SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NOVEL N-ACYLHYDRAZONE DERIVATIVES

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ABSTRACT

A series of novel N-acylhydrazone derivatives was synthesized via condensation of acid hydrazide with different substituted acetophenone in the presence of catalytically acetic acid using methanol or ethanol as a solvent. The structures of synthesized compounds were confirmed by using elemental analysis, Mass, IR and ¹H NMR spectroscopy. Also compounds were tested for their antibacterial and antifungal activity (MIC) in vitro with two Gram-positive bacteria, two Gram-negative bacteria and three fungal strains.

KEYWORDS: N-acylhydrazone, acid hydrazide, acetophenone.

INTRODUCTION

This section presents an overview of literature survey on the natural occurrence, medicinal importance, use and synthesis of acylhyrazones as important tool in organic chemistry. Acylhyrazones are a very old class of molecules: the first example of N-acylhyrazines was mentioned in 1850¹ and a number of N-unsubstituted, mono-and disubstituted acylhyrazines are now commercially available. Acylhyrazones are a versatile class of nitrogen-substituted molecules with a high degree of chemical reactivity, used as precursors and intermediates of many important organic molecules such as heterocycles, pharmaceuticals, polymers, dyestuffs and photographic products.²

Compounds of general formula ArCONHN=C(R) Ar’ are known as N-acylhyrazones. Hyrazones containing an azomethine CH=NNH- hydrogen are obtained from the action of acid hydrazide with aldehyde or ketones either in various solvents or under solvent free
conditions. Over all, the acylhydrazone derivatives can exist in four possible forms due to collective effect of configurational stereochemistry E and Z as well as conformational stereoisomers or rotamers i.e antiperiplaner (ap) and syn periplaner conformers (sp).

![Diagram of acylhydrazone derivatives]

Figure 1

The cyclic products of acylhydrazones are an important class of heterocyclic compounds with a wide range of biological activities such as analgesic, anti-inflammatory, anti-microbial, anti-convulsant, anti-platelet, anti-tubercular, anti-viral, schistomiasis and anti-tumoral activities.[3-9] Due to the simplest reaction conditions, diversified chemical libraries may be constructed for discovering potential bioactive molecules.

**Reaction scheme**

![Reaction scheme diagram]

Scheme 1 reagents and condition (1) CCl₄, Fe Powder, Bromine, reflux (2) THF, iPr-MgCl, dimethylcarbonate (3) EtOH, NH₂NH₂H₂O, reflux (4) MeOH, AcOH.

5-bromo-2,2-difluoro-1,3-benzodioxole (2) was synthesized by the bromination of 2,2-difluoro-1,3-benzodioxole (1) by using Fe powder at 75 °C in carbon tetrachloride solution.[10,11] Compound-2 was characterized by checking boiling point of liquid compound. Compound-3 was synthesized by two methods (a) Grignard reaction and (b) cross-grignard.[12] Cross-grignard reaction is easy to handle. In this method compound-2 first react with isopropyl magnesium chloride to generate grignard reagent finally quinched with dimethyl carbonate to yield ester (3). Compound-3 reacts with hydrazine hydrate in ethanolic solution to give acylhydrazine (4).[13] This acylhydrazine (4) in acidic media reacts...
with different substituted acetophenone 5(a-k) using methanol as a solvent to give library of Fluorine containing novel N-acylhydrazones 6(a-k).\(^{[14-16]}\)

