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Synthesis and Biological Evaluation of 4-(4-Chlorophenyl)cyclohexane Carbohydrazide Derivatives as Anti-Bacterial Agents

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The present paper describes the synthesis and antibacterial activity of novel hydrazone derivatives **4a-s** derived from 4-(4-chlorophenyl)-cyclohexane carboxylic acid. All the nineteen newly synthesized novel hydrazone derivatives **4a-s** were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *S. pyogenes* (Gram-positive bacteria) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria). Antibacterial activity data revealed that the basic scaffold with R = nitrogen heterocyclic ring such as pyridine, quinoline, imidazole and indole showed significant antibacterial activity (excellent activity), whereas the hetrocyclic ring like benzo[*b*]furan, furan, thiophene moiety showed good antibacterial activity.

Keywords: Atovaquone, Cyclohexanecarbohydrazide, Hydrazones, Antibacterial Activity.

INTRODUCTION

The emergence of new infectious diseases, the resurgence of several infections that appeared to have been controlled and the increase in bacterial resistance have created the necessity for studies directed towards the development of new antimicrobials agents[1]. The chemical properties of hydrazones have been intensively studied in several research fields because of their high physiological activity and chelating capability[2]. Extensive investigations have revealed that the lone pair on trigonally hybridized nitrogen atom of the azomethine group is responsible for the chemical and biological activity[3-7]. Several hydrazide-hydrazone derivatives exhibited broad spectrum of biological activities such as antimicrobial[8,9], antitubercular[10-12], anticandida, anticancer, antiviral, antioxidant and antiinflammatory[13-17].

The starting material 4-(4-chlorophenyl)cyclohexane carboxylic acid is an important precursor used for the synthesis of atovaquone[18]. Atovaquone is the hydroxyl-1,4-naphthoquinone analogue of coenzyme Q10 and has an antipneumocystic activity[19] and it is approved and marketed as a prescription drug for the treatment of *Pneumocystis carinii* pneumonia (PCP), a common parasitic lung infection of immune compromized patients[20,21]. It is not only used for the treatment of *Pneumocystis carinii* pneumonia, but also displays potent activity as an antimalarial agent and has been used in the treatment of toxoplasmosis and babesiosis[22]. Due to the broad range of applications and encouraged by these reported biological activities the present research work is aspired to describe the synthesis and antibacterial activity of novel hydrazone derivatives (**4a-s**) derived from 4-(4-chlorophenyl)cyclohexane carboxylic acid.

EXPERIMENTAL

Chemical and solvents used were purchased either from Fluka or Merck. All the reagents were of analytical grade. Thin-layer chromatography (TLC) was performed on E. Merck AL silica gel 60 F254 plates and visualized under UV light. IR spectra were recorded as KBr pellet with a perkin-elmer spectrum gx FTIR instrument and only diagnostic and/or intense peaks are reported. ^{1}H NMR spectra were recorded in DMSO-d_6 with a Varian Mercury plus 400 MHz instrument. ^{13}C NMR spectra were recorded in DMSO-d_6 with a Varian Gemini 100 MHz instrument. Signals due to the residual protonated solvent (^{1}H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The ^{1}H NMR chemical shifts and coupling constants were determined assuming first-order behaviour. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under argon atmosphere. 4-(4-Chlorophenyl)cyclohexane carboxylic acid

(**1**) and all the benzaldehydes used for the preparation of **4a-s** were purchased from commercial sources.

Synthesis of methyl 4-(4-chlorophenyl)cyclohexane carboxylate (2): To a solution of compound **1** (2 g, 8.4 mmol) in methanol (20 mL) was added sulphuric acid (0.1 mL) and refluxed for 10 h. After completion of the reaction, methanol was evaporated under reduced pressure and the obtained residue was taken in ethyl acetate (30 mL,), washed with 10 % aq. $NaHCO_3$ solution (2 × 10 mL) followed by water and brine solution. The organic layer was separated, dried over Na_2SO_4, filtered and evaporated to afford compound **2**. Yellow oily liquid, Yield: 2 g, 94 %; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.32 (d, *J* = 12.0 Hz, 2H), 7.24 (d, *J* = 12.0 Hz, 2H), 3.61 (s, 3H), 2.51-2,42 (m, 1H), 2.39-2.35 (m, 1H), 1.97 (d, *J* = 12 Hz, 2H), 1.82 (d, *J* = 12 Hz, 2H), 1.82 (d, *J* = 12 Hz, 2H), 1.54-1.40 (m, 4H).

