Synthesis, characterization and antimicrobial evaluation of (E)-4-(substitutedphenyl)-N'(1-phenylethylidene)cyclohexane carbohydrazide derivatives

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Encouraged by the various biological activities associated with hydrazone derivatives, the present paper describes the synthesis, characterization and antimicrobial evaluation of (E)-4-(substituted phenyl)-N'(1-phenylethylidene) cyclohexanecarbohydrazide derivatives 4a-k from commercially available 4-(4-chlorophenyl)cyclohexanecarboxylic acid. The hydrazide-hydrazone derivatives 4a-k have been synthesized via the nucleophilic addition-elimination reaction of substituted acetophenones a-k with 4-(4-chlorophenyl)cyclohexanecarbohydrazide 3. The structures of the synthesized compounds are confirmed by 1H NMR, IR and mass spectral data. All the synthesized 4-(4-chlorophenyl)cyclohexane carbohydrazide derivatives 4a-k have been evaluated for their in vitro antibacterial activity against *Staphylococcus aureus* and *Staphylococcus pyogenes* (gram positive bacteria) and *Escherichia coli* and *Pseudomonas aeruginosa* (gram negative bacteria). The antibacterial screening results reveal that compounds 4f-j exhibit good antibacterial activity with zone of inhibition 21-25 mm while the compounds 4a-c show moderate antibacterial activity with zone of inhibition 16-20 mm whereas the compounds 4d, 4e and 4k show nil activity.

Keywords: Antibacterial activity, synthesis, hydrazones, gram positive bacteria, atovaquone

Hydrazones bearing an azometine −NHN=CH− proton comprise an important class of compounds for new drug development. Therefore, many researchers have synthesized these compounds as target structures. These observations have been the guiding principle for the development of new hydrazones that possess varied biological activities viz., anticonvulsant1, anti-inflammatory2, antimalarial3, antibacterial4 and antituberculosis activities5, 6. Multi-drug resistance problem has increased since the last decade. Gradual increase in bacterial resistance towards penicillin and tetracycline has inspired many researchers to design new molecules possessing good antibacterial activity. Strains have an ability to resist current antimicrobial drugs, posing a threat to the human population7-9. With the aim of obtaining novel hydrazide-hyrazones with a wide spectrum of pharmaceutical applications, herein is reported the synthesis, characterization and antibacterial activity of a series of hydrazide-hyrazones 4a-k from commercially available 4-(4-chlorophenyl) cyclohexanecarboxylic acid. This starting material is utilized as one of the key raw materials in the preparation of Atovaquone10.

Results and Discussion

The synthesis of 4-(4-chlorophenyl)cyclohexane carbohydrazide-hydrazone derivatives 4a-k is presented in Scheme I and they were characterized by 1H NMR, mass and IR spectral data. The synthesis of compounds 4a-k was carried out utilizing the commercially available 4-(4-chlorophenyl) cyclohexanecarboxylic acid 1. Esterification of carboxylic acid 1 was done in presence of conc. sulphuric acid in methanol at reflux for 10 h. Reaction of methyl ester 2 with hydrazine-hydrate in ethanol at reflux for 3 h resulted in the formation of 4-(4-chlorophenyl)cyclohexanecarbohydrazide 3. The hydrazide-hydrazone derivatives 4a-k were synthesized via the nucleophilic addition-elimination reaction of substituted acetophenones a-k with 4-(4-chlorophenyl) cyclohexane carbohydrazide 3.

The structures of the synthesized compounds were confirmed by 1H NMR, IR and mass spectral data.
The $^1$H NMR spectrum of compound 4a-k in DMSO-$d_6$, recorded immediately after dissolution, indicated two sets of signals assignable to two stereoisomers (with different configurations with respect to the C=N bond ($Z$-$E$ isomerism) and C–N bond in the amide fragment (conformational $Z$–$E$ isomerism)$^{11-13}$.

Interpretation of $^1$H NMR spectra of ($E$)-N'-(1-(4-nitrophenyl)ethylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide 4f is as follows, the broad singlets at $\delta$ 10.70 (* 10.56) and $\delta$ 8.22 (* 8.02) correspond to the proton representing to –CO-NH–C-(CH$_3$)-R group. The doublet signals resonating at $\delta$ 8.28 and 8.26 correspond to the aromatic ring having 4-NO$_2$ substituent while the mutiplet resonating at $\delta$ 7.34-7.30 corresponds to the protons attached to the phenyl ring having chloro substituent. The multiplet signals at $\delta$ 3.28 (* 2.66), 2.58 (* 2.56), 1.98-1.86 and 1.62-1.46 corresponds to the cyclohexyl ring protons. The signal at $\delta$ 2.34 (* 2.31) with three proton integration corresponds to the methyl group. 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. The FT-IR spectra of target compounds 4a-k showed absorption bands at 1649 - 1670 cm$^{-1}$ due to the presence of C=O functional group while the absorption bands at 1528 - 1597 cm$^{-1}$ corresponds to C=N linkage and the band appearing at 3176 - 3283 cm$^{-1}$ corresponds to –NH group.