**RESULTS AND DISCUSSION**

**Table-1 Acetophenone coupled N’-acylhydrazone derivatives**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acetophenone</th>
<th>Product</th>
<th>Time (h)</th>
<th>Yield(^a) (%)</th>
<th>Mp (ºC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>O(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>1</td>
<td>82</td>
<td>190-193</td>
</tr>
<tr>
<td>b</td>
<td>O(\cdot)F(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>1</td>
<td>84</td>
<td>179-182</td>
</tr>
<tr>
<td>c</td>
<td>O(\cdot)Br(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>2</td>
<td>69</td>
<td>205-208</td>
</tr>
<tr>
<td>d</td>
<td>O(\cdot)O(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>3</td>
<td>76</td>
<td>171-173</td>
</tr>
<tr>
<td>e</td>
<td>O(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>2</td>
<td>81</td>
<td>194-197</td>
</tr>
<tr>
<td>f</td>
<td>O(\cdot)Cl(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>1</td>
<td>78</td>
<td>209-212</td>
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<tr>
<td>g</td>
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<td>2</td>
<td>69</td>
<td>167-169</td>
</tr>
<tr>
<td>h</td>
<td>O(\cdot)F(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>1</td>
<td>80</td>
<td>186-189</td>
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<tr>
<td>i</td>
<td>O(\cdot)F(\cdot)</td>
<td><img src="image" alt="Fluorine-containing N-acylhydrazone" /></td>
<td>1</td>
<td>72</td>
<td>186-188</td>
</tr>
</tbody>
</table>
CONCLUSIONS
In conclusion, we have synthesized a library of fluorine containing acylhydrazones using simple and convenient method. This method produces these products in good yields, with a short reaction time and easy workup. Product is isolated by simple vacuum filtration. The isolated products are very pure and do not need any column purification. This study opens up a new area of cost-effective synthesis of potentially biologically active fluorine based acylhydrazone compounds.

EXPERIMENTAL SECTION
General. Reactions were monitored by TLC using silica-gel coated plates and ethyl acetate/hexanes solutions as the mobile phase, spots were located by iodine and UV. Melting points are uncorrected. FT-IR spectra were recorded using KBr disks on a Bruker Vector-22 infrared spectrometer and absorptions are reported as wave numbers (cm\(^{-1}\)). 1H NMR and \(^{13}\)C NMR spectra were obtained on a FT-NMR Bruker Ultra ShieldTM (400 MHz) instrument as DMSO-\textit{d}_6 solutions and the chemical shifts are expressed as units with Me\(_4\)Si as the internal standard. Mass spectra were recorded on direct inlet probe on a GCMS-QP 2010 mass spectrometer (Shimadzu). Elemental analyses were performed using a Thermo Finnigan Flash EA 1112 instrument. All other chemicals were purchased from commercial sources and were used after being freshly purified by standard procedures.

General preparation of 5-bromo-2,2-difluorobenzo[d][1,3]dioxole (2).
A mixture of 2,2-difluoro-1,3-benzodioxole (100 mmole) in 200 mL of carbon tetrachloride was stirred, and iron powder (50 mmole) was added. The reaction mixture was cooled to 0\(^\circ\) C, and bromine (100 mmole) was added during a 20 minute period. Upon completion of addition the reaction mixture was cautiously warmed to 75\(^\circ\) C. where it was stirred for one hour and then was allowed to cool to ambient temperature where it was stirred for 18 hours. The mixture was filtered through celite and the filtrate was washed with 0.1N sodium...
thiosulfate solution, water and saturated sodium chloride solution. The organic layer was dried with sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to a residue. The residue was distilled under high vacuum to yield 5-bromo-2,2-difluoro-1,3-benzodioxole (2) as an oil. Yield: 57%, bp 78-79°C at 20 mm; 1H NMR (400 MHz, CDCl3): \( s=6.96 \text{ (m, 1H)}, 7.22 \text{ (m, 2H)}; \) MS (m/z): 236 (M⁺); Anal. calc. for C₇H₃BrF₂O₂: C, 35.47; H, 1.28; Br, 33.72; F, 16.03; O, 13.50; Found: C, 35.56; H, 1.34; Br, 33.61; F, 16.08; O, 13.42.

