry
3-(4'-hydroxy-3'-methylphenyl)-5-[(substituted phenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone derivatives IXI described in this study are shown in Table 1 and 2, and a reaction sequence for the preparation is outlined in Scheme I. The chalcone (E)-1-(4'-hydroxy-3'-methylphenyl)-3-(substituted phenyl)-2-propen-1-one IXI derivatives were prepared by reacting 3-methyl-4-hydroxy-acetophenone with appropriate aldehydes in presence of base by conventional Claisen-Schmidt condensation. Reaction between chalcone with isonicotinyl hydrazide in ethanolic solution in the presence of glacial acetic acid afforded pyrazolines IXI in 65-94% yield. The purity of the compounds was checked by TLC and elemental analyses. Both analytical and spectral data (IR and 'H-NMR) of all the synthesized compounds were in full agreement with the proposed structures.

Antimicrobial activity
The synthesized compounds IXI were tested for their in vitro antimicrobial activity against bacterial and fungal strains using the well method (8). The results are summarized in Table 2 with standard drugs, Ofloxacin and Fluconazole, for comparison. Among the eleven newly synthesized compounds all were found to have moderate to high activity against the used bacterial strains and Aspergillus fumigatus but compound IX and IX showed good activity in both bacterial and fungal strains. Among them, compounds with 4-dimethylaminophenyl and 2,6-dichlorophenyl substituents were found to be the most active and were equally active as Ofloxacin and Fluconazole against the microorganisms used.

CONCLUSION
Among the newer derivatives, compounds 5-(4'-dimethylaminophenyl)-3-(4'-hydroxy-3'-methylphenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridylmethanone (IX) and 5-(2',6'-dichlorophenyl)-3-(4'-hydroxy-3'-methyl phenyl)-4,5-dihydro-1H-1-pyrazolyl-4-pyridyl methanone (IX) showed a promising antimicrobial activity in-vitro. Further it is conceived that derivatives showing anti-microbial activity can be further modified to exhibit better potency than the standard drugs. Further studies to acquire more information about structure-activity relationships are in progress in our laboratory.

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REFERENCES

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