Synthesis of 4-(4-chlorophenyl)cyclohexane carbohydrazide (3): To a solution of compound **2** (1.5 g, 5.95 mmol) in ethanol (15 mL) was added hydrazine hydrate (24 m mol) and heated to reflux for 3 h. After completion of the reaction, ethanol was concentrated under reduced pressure to obtain crude compound **3**. The crude compound was slurred in *n*-hexane, filtered at the high vaccum pump and dried to obtain compound **3**. White solid, Yield: 1.35 g, 86 %; m.p.: 121-122 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (br.s, 1H), 7.32 (d, *J* = 12.0 Hz, 2H), 7.24 (d, *J* = 12.0 Hz, 2H), 4.16 (br.s, 2H), 2.55-2,47 (m, 1H), 2.16-2.08 (m, 1H), 1.82-1.60 (m, 4H), 1.32-1.56 (m, 4H).

General experimental procedure for the synthesis of hydrazone derivatives (4a-s): To a stirred solution of compound **3** (100 mg, 0.40 mmol) in ethanol was added corresponding benzaldehydes (1 mmol) and refluxed for 1 h. The reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 80 and 94 %.

(*E*)-*N*'-(2,4-Dimethoxybenzylidene)-4-(4-chlorophenyl)-cyclohexane carbohydrazide (4a): White solid; Yield: 82 %; m.p.: 102-103 °C; IR (KBr, ν_{max}, cm^{-1}): 3225.4, 2933.2, 1655.6, 1608.3, 1595.8, 1554.3, 1503.2, 1209.1, 1159.0; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.23 (*11.00, s, 1H), 8.44 (*8.23, s, 1H), 7.70-7.75 (m, 1H), 7.27-7.42 (m, 4H), 6.90-6.60 (m, 2H), 3.81 (s, 3H), 3.91 (s, 3H), 3.35-3.18 (*2.57-2.50, m, 1H), 2.27-2.19 (m, 1H), 1.84-1.87 (m, 4H), 1.65-1.38 (m, 4H); ESI-MS: *m/z*, 400.90.

(*E*)-*N*'-(2,5-Dimethoxybenzylidene)-4-(4-chlorophenyl)-cyclohexane carbohydrazide (4b): Yellow solid; Yield: 80 %; m.p.: 112-113 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (*11.17, s, 1H), 8.52 (*8.29, s, 1H), 7.40-7.24 (m, 5H), 6.99-6.96 (m, 2H), 3.79 (s, 6H), 3.19 (*2.58, m, 1H), 2.58-2.22 (m, 1H), 1.91-1.84 (m, 4H), 1.66-1.46 (m, 4H); ESI-MS: *m/z*, 400.90.

(*E*)-*N*'-(3,4-Dimethoxybenzylidene)-4-(4-chloro-phenyl)-cyclohexane carbohydrazide (4c): Yellow solid; Yield: 82 %; m.p.: 118-119 °C; IR (KBr, ν_{max}, cm^{-1}): 3233.1, 2935.1, 1659.4, 1600.6, 1556.3, 1512.90, 1268.0; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.41 (*11.17, s, 1H), 8.52 (*8.29, s, 1H), 7.27-7.40 (m, 5H), 6.99-6.96 (m, 2H), 3.79 (s, 6H), 3.19 (*2.58, m, 1H), 2.58-2.22 (m, 1H), 1.84-1.91 (m, 4H), 1.66-1.46 (m, 4H); ESI-MS: *m/z*, 400.90.

