Synthesis, characterization and antibacterial activity of some new 3-(3-(trifluoromethyl)-phenyl)-3-(2-hydroxy-5-methylphenyl)-propanehydrazones

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The present paper describes the synthesis of some new potent antibacterial hydrazone-hydrazone derivatives 6a-j. The synthetic methodology involves preparation of substituted chromen-2-one 3 from the starting materials (E)-3-(3-(trifluoromethyl)phenyl)acrylic acid 1 with p-cresol 2, which in turn on treatment with hydrazine-hydrate in methanol results in the substituted propane hydrazide 4. Finally, the hydrazones 6a-j have been obtained in 80-88% yield on further condensation with various aromatic aldehydes 5a-j. Compounds 6d, 6e, 6f and 6j exhibit excellent antibacterial activity (zone of inhibition: 26-32 mm) against both the Gram positive (E. coli, P. aeruginosa) and Gram negative bacteria (S. aureus, S. pyogenes) and the compounds 6a, 6b, 6c show good activity (zone of inhibition: 19-27 mm), while the remaining compounds viz., 6g, 6h, 6i show weak activity (zone of inhibition: 10-15 mm).

**Keywords:** (E)-3-(3-(Trifluoromethyl)phenyl)acrylic acid, p-cresol, antibacterial activity

Hydrazones are a class of organic compounds in the Schiff base family\textsuperscript{12}. Hydrazones constitute a versatile class of organic compounds having the basic structure \textsuperscript{R}1\textsuperscript{R}2C=NNR\textsuperscript{R}3\textsuperscript{R}4 (Ref 3,4). Hydrazones and their derivatives are known to exhibit interesting diverse biological activities like antioxidant\textsuperscript{5}, anti-inflammatory\textsuperscript{6,7}, anti-convulsant\textsuperscript{8}, analgesic\textsuperscript{9,10}, antimicrobial\textsuperscript{11,12,14}, anticancer\textsuperscript{15,16}, antiprotozoal\textsuperscript{17}, antiparasitic\textsuperscript{18}, cardioprotective\textsuperscript{19}, anti-depressant\textsuperscript{20}, antitubercula\textsuperscript{21,22}, and anti-HIV\textsuperscript{23}. Hydrazones are also used to couple with certain drugs and the bonds based on hydrazones are stable at neutral pH (Ref 24). Some hydrazones have been introduced by the researchers as potent drugs, such as nifuroxazide, an intestinal antiseptic\textsuperscript{25,26}, dihydralazine as hypertensive, and gyromitrin, a toxin.

During the last decade, antimicrobial resistance represents the major problem facing the world, so that several new antibiotics and antifungal agents are introduced each year to help in the treatment of infectious diseases. Resistance to antimicrobial drugs has become an increasingly important global problem\textsuperscript{27,28}. Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the lifespan of antifungal agents such as the azoles, the antiviral agents such as the non-nucleoside reverse transcriptase inhibitors\textsuperscript{29}, and various antibacterial agents including lactams and quinolones\textsuperscript{30}. Broad empirical screening of chemical entities for antimicrobial activity represents a strategy for the development of novel drugs.

Encouraged by the various pharmacological activities associated with hydrazone derivatives, herein is report the synthesis, characterization and antibacterial activity of some new 3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propanehydrazone derivatives.