**General preparation of methyl-2,2-difluorobenzo[d][1,3]dioxole-5-carboxylate (3).**

To a oven-dried 500 mL round bottom flask equipped with a stirring bar was added 5-bromo-2,2-difluorobenzo[d][1,3]dioxole (32 mmol) and anhydrous THF (200 mL). The flask was placed under nitrogen and cooled in an ice bath for 10 min. A solution of 1.3 M isopropylmagnesium chloride-lithium chloride complex (38.6 mmol) was added over 15 min. The ice bath was removed after 1 hour, and the reaction was warmed to room temperature and stirred for 2 hours. Dimethyl carbonate (35.3 mmol) was added in a slow, steady stream. The reaction was warmed slightly during the addition and was stirred at room temperature overnight. The reaction was quenched with saturated ammonium chloride and extract with EtOAc. Aqueous layer was extract with EtOAc. The combine organic layer was washed with saturated sodium chloride solution. Organic layer dried over anhydrous sodium sulfate, filtered and concentrated to yield crude viscous oil. It was distilled under high vacuum to yielded methyl-2,2-difluorobenzo[d][1,3]dioxole-5-carboxylate (3) as colorless oil. Yield: 51%; 1H NMR (400 MHz, CDCl3): \( s= 7.91 \text{ (dd, J= 6.8Hz, J=2Hz, 1H)}, 7.76 \text{ (d, J= 1.6Hz, 1H)}, 7.13 \text{ (d, J= 8.4Hz, 1H)}, 3.95 \text{ (s, 3H)}; \) MS (m/z): 216 (M⁺); Anal. calc. for C₉H₆F₂O₄: C, 50.01; H, 2.80; F, 17.58; O, 29.61; Found: C, 50.14; H, 2.87; F, 17.46; O, 29.52.

**General preparation of 2,2-difluorobenzo[d][1,3]dioxole-5-carbohydrazide (4).**

To a solution of methyl-2,2-difluorobenzo[d][1,3]dioxole-5-carboxylate (16 mmol) in EtOH (16mL) was added hydrazine monohydrate (40 mmol) and the reaction was stirred under reflux temperature for 8 hours. Cool the reaction mixture up to ambient temperature. Reaction mixture concentrated in rotator evaporator under reduces pressure to remove ethanol. Dilute reaction mass with purified water and extract with ethyl acetate. The organic phase is separated and subsequently washed with brine. Organic phase is dried over sodium sulphate and filtered. The filtrate is evaporated under reduce pressure to afforded 2,2-difluorobenzo[d][1,3]dioxole-5-carbohydrazide (4) as white crystalline solid. Yield: 91%;
General preparation of N-acylhydrazone derivatives (6a-k).
To the mixture of 2,2-difluorobenzo[d][1,3]dioxole-5-carbohydrazide (1 mmol) and substituted acetophenone (1 mmol) in 20 mL methanol was added three drops of acetic acid with stirring for 3 h at ambient temperature. Insoluble solid was gradually generated, then filter and wash with ethanol. After drying pure target compound was afforded as crystalline solid. The product was identified based on its physical and spectral characteristics.

2,2-difluoro-N’-(1-phenylethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6a). 
\(^1\)H NMR (400 MHz, DMSO): \(\delta = 11.26\) (s, 1H), 7.92 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.48-7.23 (m, 5H), 3.21 (s, 3H); IR (cm\(^{-1}\)): 3234 (N-H), 3052 (C-H aromatic ring), 2982 (C-H), 2847 (C-H), 1654 (C=O), 1523 (C=N), 1342 (C-H), 1284 (C-N), 1172 (C-O), 1023 (C-F) cm\(^{-1}\). Yield: 82%; mp 190-193°C; MS (m/z): 318 (M\(^+\)); Anal. calcd for C\(_{16}\)H\(_{12}\)F\(_2\)N\(_2\)O\(_3\): C, 60.38; H, 3.80; F, 11.94; N, 8.80; O, 15.08; Found: C, 60.12; H, 3.74; F, 11.63; O, 15.02.