(*E*)-*N*'-(3,4,5-Trimethoxybenzylidene)-4-(4-chlorophenyl)cyclohexane carbohydrazide (4d): White solid; Yield: 82 %; m.p.: 115-116 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.42 (*11.2, s, 1H), 8.12 (*7.9, s, 1H), 7.34-7.30 (m, 4H), 6.99 (s, 2H), 3.83 (s, 6H), 3.86 (s, 3H), 2.54-2.52 (m, 1H), 2.23-2.20 (m, 1H), 1.70-1.40 (m, 4H), 1.90-1.80 (m, 4H); ESI-MS: *m/z*, 431.1.

(*E*)-*N*'-(4-Ethoxy-3-methoxybenzylidene)-4-(4-chlorophenyl)cyclohexane carbohydrazide (4e): Yellow solid; Yield: 75 %; m.p.: 120-121 °C; IR (KBr, ν_{max}, cm^{-1}): 3207.0, 2933.2, 1655.5, 1598.7, 1556.3, 1511.9, 1270.9; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.26 (*11.08, s, 1H), 8.10 (*7.91, s, 1H), 7.28-7.36 (m, 5H), 7.12 (d, 1H, *J* = 9.0 Hz), 6.98 (d, 1H, *J* = 6.0 Hz), 4.04 (q, 2H, *J* = 6.0 Hz), 3.80 (s, 3H), 3.42 (* 2.56, m, 1H), 2.25 (m, 1H), 1.88-1.85 (m, 4H), 1.66-1.46 (m, 4H), 1.32 (t, 2H, *J* = 6.4 Hz); ESI-MS: *m/z*, 415.1.

(*E*)-*N*'-(3-Methoxy-4-propoxybenzylidene)-4-(4-chlorophenyl)cyclohexane carbohydrazide (4f): Brown solid; Yield: 78 %; m.p.: 122-123 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.26 (*11.09, s, 1H), 8.10 (*7.9, s, 1H), 7.36-7.25 (m, 5H), 7.12 (d, 1H, *J* = 9.0 Hz), 6.98 (d, 1H, *J* = 9.0 Hz), 3.93 (q, 2H, *J* = 3.0 Hz), 3.83 (s, 3H), 2.58-2.56 (m, 1H), 2.2 (m, 1H),1.88-1.85 (m, 4H), 1.78-1.71 (m, 4H), 1.63-152 (q, 2H, *J* = 9.0 Hz), 0.95-1.0 (t, 3H, *J* = 9.0 Hz); ESI-MS: *m/z*, 429.2.

(*E*)-*N*'-(4-(Dimethylamino)benzylidene)-4-(4-chlorophenyl)cyclohexane carbohydrazide (4g): Pale brown solid; Yield: 71 %; m.p.: 102-103 °C; IR (KBr, ν_{max}, cm^{-1}): 3287.1, 2934.2, 1692.2, 1539.9, 1492.6, 1310.4, 1300.8, 1144.5; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.06 (*10.9, s, 1H), 8.03 (*7.86, s, 1H), 7.49-7.46 (d, 1H, *J* = 9.0 Hz), 7.29 (m, 4H), 6.75-6.72 (d, 1H, *J* = 9.0 Hz), 2.95 (s, 6H), 3.34-3.20 (m, 1H), 2.27-2.25 (m, 1H), 1.85-1.84 (m, 4H), 1.58-1.55 (m. 4H); ESI-MS: *m/z*, 384.1.

(*E*)-*N*'-(4-(Methanesulfonyl)benzylidene)-4-(4-chlorophenyl)cyclohexane carbohydrazide (4h): Yellow solid; Yield: 68 %; m.p.: 131-132 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.65 (*11.44, s,1H), 8.28 (*8.16, s, 1H), 7.92-8.08 (m, 4H), 7.27-7.37 (m, 4H), 3.26 (s, 3H), 3.46 (*2.57, m, 1H), 2.36-2.23 (m, 1H), 1.85-1.94 (m, 4H), 1.4-1.67 (m. 4H); ESI-MS: *m/z*, 419.1.