**Results and Discussion**

**Chemistry**

Scheme I depicts the synthetic sequence for hydrazide-hydrazone derivatives 6a-j. Lactone 4-(3-(trifluoromethyl)phenyl)-3,4-dihydro-6-methylchromen-2-one 3 was isolated in 85% yield by coupling of (E)-3-(3-(trifluoromethyl)phenyl)acrylic acid 1 with p-cresol 2 in presence of catalytic quantity of conc. H\textsubscript{2}SO\textsubscript{4}. Reaction of 4-(3-(trifluoromethyl)phenyl)-3,4-dihydro-6-methylchromen-2-one 3 with hydrazine-hydrate in methanol at 50°C for 5 h resulted in 3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propanehydrazone 4 in 83% yield. The structural elucidation of the newly synthesized compounds 6a-j was done on the basis of their spectral data. Each compound was characterized by \textsuperscript{1}H NMR, IR and mass spectrometry (MS). Interpretation of IR spectra: all the hydrazo-derivatives of some new 3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propanehydrazone derivatives.
compounds 6a-j, observed at 3200-3600 cm$^{-1}$ (H-bonded) and 3500-3700 cm$^{-1}$ (free, H bonded) respectively. As a representative example, the interpretation of the $^1$H NMR spectra of (E)-N'-(4-methoxybenzylidene)-3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methyl-phenyl)propanehydrazide is described as follows, aromatic protons were observed at about $\delta$ 8.05-6.60. Because hydrazones exist as couple of diastereoisomers E/Z (Ref 30) –OH,-N-H,-N=CH-Ar,-CH$_2$-CH-Ar, –CH$_2$=O, OCH$_3$ and –CH$_3$ protons were observed as couples$^{31}$ of peaks at $\delta$ 11.30 (*11.08), 9.28 (*9.24), 8.05 (*7.90), 4.96 (*4.90), 3.54 (*3.30), 3.10 (*2.96), 3.78 (3.72) and 2.18 (*2.14, s, 3H, CH$_3$) respectively. To confirm the identification of each hydrazide-hydrazone 6a-j, ESI-MS analysis was performed in the positive ion mode, showing peaks at $m/z$ corresponding to the expected monoisotopic mass of the [M+H]$^+$ ion.

**Antibacterial activity**

The antibacterial evaluation of the synthesized hydrazide-hydrazone derivatives 6a-j is depicted in Table I. The antibacterial activity was measured in terms of zone of inhibition (ZI, in mm). Compounds 6d, 6e, 6f and 6j exhibited excellent activity (zone of inhibition: 26-32 mm) against both the Gram positive (E.coli, P.aeruginosa) and Gram negative bacteria (S.aureus, S.pyogenes) and the compounds 6a, 6b, 6c showed good activity (zone of inhibition: 19-27 mm), while the remaining compounds viz., 6g, 6h, 6i showed weak activity (zone of inhibition: 10-15 mm). It may
be put forth from Table I, that compounds having R = 4-NO$_2$, 2-hydroxy-3-methoxy, 3-methoxy-4-ethoxy and 3,4-diethoxy exhibited excellent antibacterial activity and the compounds with substitution R = H, 2-OMe, 4-OMe displayed good antibacterial activity, while the compounds with R = 2-Cl, 3-Cl and 3-Br weak antibacterial activity.

**Experimental Section**

Solvents and reagents were obtained from commercial sources and used without purification. The IR spectra ($\nu_{max}$, cm$^{-1}$) were recorded in solid state KBr dispersion using Perkin-Elmer FT-IR spectrometer. The $^1$H NMR spectra was recorded on Varian 500 MHz spectrometer. The chemical shifts were reported in $\delta$ (ppm) relative to TMS. The mass spectra were recorded on API 2000 Perkin-Elmer PE-Sciex mass spectrometer. The reactions were monitored by Thin Layer Chromatography (TLC).

Melting points were determined on Polman melting point apparatus (Model No MP96) by open capillary method and are uncorrected. All the reactions were carried out under nitrogen atmosphere.

4-(3-(Trifluoromethyl)phenyl)-3,4-dihydro-6-methylchromen-2-one, 3. A mixture of (E)-3-(3-(trifluoromethyl)phenyl)acrylic acid 1 (20 g, 0.135 mol), p-cresol (15.32 g, 0.142 mol) and a catalytic amount of conc. H$_2$SO$_4$ (4.63 g, 0.0472 mol) was heated to 110°C for 12 h. After completion of reaction (judged by TLC), the reaction mixture was cooled to 0-5°C and filtered to obtain pure compound 3. The crude compound was purified by recrystallization from isopropyl alcohol. White solid, Yield 27.33 g, 85%; m.p. 48-50°C.