2,2-difluoro-N’-(1-(3,4-difluorophenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6b).
\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 11.77\) (s, 1H), 7.93 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 8.4 Hz, 1H), 7.45 (s, 1H), 7.29 (d, J = 8 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 3.29 (s, 3H); IR (KBr): 3247 (N-H), 3070 (C-H aromatic ring), 2863 (C-H), 1637 (C=O), 1590 (C=N), 1556 (C=C), 1494 (C=C), 1298 (C-H), 1232 (C-N), 1085 (C-O), 1027 (C-F), 852 cm\(^{-1}\). Yield: 84%; mp 179-182°C; MS (m/z): 354 (M\(^+\)); Anal. calcd for C\(_{16}\)H\(_{12}\)F\(_4\)N\(_2\)O\(_3\): C, 54.25; H, 2.85; F, 21.45; N, 7.91; O, 13.55; Found: C, 54.49; H, 2.92; F, 21.32; N, 7.73; O, 13.27.

2,2-difluoro-N’-(1-(4-bromophenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6c).
\(^1\)H NMR (400 MHz, DMSO): \(\delta = 11.27\) (s, 1H), 7.92 (s, 1H), 7.84 (dd, J = 7.6, 1.2 Hz, 2H), 7.83 (d, J = 8Hz, 1H), 7.63 (dd, J = 8.4, 8 Hz, 2H), 7.59 (d, J = 8.4Hz, 1H), 3.27 (s, 1H); IR (cm\(^{-1}\)): 3289 (N-H), 3057 (C-H aromatic ring), 2924 (C-H), 2815 (C-H), 1651 (C=O), 1548 (C=N), 1462 (C=C), 1369 (C-H), 1278 (C-N), 1165 (C-O), 1028 (C-F) cm\(^{-1}\). Yield: 69%; mp 205-208°C; MS (m/z): 396 (M\(^+\)); Anal. calcd for C\(_{16}\)H\(_{11}\)BrF\(_2\)N\(_2\)O\(_3\): C, 48.39; H, 2.79; Br, 20.12; F, 9.57; N, 7.05; O, 12.09; Found: C, 48.51; H, 2.63; Br, 20.08; F, 9.42; N, 7.01; O, 12.04.
2,2-difluoro-N’-(1-(4-methoxyphenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6d). 1H NMR (400 MHz, DMSO): δ = 11.29 (s, 1H), 7.93 (d, J = 1.2Hz, 1H), 7.83 (dd, J = 8.4, 8 Hz, 1H), 7.72 (d, J = 8.8, 1.2 Hz, 2H), 7.59 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 8.8Hz, 2H), 3.84 (s, 3H), 3.28 (s, 3H); IR (cm⁻¹): 3272 (N-H), 3059 (C-H aromatic ring), 2915 (C-H), 2881 (C-H), 1678 (C=O), 1541 (C=N), 1457 (C=C), 1348 (C-H), 1264 (C-N), 1169 (C-O), 759 cm⁻¹. Yield: 76%; mp 171-173°C; MS (m/z): 348 (M⁺); Anal. calcd for C₁₇H₁₄F₂N₂O₆: C, 58.62; H, 4.05; F, 10.91; N, 8.04; O, 18.37; Found: C, 58.52; H, 4.15; F, 10.64; N, 8.10; O, 18.14.

2,2-difluoro-N’-(1-p-tolylethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6e). 1H NMR (400 MHz, DMSO): δ = 11.30 (s, 1H), 7.92 (s, 1H), 7.84 (d, J = 8Hz, 1H), 7.59 (d, J = 8.4Hz, 1H), 7.47 (dd, J = 8.4, 2 Hz, 2H), 7.19 (dd, J = 8, 1.2 Hz, 2H), 3.28 (s, 3H), 2.42 (s, 3H); IR (cm⁻¹): 3268 (N-H), 3082 (C-H aromatic ring), 2958 (C-H), 2829 (C-H), 1657 (C=O), 1573 (C=N), 1478 (C=C), 1364 (C-H), 1308 (C-H), 1264 (C-N), 1132 (C-O), 1032 (C-F) cm⁻¹. Yield: 81%; mp 194-197°C; MS (m/z): 332 (M⁺); Anal. calcd for C₁₇H₁₄F₂N₂O₆: C, 61.44; H, 4.25; F, 11.43; N, 8.43; O, 14.44; Found: C, 61.52; H, 4.37; F, 11.38; N, 8.31; O, 14.40.