(*E*)-*N*'-(4-(Methyl)benzylidene)-4-(4-chlorophenyl)-cyclohexane carbohydrazide (4i): Brown solid; Yield: 68 %; m.p.: 125-126 °C; IR (KBr, ν_{max}, cm^{-1}): 3209.9, 2926.4, 1660.4, 1604.5, 1563.0, 1491.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.38 (*11.12, s, 1H), 8.47 (*8.27, s, 1H), 7.78-7.70 (m, 1H), 7.37-7.27 (m, 7H), 3.18 (*2.6, m, 1H), 2.50 (s, 3H), 2.20 (m, 1H), 1.94-1.85 (m, 4H), 1.63-1.44 (m. 4H); ESI-MS: *m/z*, 355.2.

(*E*)-4-(4-Chlorophenyl)-*N*'-((quinoline-4-yl)methylene)cyclohexane carbohydrazide (4j): Brown solid; Yield: 72 %; m.p.: 134-135 °C; IR (KBr, ν_{max}, cm^{-1}): 3210.9, 2927.4, 1668.1, 1558.2; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (*11.55, s, 1H), 8.99 (d, *J* = 8.0 Hz, 1H), 8.81-8.75 (*8.09, s, 1H), 8.64 (d, *J* = 8.0 Hz, 1H), 7.86-7.71 (m, 3H), 7.34-7.29 (m, 5H), 3.36 (*2.59, m, 1H), 2.36-2.34 (m, 1H), 1.94-1.91 (m, 4H), 1.65-1.43 (m. 4H); ESI-MS: *m/z*, 392.4.

(*E*)-4-(4-Chlorophenyl)-*N*'-((pyridine-2-yl)methylene)-cyclohexane carbohydrazide (4k): Brown solid; Yield: 67 %; m.p.: 127-128 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (*11.28, s,1H), 8.69 (d, *J* = 8.0 Hz, 1H), 8.16 (*7.96, m, 1H), 7.82-7.77 (m, 2H), 7.42-7.28 (m, 5H), 3.34 (*2.59, m, 1H), 2.38- 2.36 (m, 1H), 1.92-1.90 (m, 4H), 1.64-1.44 (m. 4H); ESI-MS: *m/z*, 342.40.

(*E*)-4-(4-Chlorophenyl)-*N*'-((pyridin-4-yl)methylene)-cyclohexane carbohydrazide (4l): Yellow solid; Yield: 66 %; m.p.: 122-123 °C; IR (KBr, ν_{max}, cm^{-1}): 3241.8, 2929.3, 1668.1, 1550.5; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.50 (*11.6, s, 1H), 8.64 (s, 2H), 8.30 (*8.17, s, 1H), 7.67 (d, *J* = 8.0 Hz, 2H), 7.30-7.28 (m, 4H), 3.42-3.40 (m, 1H), 2.54-2.51 (m, 1H), 1.27-1.25 (m, 4H), 1.82-1.80 (m. 4H); ESI-MS: *m/z*, 342.40.

(*E*)-*N*'-(Benzofuran-2-yl)methylene)-4-(4-chloro-phenyl)-cyclohexane carbohydrazide (4m): Dark yellow solid; Yield: 95 %; m.p.: 136-137 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.3 (*11.6, s, 1H), 8.10 (*8.30, s, 1H), 7.64-7.60 (m, 2H), 7.40-7.20 (m, 7H), 3.62-3.58 (m, 1H), 2.54-2.52 (m, 1H), 1.82-1.80 (m, 4H), 1.62-1.60 (m. 4H); ESI-MS: *m/z*, 381.50.

(*E*)-4-(4-Chlorophenyl)-*N*'-((5-methylfuran-2-yl)-methylene)cyclohexane carbohydrazide (4n): Brown solid; Yield: 86 %; m.p.: 130-132 °C; IR (KBr, ν_{max}, cm^{-1}): 3233.1, 2934.2, 1655.6, 1624.0, 1561.1, 1450.2, 1260.3, 1241.0; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.25 (*11.03, s,1H), 7.97 (*7.81, s, 1H), 7.36-7.23 (m, 5H), 6.76-6.73 (m, 1H), 6.24 (s, 1H), 3.66 (*3.01, m, 1H), 2.82-2.80 (m, 1H), 1.87-1.85 (m, 4H), 1.67-1.65 (m. 4H); ESI-MS: *m/z*, 345.4.