3-(3-(Trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propanehydrazide, 4. To a solution of 4-(3-(trifluoromethyl)phenyl)-3,4-dihydro-6-methylchromen-2-one 3 (20 g, 0.084 mol) in methanol (100 mL) was added hydrazine hydrate (12.5 g, 0.252 mol) and the reaction mixture heated to 50°C for 5 h. After completion of the reaction (checked by TLC), the reaction mixture was cooled to 0-5°C and filtered to obtain the crude compound. The crude compound was dissolved in ethanol (100 mL) at 45°C to get a clear solution and cooled to 10°C and the crystals obtained were filtered at the pump to obtain pure compound 4. White solid, Yield 16.5 g, 83%, m.p. 136-38°C; IR (KBr): 3311, 3301, 3269, 3082, 3049, 2967, 2867, 2811, 1647, 1574, 1509, 1475, 1452, 1440, 1293, 1273, 1262, 1256, 1239, 1221, 1098, 1084 cm$^{-1}$; $^1$H NMR (500 MHz, DMSO-d$_6$): $\delta$ 9.23 (s, 1H,OH), 9.01 (s, 1H, NH), 7.53 (t, $J = 5.0$ Hz, 2H-CF$_3$Ar ring), 7.47 (d, $J = 7.0$ Hz, 2H-CF$_3$Ar ring), 6.96 (d, $J = 2.0$ Hz, 1H-CF$_3$Ar ring), 6.80 (q, $J = 1.5$ Hz, 1H-CF$_3$Ar ring), 6.63 (d, $J = 8.0$ Hz, 1H-CF$_3$Ar ring), 4.84 (dd, $J = 7.0, 9.5$ Hz, 1H-CF$_3$Ar ring), 4.09 (brs, 2H), 2.86 (dd, $J = 8.5, 14.5$ Hz, 1H,-CH-CH$_2$-CO, H$_2$), 2.70 (dd, $J = 8.0, 15.0$ Hz, 1H,-CH-CH$_2$-CO, H$_3$), 2.18 (s, 3H,-CH$_3$); ESI-MS: $m/z$ 338.9 (M+H)$^+$. General experimental procedure for the synthesis of hydrazones derivatives, 6a-j

To a suspension of 3-(2-hydroxy-5-methylphenyl)-3-phenylpropanehydrazone (1g, 3.70 mmol) in methanol (10 mL) at 25-27°C was added benzaldehydes 5a-j (0.411 g, 3.80 mmol) and heated to 50°C for 5 h. After completion of the reaction (checked by TLC), the reaction mixture was concentrated at 45°C, then the crude product was dissolved in dichloromethane (10 mL) and n-hexane (20 mL) added slowly at 20-25°C. The precipitated solids were filtered at the pump and dried. The yields of the products varied from 80-88%.

(3-(1-Benzyliden-3-(3-(trifluoromethyl)phenyl)acetyl)-3-(2-methoxybenzylidene)-3-(3-(trifluoromethyl)phenyl)propanehydrazide, 4a. To a solution of 3-(2-hydroxy-5-methylphenyl)-3-phenylpropanehydrazone (1g, 3.70 mmol) in methanol (10 mL) at 25-27°C was added benzaldehydes 5a-j (0.411 g, 3.80 mmol) and heated to 50°C for 5 h. After completion of the reaction (checked by TLC), the reaction mixture was concentrated at 45°C, then the crude product was dissolved in dichloromethane (10 mL) and n-hexane (20 mL) added slowly at 20-25°C. The precipitated solids were filtered at the pump and dried. The yields of the products varied from 80-88%.
(E)-N'-(4-Methoxybenzylidene)-3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propenylhydrazide, 6c: White solid; Yield 86%; m.p. 152-54°C; IR (KBr): 3171, 3097, 3077, 3000, 2985, 2872, 2853, 2839, 1643, 1623, 1607, 1539, 1514, 1287, 1101, 1084 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 13.80 (s, 1H, NH), 11.61 (s, 1H, OEt Ar ring), 7.90 (d, J = 8.5 Hz, 2H, -CH₂-Ar ring), 7.06-6.96 (m, 3H, -CH₃Ar ring / -OEt Ar ring), 6.89 (d, J = 8.0 Hz, 1H, -OMeAr ring), 6.66 (dd, J = 8.5 Hz, 1H, -2-OMeAr ring), 4.96 (*4.90, dd, J = 9.0 Hz, 1H, -CF₃ArCH ring), 3.96 (*4.90, dd, J = 9.0 Hz, 1H, -CF₃ArCH ring), 3.78 (*3.72, s, 3H, OMe), 3.54 (*3.30, dd, J = 9.0, 15.0 Hz, 1H, -CH₂CH₂-CO, H₂), 3.10 (*2.96, dd, J = 9.0, 15.5, 1H, -CH₂CH₂-CO, H₂), 2.18 (*2.14, s, 3H, CH₃); ESI-MS: m/z 457.5 (M+H⁺).