2,2-difluoro-N’-(1-(4-chlorophenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6f). 1H NMR (400 MHz, DMSO): δ = 11.28 (s, 1H), 7.92 (s, 1H), 7.82 (d, J = 8Hz, 1H), 7.73 (dd, J = 8, 1.6 Hz, 2H), 7.58 (d, J = 8.4Hz, 1H), 7.46 (dd, J = 7.6Hz, 2H), 3.21 (s, 3H); IR (cm⁻¹): 3281 (N-H), 3079 (C-H aromatic ring), 2918 (C-H), 2875 (C-H), 1662 (C=O), 1536 (C=N), 1439 (C=C), 1336 (C-H), 1308 (C-H), 1274 (C-N), 1142 (C-O), 1019 (C-F) cm⁻¹. Yield: 78%; mp 209-212°C; MS (m/z): 352 (M⁺); Anal. calcd for C₁₆H₁₁ClF₂N₂O₃: C, 54.48; H, 3.14; Cl, 10.05; F, 10.77; N, 7.94; O, 13.61; Found: C, 54.57; H, 3.21; Cl, 10.09; F, 10.68; N, 7.86; O, 13.57.

2,2-difluoro-N’-(1-(2-chlorophenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6g). 1H NMR (400 MHz, DMSO): δ = 11.23 (s, 1H), 7.93 (s, 1H), 7.84 (d, J = 8Hz, 1H), 7.58 (d, J = 8Hz, 1H), 7.43-7.29 (m, 4H), 3.26 (s, 3H); IR (cm⁻¹): 3294 (N-H), 3059 (C-H aromatic ring), 2956 (C-H), 2875 (C-H), 1657 (C=O), 1563 (C=N), 1482 (C=C), 1328 (C-H), 1308 (C-H), 1281 (C-N), 1174 (C-O), 1016 (C-F) cm⁻¹. Yield: 69%; mp 167-169°C; MS (m/z): 352 (M⁺); Anal. calcd for C₁₆H₁₁ClF₂N₂O₃: C, 54.48; H, 3.14; Cl, 10.05; F, 10.77; N, 7.94; O, 13.61; Found: C, 54.52; H, 3.18; Cl, 10.07; F, 10.71; N, 7.86; O, 13.53.

2,2-difluoro-N’-(1-(2-fluorophenyl)ethylidene)benzo[d][1,3]dioxole-5-carbohydrazide (6h). 1H NMR (400 MHz, DMSO): δ = 11.29 (s, 1H), 7.93 (s, 1H), 7.84 (d, J = 8Hz, 1H), 7.59 (d, J =
8.4Hz, 1H), 7.38-7.26 (m, 4H), 3.29 (s, 3H); IR (cm⁻¹): 3282 (N-H), 3069 (C-H aromatic ring), 2947 (C-H), 2852 (C-H), 1653 (C=O), 1542 (C=N), 1478 (C=C), 1356 (C-H), 1319 (C-H), 1275 (C-N), 1161 (C-O), 1009 (C-F) cm⁻¹. Yield: 80%; mp 186-189°C; MS (m/z): 336 (M⁺); Anal. calcd for C₁₆H₁₁F₃N₂O₃: C, 57.15; H, 3.30; F, 16.95; N, 8.33; O, 14.27; Found: C, 57.18; H, 3.36; F, 16.84; N, 8.29; O, 14.21.