(*E*)-4-(4-Chlorophenyl)-*N*'-((5-nitrothiophen-3-yl)-methylene)cyclohexane carbohydrazide (4o): Yellow solid; Yield: 82 %; m.p.: 140-141 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.80 (*11.63, s,1H), 8.47 (*8.17, s, 1H), 8.14-8.12 (m, 1H), 7.55-7.52 (m, 1H), 7.36-7.27 (m, 4H), 3.31-3.29 (m, 1H), 2.52-2.50 (m, 1H), 1.86-1.85 (m, 4H), 1.55-1.52 (m. 4H); ESI-MS: *m/z*, 392.3.

(*E*)-4-(4-Chlorophenyl)-*N*'-((5-nitrothiophen-2-yl)-methylene)cyclohexane carbohydrazide (4p): Yellow solid; Yield: 85 %; m.p.: 145-146 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.53 (*11.30, s, 1H), 8.25 (d, *J* = 12.0 Hz, 2H), 8.21 (*7.99, s, 1H), 8.12 (m, 1H), 7.36-7.27 (m, 4H), 3.31-3.29 (m, 1H), 2.52-2.50 (m, 1H), 1.88-1.85 (m, 4H), 1.54-1.52 (m. 4H); ESI-MS: *m/z*, 392.3.

(*E*)-*N*'-((1*H*-Indole-2-yl)methylene)-4-(4-chlorophenyl)-cyclohexane carbohydrazide (4q): White solid; Yield: 79 %; m.p.: 138-139 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.54 (s, 1H), 11.06 (*10.89, s,1H), 8.37 (*8.25, s, 1H), 8.23 (s, 1H), 7.77 (s, 1H), 7.12-7.10 (m, 7H), 3.32-3.29 (*2.3, m, 1H), 2.60-2.57 (m, 1H), 1.92-1.89 (m, 4H), 1.46-1.44 (m. 4H); ESI-MS: *m/z*, 380.4.

(*E*)-*N*'-(4-Pyridin-2-yl)benzylidene)-4-(4-chlorophenyl)-cyclohexane carbohydrazide (4r): White solid; Yield: 75 %; m.p.: 128-129 °C; IR (KBr, ν_{max}, cm^{-1}): 3197.4, 2934.2, 2853.2, 1655.6, 1555.3, 1491.7, 1463.7; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.48 (*11.28, s,1H), 8.36 (s, 2H), 8.24 (*8.04, s, 1H), 7.83-7.77 (m, 5H), 7.34-7.24 (m, 4H), 7.14 (s, 1H), 3.24 (*2.31, m, 1H), 2.53-2.51 (m, 1H), 1.88-1.85 (m, 4H), 1.46-1.44 (m. 4H); ESI-MS: *m/z*, 417.93.

(*E*)-4-(4-Chlorophenyl)-*N*'-((5-phenyl-1*H*-imidazol-2-yl)methylene)cyclohexane carbohydrazide (4s): White solid; Yield: 76 %; m.p.: 144-145 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H), 11.47 (*11.27, s, 1H), 8.35 (*8.24, s, 1H), 8.04 (s, 1H), 7.14-7.18 (m, 9H), 3.60 (*2.31, m, 1H), 2.51 (m, 1H), 1.89 (m, 4H), 1.44 (m. 4H); ESI-MS: *m/z*, 407.20

