Antibacterial and antitubercular activities of some diphenyl hydrazones and semicarbazones

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Condensation of phenoxy or 4-bromophenoxy acetic acid hydrazide and substituted aryl semicarbazides with appropriate carbonyl compounds yield corresponding hydrazones and semicarbazones, respectively. The chemical structure of the synthesized compounds has been confirmed by UV, IR and 1H NMR spectral data. All the compounds have been investigated for their antibacterial activity against ten pathogenic strains and antitubercular activity against Mycobacterium tuberculosis H37 Rv. Compound 4f, Acetic acid, phenoxy- [1-(4-chlorophenyl) ethylidene] hydrazide has been found to exhibit 80% inhibition in the preliminary antitubercular screening (MIC > 6.25 μg/mL).

Keywords: Hydrazones, semicarbazones, synthesis, antibacterial, antitubercular

Tuberculosis (TB) ranks among the most important burdens on human health, not only due to the large number of cases (~9 million/year worldwide) but also because about one quarter of sufferers dies most of them young adults. Drug resistance and multidrug-resistant tuberculosis is perceived as a growing hazard to human health worldwide1. The perceived threat of drug-resistant TB is enormous.

The biggest menace is multidrug-resistant TB caused by strains resistant to at least INH and rifampicin, the two principal first line drugs used in combination chemotherapy. Therefore, the development of potent new antitubercular drugs without cross resistance with known antimycobacterial properties is urgently needed2. Semicarbazones with NHCONHN=C< pharmacophore have been reported to possess a wide range of biological properties such as antimicrobial3,4; antiviral5; anti-HIV6 and anticonvulsant7 actions. Several thiosemicarbazides8, hydrazides9 and hydrazones10,11 and its cyclic derivatives12 were identified as potent antitubercular agents. In view of these facts and in continuation of our research work13-15, we prepared a new series of hydrazones and semicarbazones bearing R1-OCH2CONHN=CR2-R3 and R1R2-NCONHN=CHR3 functionalities, respectively. Recently, we reported the anticonvulsant activity of some of these diphenyl hydrazones and 4-nitrophenyl semicarbazones16. The diphenyl hydrazones were prepared in a study of structural modification of semicarbazones and found to possess less or no anticonvulsant activity than semicarbazones. In extension of our earlier work, the diphenyl hydrazones and semicarbazones were further evaluated for their antimicrobial properties and the results are presented here.

The diphenyl hydrazones (4a-r) were synthesized from phenoxy or 4-bromophenoxy acetic acid as depicted in Scheme I. The semicarbazones were obtained from substituted anilines as shown in Scheme II. The synthesized compounds were investigated for their antimicrobial activity against Staphylococcus aureus, Aeromonas hydrophilia, Bacillus subtilis, Vibrio cholerae, Enterococcus faecalis, Shigella flexeneri, Enterobacter, Plesiomonas shigelloides, Klebsiella pneumoniae, Escherichia coli and Mycobacterium tuberculosis H37 Rv.

Results and Discussion

Synthesis of phenoxy or 4-bromophenoxy acetic acid hydrazones (Scheme I) was carried out by following the method reported by us earlier16. The 4-substituted phenyl semicarbazones were obtained by following the Scheme II. A solution of 4-substituted aniline and sodium cyanate was stirred together to precipitate substituted phenyl urea. Hydrazinolysis of the urea with hydrazine hydrate gave good yield (78%) of semicarbazides, which were further on treatment with appropriate carbonyl compound yielded the semicarbazones (8a-d). The spectral analysis of all the compounds was done by IR and 1H NMR and the spectral data were consistent with the assigned structures.

The MIC data of the compounds, which have shown activity against the tested microbes, are
Scheme I

4a: R, R₁, R₃ = H; R₂ = OH
4b: R, R₁, R₂, R₃ = H
4c: R, R₁, R₂ = H; R₃ = OCH₃
4d: R, R₁, R₃ = H; R₂ = CH₃
4e: R, R₁, R₃ = H; R₂ = Cl
4f: R, R₂ = H; R₃ = CH₃; R₄ = Cl
4g: R, R₂ = H; R₁ = CH₃; R₃ = NH₂
4h: R, R₁, R₂ = H; R₃ = N(CH₃)₂
4i: R, R₂ = H; R₁ = CH₃; R₃ = OH
4j: R, R₂ = H; R₁ = CH₃; R₃ = OPh
4k: R = Br; R₁, R₃ = H; R₂ = OH
4l: R = Br; R₁, R₂, R₃ = H
4m: R = Br; R₁, R₂ = H, R₃ = H
4n: R = Br; R₁ = CH₃; R₂, R₃ = H
4o: R = Br; R₂ = Cl; R₁, R₃ = H
4p: R = Br; R₁ = CH₃; R₂ = H; R₃ = OPh
4q: R = Br; R₁ = CH₃; R₂ = H; R₃ = NO₂
4r: R = Br; R₁ = CH₃; R₂ = H, R₃ = Cl