2,2-difluoro-N’-(1-(4-fluorophenyl)ethyldene)benzo[d][1,3]dioxole-5-carbohydrazide (6i). ¹H NMR (400 MHz, DMSO): δ= 11.37 (s, 1H), 7.90 (s, 1H), 7.81 (d, J= 8Hz, 1H), 7.60 (d, J= 8.4Hz, 1H), 7.59 (d, J= 8Hz, 2H), 7.57 (d, J= 8Hz, 1H), 7.56 (d, J= 8.4Hz, 2H), 7.54 (d, J= 8Hz, 1H); IR (cm⁻¹): 3277 (N-H), 3047 (C-H aromatic ring), 2932 (C-H), 2871 (C-H), 1683 (C=O), 1565 (C=N), 1468 (C=C), 1359 (C-H), 1316 (C-H), 1282 (C-N), 1157 (C-O), 1032 (C-F) cm⁻¹. Yield: 72%; mp 186-188°C; MS (m/z): 336 (M⁺); Anal. calcd for C₁₆H₁₁F₃N₂O₃: C, 57.15; H, 3.30; F, 16.95; N, 8.33; O, 14.27; Found: C, 57.23; H, 3.38; F, 16.92; N, 8.27; O, 14.19.

2,2-difluoro-N’-(1-(3-bromophenyl)ethyldene)benzo[d][1,3]dioxole-5-carbohydrazide (6j). ¹H NMR (400 MHz, DMSO): δ= 11.28 (s, 1H), 8.23 (s, 1H), 8.05 (d, J= 8Hz, 1H), 8.03 (d, J= 8Hz, 1H), 7.92 (s, 1H), 7.87 (d, J= 8Hz, 1H), 7.83 (d, J= 8Hz, 1H), 7.81 (d, J= 8Hz, 1H), 7.59 (d, J= 8.4Hz, 1H), 3.3 (s, 3H); IR (cm⁻¹): 3239 (N-H), 3078 (C-H aromatic ring), 2932 (C-H), 2854 (C-H), 1647 (C=O), 1568 (C-N), 1538 (C=C), 1393 (C-H), 1316 (C-H), 1288 (C-N), 1172 (C-O), 1084 (C-F), 784 (C-Br) cm⁻¹. Yield: 70%; mp 232-235°C; MS (m/z): 396 (M⁺); Anal. calcd for C₁₆H₁₃BrF₂N₂O₃: C, 48.39; H, 2.79; Br, 20.12; F, 9.57; N, 7.05; O, 12.09; Found: C, 48.47; H, 2.84; Br, 20.10; F, 9.52; N, 7.01; O, 12.14.

2,2-difluoro-N’-(1-o-tolylethylene)benzo[d][1,3]dioxole-5-carbohydrazide (6k). ¹H NMR (400 MHz, DMSO): δ= 11.10 (s, 1H), 7.92 (s, 1H), 7.83 (d, J= 8Hz, 1H), 7.59 (d, J= 8.4Hz, 1H), 7.29-7.11 (m, 4H), 3.25 (s, 3H), 2.47 (s, 3H); IR (cm⁻¹): 3284 (N-H), 3069 (C-H aromatic ring), 2957 (C-H), 2812 (C-H), 1657 (C-O), 1538 (C-N), 1436 (C=C), 1393 (C-H), 1319 (C-H), 1268 (C-N), 1128 (C-O), 1022 (C-F) cm⁻¹. Yield: 76%; mp 156-158°C; MS (m/z): 332 (M⁺); Anal. calcd for C₁₇H₁₄F₂N₂O₃: C, 61.44; H, 4.25; F, 11.43; N, 8.43; O, 14.44; Found: C, 61.39; H, 4.29; F, 11.38; N, 8.46; O, 14.51.

**BIOLOGICAL ACTIVITY**

**Pharmacology**

The minimum inhibitory concentrations (MICs) of synthesized compounds were carried out by broth microdilution method as described by Rattan. Antibacterial activity was screened...
against two gram positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenus* MTCC 443) and two gram negative (*Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441) bacteria, ampicillin was used as a standard antibacterial agent. Antifungal activity was screened against three fungal species *Candida albicans* MTCC 227, *Aspergillus niger* MTCC 282 and *Aspergillus clavatus* MTCC 1323, Griseofulvin was used as a standard antifungal agent.