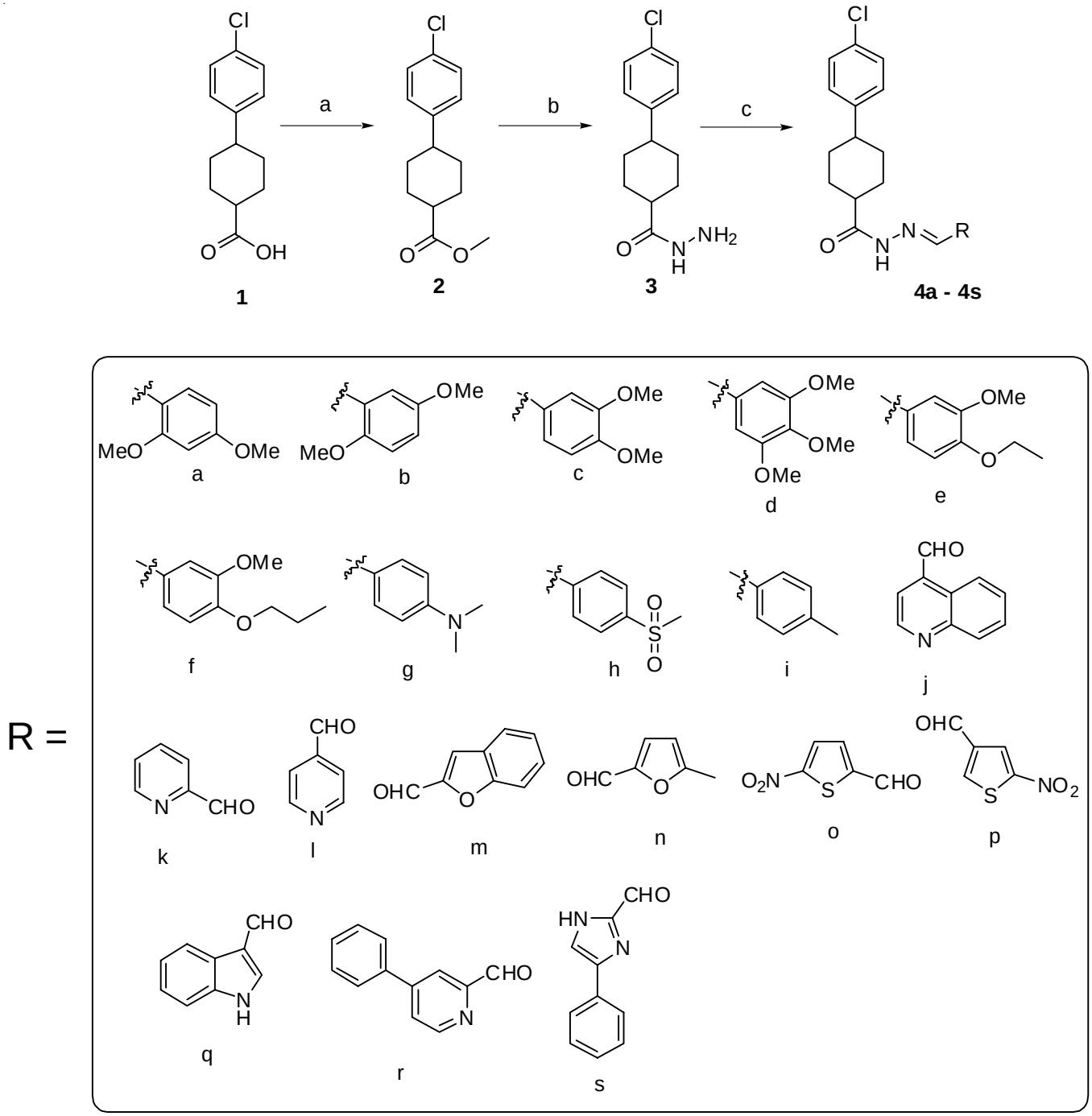
***in vitro* Antibacterial assay:** All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 × 10^8 cfu/mL[23]. The antibacterial activity of newly synthesized 4-(4-chlorophenyl)cyclohexane carbohydrazide derivatives was evaluated by agar well diffusion method[24,25]. Into the each petri plate, 20 mL of Mueller Hinton agar medium was poured and the agar plates were swabbed with 100 µL inocula of each test bacterium and kept for 15-20 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into seeded agar plates and these were loaded with a 100 µL volume with concentration of 4 mg/mL of each compound reconstituted in dimethyl sulphoxide (DMSO). All the plates were incubated at 37 °C for 24 h.