Scheme II

8a: R = Cl; R₁ = CH₃; R₂, R₄ = H; R₃ = OCH₃
8b: R = F; R₁, R₂, R₄ = H; R₃ = OCH₃
8c: R = F; R₁ = H; R₂, R₃, R₄ = OCH₃
8d: R = NO₂; R₁ = H; R₂, R₃, R₄ = OCH₃
Table I — MIC (μg/mL) data of the compounds tested against bacterial strain(s)

<table>
<thead>
<tr>
<th>Micro organisms</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>4a 4b 4c 4d 4f 4h 4i 4n 4r 8b TM SM</td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td>625 2500 1250 312.5 1250 5000 1250 2500</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>2500 — 1250 1250 — 1250 — 5000 —</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>312.5 — 1250 — 5000 — 1250 —</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>— 5000 1250 5000 — 625 5000 78.12 5000</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>— 2500 1250 1250 — 5000 — 156.25 2500</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>— — 5000 1250 1250 — 5000 — 4.88 1250</td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td>2500 625 5000 5000 5000 5000 —</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>— 5000 1250 — 5000 5000 5000 2500</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>— — 312.5 1250 — 1250 5000 19.53 2500</td>
</tr>
</tbody>
</table>

*The (—) sign indicates that the compounds were active at MIC of more than 5000 μg/mL. Compounds 4e, 4g, 4j, 4k, 4l, 4m, 4o, 4p, 8a, 8c and 8d have shown no activity in the initial screening against all strains tested.

reported in Table I. It has been observed that most of the diphenyl hydrazones have exhibited mild to moderate antibacterial activity towards the selected bacterial strains and the results were comparable to standard drugs used. Compounds 4a, 4c, 4f, 4n and 8b have exhibited broad spectrum activity by showing activity against both Gram-negative and Gram-positive bacteria at MIC ranging from 312.5 to 5000 μg/mL. In general, it was observed that the hydrazones (4a-r) exhibited better antibacterial profile than that of semicarbazones (8a-d).

The in vitro antitubercular screening was conducted at 6.25 μg/mL against Mycobacterium tuberculosis H37 Rv (ATCC 27294) in BACTEC 12 B medium using a broth micro dilution method, the Microplate Alamar Blue Assay (MABA). The MIC and percentage inhibition data of the compounds, which have shown activity against the pathogenic bacterium, are presented in Table II. The compounds 4f, 4c and 4d exhibited substantial antitubercular activity in the initial screening, particularly 4f Acetic acid, phenoxy-[1-(4-chlorophenyl) ethylidene] hydrazide showed 80% inhibition (MIC > 6.25 μg/mL) and emerged as the most active compound in the present series.

Experimental Section

The melting points were determined in open capillary tubes using Thomas Hoover melting point apparatus and are uncorrected. The purity of the compounds was checked by TLC using chloroform and methanol (9:1). UV spectra were scanned on JASCO model 7800 UV/VIS spectrophotometer.
Infra-red spectra were determined on JASCO FT/IR-5300 Infrared spectrophotometer by KBr disc method. $^1$H NMR spectral studies were done on Jeol FX90Q FT spectrophotometer using DMSO-$d_6$ and CDCl$_3$ as solvents and TMS as internal reference.

**Synthesis**

The synthesis of diphenyl hydrazones (4a-r) was accomplished by the condensation of phenoxy or 4-bromophenoxy acetic acid hydrazide with appropriate carbonyl compound (Scheme I). The physicochemical characterization data of diphenyl hydrazones were reported by us earlier$^{16}$. The semicarbazones (8a-d) were obtained from appropriate 4-substituted aniline by the reaction with sodium cyanate and followed by treatment with hydrazine hydrate to yield corresponding phenyl urea and phenyl semicarbazide respectively. The treatment of later with appropriate aryl aldehyde yielded the corresponding semicarbazones (Scheme II). The representative synthetic procedures along with the chemical data of intermediates and final products are described below.