(E)-N'-(4-Methoxybenzylidene)-3-(3-trifluoromethylphenyl)-3-(2-hydroxy-5-methylphenyl)propenylhydrazide, 6d: Pale yellow solid; Yield 88%; m.p. 178-79°C; IR (KBr): 3347, 3311, 3269, 3111, 3070, 3002, 2961, 2942, 2877, 2843, 1672, 1626, 1613, 1590, 1513, 1250, 1212, 1103, 1095, 1075, 1020 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 11.64 (*11.18, s, 1H, OH), 10.70 (*9.41, s, 1H, OH), 9.28 (*9.23, s, 1H, NH), 8.32 (*8.28, s, 1H, N=CH-Ar), 7.58-7.54 (m, 2H, -CF₃Ar ring), 7.50-7.48 (m, 2H, -CF₃Ar ring), 7.10-6.98 (m, 3H, -CH₃Ar ring / -OEt Ar ring), 6.85-6.73 (m, 2H, -CH₃Ar ring / -OEt Ar ring), 6.66 (t, J = 4.0 Hz, 1H, -OMeAr ring), 4.94 (*4.89, t, J = 7.0 Hz, 1H, -CF₃ArCH ring), 3.82 (*3.78, s, 3H, OMe), 3.50-3.45 (*3.31-3.24, m, 1H, -CH₂CH₂-CO, H₃), 3.11-3.08 (*3.06-2.95, m, 1H, -CH₂CH₂-CO, H₂), 2.18 (*2.14, s, 3H, CH₃); ESI-MS: m/z 473.0 (M+H⁺).
2.20 (*2.14, s, 3H, CH
3.09 (*2.98, dd, 3H, CH
1566, 1509, 1286, 1281, 1161, 1142, 1096, 1002 cm
IR (KBr): 3368, 3139, 3126, 3078, 3001, 2964, 2881,
7.0 Hz, 1H,-CH,-CO, H
9.0, 15.5 Hz,-CH-C
11.75 (*11.52, s, 1H, OH), 9.28 (*9.27, s, 1H, NH), 8.38-8.23 (m, 2H), 8.08 (s, 1H,-N=CH-Ar), 7.98 (d, J = 5.0 Hz, 1H), 7.92 (d, J = 5.5 Hz, 1H), 7.62-7.58 (m, 2H), 7.50-7.48 (m, 2H), 7.05 (dd, J = 1.5, 7.0 Hz, 1H), 6.82 (dd, J = 2.5, 6.5 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 4.97 (*4.90, t, J = 7.0 Hz, 1H,-CF3ArCH ring), 3.54 (*3.35, dd, J = 9.0, 15.5 Hz, 1H,-CH-CH₂-CO, H₂), 3.13 (*3.01, dd, J = 9.0, 15.5 Hz,-CH-CH₂-CO, H₃), 2.16 (*2.14, s, 3H, CH₃); ESI-MS: m/z 472.3 (M+H)⁺.

(E)-N'-(3-Chlorobenzylidene)-3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propane-hydrazide, 6b: Yellow solid; Yield 86%; m.p. 166-68°C; IR (KBr): 3341, 3286, 3189, 3126, 3090, 3066, 2997, 2936, 2874, 1650, 1622, 1611, 1601, 1578, 1566, 1509, 1286, 1281, 1161, 1142, 1096, 1002 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 11.57 (*11.32, s, 1H, OH), 9.27 (*9.23, s, 1H, NH), 8.10 (*7.90, s, 1H,-N=CH-Ar), 7.75 (*7.70, s, 1H,-CF3Ar ring), 7.62-7.58 (m, 3H,-CF₃Ar ring), 7.52-7.40 (m, 4H, 3-Cl Ar ring), 7.10 (*7.05, s, 1H,-CH₂ Ar ring), 6.84 (*6.80, m, 1H,-CH₂ Ar ring), 6.66 (*6.64, s, 1H,-CH₂ Ar ring), 4.96 (*4.89, t, J = 7.0 Hz, 1H,-CF₃ArCH ring), 3.54 (*3.29, dd, J = 9.0, 15.5 Hz, 1H,-CH-CH₂CO, H₂), 3.10 (*2.98, dd, J = 9.0, 15.5 Hz,-CH-CH₂CO, H₃), 2.18 (*2.14, s, 3H, CH₃); ESI-MS: m/z 461.3 (M+H)⁺.