Antibacterial activity of the newly synthesized 4-(4-chlorophenyl)cyclohexanecarbohydrazide derivatives was evaluated by measuring the zone of growth inhibition against the test bacteria. DMSO was used as a negative control whereas ciprofloxacin was used as a positive control. The experiments were performed in triplicates. The antibacterial activity of the compounds was compared with ciprofloxacin as standard.

RESULTS AND DISCUSSION

A series of 4-(4-chlorophenyl)cyclohexane carboxylic acid related hydrazones **4a-s** are synthesized (**Scheme-I**) and the structures of the target compounds (**4a-s**) were confirmed by ^{1}H NMR, Mass and IR spectral data. The target compounds (**4a-s**) was obtained in three steps, the methyl ester **2** was prepared from corresponding carboxylic acid **1** in presence of catalytic quantity of concentrated sulphuric acid in methanol at reflux for 10 h and the reaction of methyl 4-(4-chlorophenyl)-cyclohexane carboxylate (**2**) with hydrazine hydrate in ethanol at reflux for 3 h afforded 4-(4-chlorophenyl)cyclohexane carbohydrazide (**3**). The target compounds **4a-s** were synthesized *via* the nucleophilic addition-elimination reaction of 4-(4-chlorophenyl)cyclohexane carbohydrazide (**3**) with various aryl and heteroaryl aldehydes.

The FT-IR spectra of target compounds (**4a-s**) showed absorption bands at 1692-1655 cm^{-1} due to the presence of C=O functional group, while the bands observed at 1563-1550 cm^{-1} corresponded to C=N linkage and the band appearing at 3245-3197 cm^{-1} represented -NH group. From the ^{1}H NMR spectra, the structures of the synthesized compounds (**4a-s**) were confirmed on the basis of the fact that the aldehydic proton (which was visible at δ 10.55) present in the corresponding aldehydes disappeared and a new singlet due to the azomethine (CH=N) group appeared at δ values between 7.86-8.52 ppm in all the compounds. Also, the protons of CONH and CH=N exhibited two separate signals in ^{1}H NMR spectra at 11, 11.80 and 7.86, 8.52 ppm, respectively due to the nitrogen inversion. Furthermore, all the synthesized hydrazones derivatives **4a-4s** were found to exist as a mixture of two rotameric forms in solution[26] *e.g*. *anti*-periplanar (*ap*) and *syn*-periplanar (*sp*) as indicated by their ^{1}H NMR spectra. Two sets of signals were observed for all groups in ^{1}H NMR spectra of each



Scheme-I: Synthesis of hydrazone derivatives of 4-(4-chlorophenyl)cyclohexane carboxylic acid (**4a-s**); Experimental conditions: (a) Conc; H_2SO_4, methanol, reflux, 10 h; (b) $NH_2NH_2 \cdot H_2O$, ethanol, reflux, 3 h; (c) Benzaldehydes (**a-s**), ethanol, reflux, 1 h

compound indicating the possibility of equilibrium and interconversion between rotamers (and/or configurationally isomers) in solution[26].

As a representative example, the ^{1}H NMR spectra of the compound **4a** is as follows, the broad singlets at 11.23 (*11.00 ppm) and 8.44 ppm (*8.23 ppm) corresponds to the protons representing to -CONH-N=C- -N=CH- groups, respectively. The phenyl ring protons appears at 6.6-7.75 ppm and the cyclohexy protons were observed at 1.38-1.84 ppm and all the other aliphatic protons were observed at expected regions. The ^{1}H NMR data for the remaining hydrazone derivatives in the series are in agreement with the assigned structures. The mass spectra of compounds showed (M+1) peaks and are in agreement with their molecular formula.