**Synthesis of substituted phenylurea**

The substituted aniline (0.1 mole) was dissolved in glacial acetic acid (10 mL) and diluted with water (100 mL). To this, an equimolar quantity (0.1 mole) of sodium cyanate in warm water (50 mL) was added with constant stirring. The reaction-mixture was allowed to stand for 1 hr and the solid precipitate formed was filtered off and dried after recrystallization from boiling water.

4-Chloro-N-methylphenylurea 6a: Yield 78%, m.p. 81°C; 4-Nitrophenylurea 6b: Yield 78%, m.p. 140°C; 4-Fluorophenylurea 6c: Yield 74%, m.p. 167°C; IR (KBr): 3370 (2\text{-NH, amide}), 2815 (CH$_3$-N), 1682 (-C=O), 830 cm$^{-1}$ (C$_6$H$_5$-F); $^1$H NMR (CDCl$_3$): δ 6 (s, 1H, C$_6$H$_5$-NH$_2$), 7.52-7.59 (m, 4H, -O-C$_6$H$_5$), 3.42 (s, 2H, -CONH-), 10.09 (s, 1H, -CONH-, D$_2$O exchangeable).

**Synthesis of substituted phenyl semicarbazides$^{17,18}$**

To a solution of substituted phenurea (0.01 mole) in ethanol (20 mL), an equimolar quantity of hydrazine hydrate was added. The reaction-mixture was made alkaline by adding sodium hydroxide. The reaction-mixture was refluxed for 1-2 hr and the precipitate obtained after cooling was filtered under suction and recrystallized from ethanol.

4-Chloro-(N-methyl)-phenylsemicarbazide 7a: Yield 78%, m.p. 190°C; (KBr): 3370 (2\text{-NH}), 2815 (CH$_3$-N), 1682 (-C=O), 1312 (C=N), 1035 (C$_6$H$_5$-Cl), 845 cm$^{-1}$ (C$_6$H$_5$-H); $^1$H NMR (CDCl$_3$): δ 3.42 (s, 2H, -N-CH$_2$(CH$_3$CO)), 7.95-8.05 (m, 4H, p-Cl-C$_6$H$_4$), 8.83 (s, 1H, -CONH-, D$_2$O exchangeable).

4-Nitrophenylsemicarbazide 7b: Yield 78%, m.p. 180-185°C; 4-Fluorophenylsemicarbazide 7c: Yield 78%, m.p. 216°C; IR (KBr): 3420 (2\text{-NH}), 1755 (C=O), 845 cm$^{-1}$ (C$_6$H$_4$-F); $^1$H NMR (CDCl$_3$): δ 6 (s, 2H, C$_6$H$_4$-NH$_2$), D$_2$O exchangeable), 7.52-7.59 (m, 4H, p-F-C$_6$H$_4$), 10.09 (s, 1H, -CONH-, D$_2$O exchangeable).

**Synthesis of semicarbazones 8a-d**

A mixture of substituted phenyl semicarbazide (0.01 mole) and appropriate carbonyl compound (0.01 mole) in ethanol (20 mL) was refluxed together in the presence of few drops of glacial acetic acid. After 2 hr, the precipitate obtained was filtered off and recrystallized from ethanol.

N-methyl-4-chlorophenyl-(4’-methoxyphenylmethylen) semicarbazide 8a: Yield 55%, m.p. 160°C, IR (KBr): 3370 (2\text{-NH, amide}), 2815 (CH$_3$-N), 1682 (-C=O), 1312 (C=N), 1035 (C$_6$H$_5$-Cl), 845 cm$^{-1}$ (C$_6$H$_5$-H); $^1$H NMR (CDCl$_3$): δ 3.42 (s, 3H, N-CH$_3$), 3.92 (s, 3H, -OCH$_3$), 7.18-7.28 (s, 4H, -OCH$_3$-C$_6$H$_4$), 9.59 (s, 1H, -CONH-, D$_2$O exchangeable), Anal. Calcd for C$_9$H$_8$ClN$_2$O$_2$: C, 50.26; H, 4.22; N, 11.00. Found: C, 50.26; H, 4.22; N, 10.90%.