(E)-N'-(4-Nitrobenzylidene)-3-(3-(trifluoromethyl)phenyl)-3-(2-hydroxy-5-methylphenyl)propane-hydrazide, 6i: Yellow solid; Yield 86%; m.p. 191-93°C; IR (KBr): 3341, 3286, 3189, 3126, 3090, 3066, 2997, 2936, 2874, 1650, 1622, 1611, 1601, 1578, 1566, 1509, 1286, 1281, 1161, 1142, 1096, 1002 cm⁻¹; ¹H NMR (400 MHz, DMSO-d₆): δ 11.68 (*11.41, s, 1H, OH), 9.28 (*9.25, s, 1H, NH), 8.52 (*8.35, s, 1H,-N=CH-Ar), 8.05 (*7.88, d, J = 7.2 Hz, 1H,-CF₃Ar ring), 7.62-7.58 (m, 2H,-CF₃Ar ring), 7.52-7.38 (m, 5H,-CF₃Ar ring/-2-Cl-Ar ring), 7.04 (t, J = 8.5 Hz, 1H,-CH₂ Ar ring), 6.80 (dd, J = 2.5, 7.5 Hz, 1H,-CH₂ Ar ring), 6.65 (dd, J = 3.0, 8.5 Hz, 1H,-CH₁ Ar ring), 4.96 (*4.90, t, J = 7.0 Hz, 1H,-CF₃ArCH ring), 3.54 (*3.32, dd, J = 9.0, 15.5 Hz,-CH-C, H₂), 3.09 (*2.98, dd, J = 9.0, 15.5 Hz,-CH-CH₂CO, H₂), 2.20 (*2.14, s, 3H, CH₃); ESI-MS: m/z 472.3 (M+H)⁺.

Antibacterial screening
The antimicrobial activity was determined using disc diffusion method by measuring zone of inhibition in mm (Ref 32). All the compounds, 6a-j were screened in vitro at a concentration of 250 µg/mL for antibacterial activity against two Gram-positive pathogenic organisms: Staphylococcus aureus and Staphylococcus pyogenes, two Gram-negative organisms: Escherichia coli and Pseudomonas aeruginosa (Table I). Standard antibacterial drug ciprofloxacin (250 µg/disc) was also tested under similar conditions against these organisms. Each experiment was carried out in triplicate and the average reading was taken. Growth inhibition was calculated with reference to positive control. Hydrazide-hydrazones 6a-j were dissolved in dimethyl sulphoxide at 250 µg/mL concentration. The inhibition zones were measured in millimeters at the end of an incubation period of 48 h at (35±2)°C. DMSO alone showed no inhibition. The composition of nutrient agar medium was Bactotryptone (10 g), yeast extract (5 g), NaCl (10 g), final pH 7.4.

Conclusion
In the present paper an efficient synthesis of some potent antibacterial hydrazones 6a-j is described utilizing commercially available (E)-3-(3-(trifluoromethyl)phenyl)-acrylic acid 1 and p-cresol 2. All the synthesized hydrazide derivatives have been well characterized by ¹H NMR, IR and mass spectral analysis. The synthesized compounds have been further screened against a panel of four selected bacterial strains viz., Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes and Staphylococcus aureus, at the concentrations 250 µg/mL with reference to the antibiotic drug Ciprofloxacin. Compounds 6d, 6e, 6f and 6j exhibit excellent activity (zone of inhibition: 26-32 mm) against both the Gram positive (E.coli, P.aeruginosa) and Gram negative bacteria (S.aureus, S.pyogenes) and the compounds 6a, 6b, 6c showed good activity (zone of inhibition: 19-27 mm), while the remaining compounds viz., 6g, 6h, 6i showed weak activity (zone of inhibition: 10-15 mm).

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