***in vitro* Antibacterial activity:** All the newly synthesized 4-(4-chlorophenyl)cyclohexane carbohydrazide derivatives (**4a-4s**) were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *S. pyogenes* (Gram-positive bacteria) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria). The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial growth surrounding the well in mm. The minimum inhibitory concentration (MIC) measurements were performed using a macrodilution tube method[27]. On the basis of MIC,

TABLE-1
ANTIBACTERIAL ACTIVITY OF INTERMEDIATES AND COMPOUNDS **4a-s**

Compound no.	Zone of inhibition in mm[b]			
	Gram-negative bacteria		Gram-positive bacteria	
	E. coli MTCC 443	*P. aeruginosa* MTCC 424	*S. aureus* MTCC 96	*S. pyogenes* MTCC 442
4a	13	13	15	14
4b	14	13	13	15
4c	15	13	16	13
4d	21	22	23	21
4e	17	15	14	14
4f	16	14	17	16
4g	22	23	23	21
4h	22	23	22	23
4i	17	16	17	15
4j	28	28	30	27
4k	26	26	29	28
4l	26	28	29	27
4m	23	22	25	24
4n	24	22	24	25
4o	23	24	23	24
4p	23	23	24	24
4r	26	27	28	27
4s	29	28	30	28
Ciprofloxacin[a]	25	25	27	26

[a]Concentration: 4 mg/mL^{-1} of DMSO; [b]Values, including diameter of the well (8 mm), are means of three replicates; [c]No activity

none of the compounds was found to have substantial antibacterial activity[28] .

The antibacterial activity data of the tested compounds **4a-s** is presented in Table-1. Interpretation of the results of the antibacterial activity data was done on the basis of zone of inhibition (measured in mm). Compounds **4j** (R = quinoline ring), **4k** (R = pyridine ring), **4l** (R = pyridine ring), **4q** (R = indole ring), **4r** (R = pyridine ring with phenyl substitution at 4th position), **4s** (R = imidazole ring) with zone of inhibition ranging between 26-30 mm, exhibited excellent antibacterial activity against all the tested bacterial strains when compared to the standard drug ciprofloxacin.

Compounds **4d** (R = -3,4,5-trimethoxy phenyl), **4g** (R = 4-*N*,*N*-dimethyl phenyl), **4h** (R = 4-methane sulphonyl phenyl), **4m** (R = benzo[b]furan ring), **4n** (2-methyl-furan ring), **4o** (R = 5-nitro-thiophene ring) and **4p** (2-nitro-thiophene ring) displayed good antibacterial activity (with zone of inhibition 21-25 mm), while the compounds **4a** (R = -2,4-dimethoxy phenyl), **4b** (R = -2,5-dimethoxy phenyl), **4c** (R = -3,4-dimethoxy phenyl), **4e** (R = -3-methoxy-4-ethoxy phenyl), **4f** (R = 2,4-dimethoxy phenyl) and **4i** (R = 4-methyl-phenyl) showed moderate antibacterial activity.

A careful observation from the above data reveals that the scaffold with R = nitrogen heterocyclic ring such as pyridine, quinoline, imidazole and indole showed significant antibacterial activity (excellent activity), whereas the hetrocyclic ring like benzo[b]furan, furan, thiophene moiety showed good antibacterial activity. Among the aromatic moieties, the phenyl ring with substitutions such as 3,4,5-trimethoxy, -4-*N*,*N*-dimethyl and 4-SO_2Me also showed good antibacterial activity.

Therefore it may be suggested that a suitable structural modification of the basic scaffold with an appropriate R substitution may pave path to the discovery of a lead compound which can be further transformed to a promising antibacterial agent.

Conclusion

The present paper describes the synthesis and antibacterial activity of novel hydrazones derivatives **4a-s** derived from 4-(4-chlorophenyl)cyclohexane carboxylic acid (**1**) as starting material. The structures of the synthesized compounds were confirmed by ^{1}H NMR, Mass and IR spectral data. The antibacterial activity of newly synthesized 4-(4-chlorophenyl)-cyclohexane carbohydrazide derivatives (**4a-s**) was evaluated by agar well diffusion method by measuring zone of inhibition. Compounds **4j** (R = quinoline ring), **4k** (R = pyridine ring), **4l** (R = pyridine ring), **4q** (R = indole ring), **4r** (R = pyridine ring with phenyl substitution at 4th position), **4s** (R = imidazole ring) with zone of inhibition ranging between 26-30 mm, exhibited excellent antibacterial activity against all the tested bacterial strains when compared to standard drug ciprofloxacin.

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