4-Fluorophenyl-(4’-methoxyphenylenemethylene) semicarbazide 8b: Yield 74%, m.p. 178°C, IR (KBr): 3370 (2\text{-NH, amide}), 1735 (-C=O), 1570 (C=N), 1245 (C$_6$H$_5$-F), 830 cm$^{-1}$ (C$_6$H$_5$-H); $^1$H NMR (CDCl$_3$): δ 3.95 (s, 3H, -OCH$_3$), 6.0 (s, 1H, C$_6$H$_5$-NH), 7.23-7.33 (s, 4H, p-CH$_2$O-C$_6$H$_4$), 7.52-7.59 (m, 4H, p-F-C$_6$H$_4$), 10.09 (s, 1H, -CONH-, D$_2$O exchangeable), Anal. Calcd for C$_{13}$H$_{16}$ClN$_2$O$_2$: C, 55.35; H, 4.30; N, 12.91. Found: C, 55.27; H, 4.20; N, 12.82%.

4-Fluorophenyl-(3’4’,5’-trimethoxyphenylenemethylene) semicarbazide 8c: Yield 82%, m.p. 140°C, IR (KBr): 3480 (2\text{-NH, C$_6$H$_5$-NH}), 3370 (2\text{-NH, amide}), 1718 (-C=O), 1620 (C=N), 1176 (C$_6$H$_5$-F), 840 cm$^{-1}$ (C$_6$H$_5$-H); $^1$H NMR (CDCl$_3$): δ 3.95 (s, 3H, -OCH$_3$), 6.0 (s, 1H, C$_6$H$_5$-NH), 7.23-7.33 (s, 4H, p-(CH$_2$O)$_2$-C$_6$H$_4$), 7.52-7.59 (m, 4H, p-F-C$_6$H$_4$), 10.09 (s, 1H, -CONH-, D$_2$O exchangeable), Anal. Calcd for C$_{18}$H$_{20}$ClN$_2$O$_3$: C, 58.73; H, 5.18; N, 12.09. Found: C, 58.66; H, 5.24; N, 12.14%.

4-Nitrophenyl-(3’4’,5’-trimethoxyphenylenemethylene) semicarbazide 8d: Yield 90%, m.p. 130°C, IR (KBr): 3400 (2\text{-NH, C$_6$H$_5$-NH}), 3270 (2\text{-NH, ...
amide), 1720 (-C=O), 1610 (C=N), 1525 (C₆H₅-NO₂), 855 cm⁻¹ (C₆H₅-H); ¹H NMR (CDCl₃): δ 3.95 (s, 3H, -OCH₃), 6.0 (s, 1H, C₆H₅-NH), 7.23-7.33 (s, 4H, p-CH₂O)-C₆H₄), 7.28-7.59 (m, 4H, p-NO₂-C₆H₄), 10.09 (s, 1H, -CONH-, D₂O exchangeable), Anal. Calcd for C₁₆H₁₆ClN₃O₂: C, 54.50; H, 4.80; N, 14.95. Found: C, 54.55; H, 4.71; N, 14.88%.

Antibacterial screening

The *in vitro* antibacterial activity against ten pathogenic strains of bacteria was performed by agar double dilution technique¹⁹. The microbial strains were procured from the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi. The stock solution of standard and test compounds was prepared in dimethyl sulfoxide (DMSO) and subsequent dilutions were made with the same solvent. Mueller Hint Agar Media (Hi Media) was used to subculture various strains of microbes. Normal saline was used to prepare the inoculum of the bacteria to be used for the antibacterial study. Under aseptic conditions, the diluted test solutions with different concentration (5000 to 78.12 μg/mL) were added to the vials (previously sterilized) containing 100 discs and the disc were placed on the numbered plates. The plates were then incubated at 37°C for 24 hr. The MIC, the lowest concentration of the test drug that completely inhibited the growth of bacteria, determined and is presented in Table I. Sulphamethoxazole (*SM*) and trimethoprim (*TM*) were used as standard drugs.

Antitubercular screening

The *in vitro* antitubercular screening was carried out at Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, Alabama. The primary screening was conducted at 6.25 μg/mL against *Mycobacterium tuberculosis* H37 Rv (ATCC 27294) in BACTEC 12 B medium using a broth micro dilution assay, the Microplate Alamar Blue Assay (MABA)²⁰. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system. The MIC and the percentage inhibition data of the compounds are reported in Table II.

**References**