All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and Mueller Hinton broth was used as nutrient media to grow and diluted the drug suspension for the test. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used to dilute to get desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. All the tubes not showing visible growth (in the same manner as control tube described above) were sub cultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized drug was diluted for obtaining 2000 μg/ml concentration, as a stock solution. In primary screening 500 μg/ml, 250μg/ml and 125 μg/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.12 μg/ml and 1.56 μg/ml concentrations. The highest dilution showing at least 99 % inhibition is taken as MIC. Results obtained are given in Table 1.

**Antibacterial activity**

The minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 1. The different compounds 6(a-k) were tested for *in vitro* against two gram positive (*S.
 aureus MTCC 96, S. pyogenus MTCC 443) and two gram negative (E. coli MTCC 442, P. aeruginosa MTCC 441) bacteria. From the screening data, some of them possessed excellent antibacterial activity compared to ampicillin (MBC, 50-250 μg/ml) against gram positive S. aureus. Compound 6b showed MBC value in the range between 100 μg/ml while ampicillin has standard MBC value of 100 μg/ml against gram negative E. coli which indicates that this compounds have excellent activity, Compound 6f exhibited very good activity against P. aeruginosa, while compounds 6b, 6d, 6g, 6i and 6j displayed moderate activity in the range of 200-250 μg/ml. Compounds 6b, 6d, and 6g possessed showed MBC value in the range between 62.5-100 μg/ml against S. aureus while ampicillin has standard MBC value of 100 μg/ml against S. aureus which indicates that this compounds have excellent activity, while other compounds 6f, 6h and 6j possessed MBC value in the range of 150-250 μg/ml against gram positive S. aureus compared with ampicillin. Compound 6a have MBC of 100 μg/ml which was comparatively good against gram negative E. coli while compounds 6b, 6c, 6g, 6h and 6j displayed moderate activity in the range of 200-250 μg/ml against gram negative E. coli compare to ampicilne. Compound 6b have MBC of 100 μg/ml which was comparatively good against S. pyogenus while compounds 6a, 6c and 6g displayed moderate activity in the range of 200-250 μg/ml against S. pyogenus as compare to ampicilne. The remaining N-acylhydrazone derivatives possessed moderate to poor activity against all four bacterial species.

Antifungal activity
The minimum inhibitory concentrations (MICs) of the synthesized compounds are shown in Table 1. For in vitro antifungal activity, three fungal species C. albicans MTCC 227, A. niger MTCC 282 and A. clavatus MTCC 1323 were used and compared with standard drugs nystatin and griseofulvin. Most of the compounds possessed very good antifungal activity against A. niger; their MFC values were in the range between 100-500 μg/ml. Compounds 6a, 6f and 6h showed excellent activity of 100-250 μg/ml which is similar to griseofulvin (100 μg/ml) and nystatin (100 μg/ml) against A. niger, compounds 6c, 6g and 6i possesses good activity of 250 μg/ml against C. albicans, while compounds 6e and 6f possesses good activity of 200-250 μg/ml against A. clavatus. whereas remaining compounds possessed moderate to poor activity against C. albicans and A. clavatus compared with griseofulvin.
Table-1: - Antimicrobial Screening Result of compounds 6(a-k)

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<thead>
<tr>
<th>Code</th>
<th>Minimal inhibition concentration (µg mL⁻¹)</th>
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<tr>
<td></td>
<td>Gram-positive</td>
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<tr>
<td></td>
<td>S.aureus</td>
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<tr>
<td>6a</td>
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</tr>
<tr>
<td>6b</td>
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<tr>
<td>6d</td>
<td>100</td>
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<tr>
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</tr>
<tr>
<td>6f</td>
<td>250</td>
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<tr>
<td>6g</td>
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<tr>
<td>6h</td>
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<td>6i</td>
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<td>Nystatin</td>
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<tr>
<td>Greseofulvin</td>
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</table>

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REFERENCES