**Antibacterial activity**

The findings of the screening results of antibacterial activity of compounds 4a-k is tabulated in Table I. It is noteworthy to observe that compounds 4f-j exhibited good antibacterial activity with zone of inhibition 21-25 mm while the compounds 4a-c showed moderate antibacterial activity with zone of inhibition 16-20 mm whereas the compounds 4d, 4e
and 4k showed nil activity. In terms of structure-activity modification by varying R group in the main scaffold with substitution viz., −NO₂, −NH₂, −CN and −OH exhibited good antibacterial activity and the compounds with substitution R = H, −OMe and –OH exhibited good antibacterial activity and residual protonated solvent (1) intense peaks are reported. 1H NMR instrument and only diagnostic and/or intense peaks are reported. 1H NMR spectra were recorded in DMSO-d₆ with a Varian Mercury plus 400 MHz instrument. 13C NMR spectra were recorded in DMSO-d₆ with a Varian Gemini 100 MHz instrument. Signals due to the solvent (13C NMR) or residual protonated solvent (1H NMR) served as the internal standard. All the chemical shifts were reported in δ (ppm) using TMS as an internal standard. The 1H NMR chemical shifts and coupling constants were determined assuming first-order behavior. Multiplicity is indicated by one or more of the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad); the list of coupling constants (J) corresponds to the order of multiplicity assignment. Mass spectra were recorded with a PE Sciex model API 3000 instrument. All the reactions were carried out under argon atmosphere.

**Experimental Section**

Chemicals 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. 1H NMR (400 MHz, DMSO-d₆): δ 7.24 (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), 1.54-1.40 (m, 4H).

### Table I — Antibacterial activity of intermediates and compounds 4a-k

<table>
<thead>
<tr>
<th>Compd</th>
<th>Gram negative bacteria</th>
<th>Gram positive bacteria</th>
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<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>P. aeruginosa</em></td>
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<td></td>
<td>MTCC 443</td>
<td>MTCC 424</td>
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<td>Zone of inhibition in mm (^b)</td>
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<tr>
<td>4a</td>
<td>17</td>
<td>18</td>
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<tr>
<td>4b</td>
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<td>19</td>
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<tr>
<td>4c</td>
<td>16</td>
<td>17</td>
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<tr>
<td>4d</td>
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<td>−</td>
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<tr>
<td>4e</td>
<td>−</td>
<td>−</td>
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<tr>
<td>4f</td>
<td>22</td>
<td>23</td>
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<tr>
<td>4g</td>
<td>23</td>
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<tr>
<td>4j</td>
<td>22</td>
<td>24</td>
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<tr>
<td>4k</td>
<td>−</td>
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\(^a\) Concentration: 4 mg/mL\(^{-1}\) of DMSO; \(^b\) Values, including diameter of the well (8 mm), are means of three replicates; \(^*\) No activity.

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 ethylacetate (30 mL), washed with 10% aq. NaHCO₃ solution (2 × 10 mL) followed by water and brine solution. The organic layer was separated, dried over anhyd. Na₂SO₄, filtered and the solvent evaporated to afford compound 2. Yellow oily liquid. Yield 2.0 g, 94%. 1H NMR (400 MHz, DMSO-d₆): δ 7.24 (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), 1.54-1.40 (m, 4H).

To a solution of compound 2 (1.5 g, 5.95 mmol) in ethanol (15.0 mL) was added hydrazine hydrate (24 mmol) and heated to reflux for 3 h. After completion of the reaction, the reaction mass was concentrated under reduced pressure to obtain crude compound 3. The crude compound was slurried in n-hexane, filtered at the high vaccum pump and dried to obtain compound 3. White solid. Yield 1.35 g, 86%. m.p.121-22°C. 1H NMR (400 MHz, DMSO-d₆): 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-k

To a stirred solution of compound 3 (100 mg, 0.40 mmol) in ethanol was added corresponding acetophenones a-k (1.0 mmol) and refluxed for 3 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 anhyd. Na₂SO₄, filtered and concentrated under reduced pressure, to obtain the pure compounds. Yields of the products varied between 80 and 94%.
(E)-4-(4-Chlorophenyl)-N'-(1-phenylethylidene)cyclohexanecarbohydrazide, 4a: White solid. Yield 85%. m.p.112-13°C. IR (KBr): 3444, 3271, 3055, 3031, 2936, 2916, 2895, 2854, 1650, 1607, 1573, 1524, 1492, 1447, 1406, 1387, 1320, 1256, 1237, 1198, 1182, 1131, 1094, 1034, 1026, 1013, 982, 952, 915, 901, 826, 765, 716, 694, 618, 569, 528, 456 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.35 (brs, 1H), 7.77 (d, J = 6.0 Hz, 2H), 7.40-7.30 (m, 7H), 3.26 (* 2.58, m, 1H), 2.56 (* 2.51, m, 1H), 2.27 (* 2.25, s, 3H), 1.93-1.88 (m, 4H), 1.63-1.47 (m, 4H); ESI-MS: m/z 355.2 (M+H)⁺.

(E)-4-(4-Chlorophenyl)-N'-(1-(2-methoxyphenyl)ethylidene)cyclohexanecarbohydrazide, 4b: White solid. Yield 85%. m.p.120-21°C. IR (KBr): 3444, 3298, 3182, 3097, 3075, 3064, 3006, 2934, 2904, 2863, 2844, 2832, 2050, 1664, 1613, 1575, 1490, 1460, 1434, 1400, 1373, 1349, 1312, 1296, 1271, 1241, 1219, 1181, 1160, 1125, 1114, 1090, 1069, 1049, 1027, 1010, 969, 933, 782, 748, 696, 687, 672, 616, 579, 531, 509, 494, 454, 424, 414 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.22 (* 10.20, s, 1H), 7.39-7.24 (m, 4H), 7.06 (d, J = 6.0 Hz, 1H), 6.98 (dd, J = 6.0, 9.0 Hz, 1H), 3.81 (s, 3H), 3.16 (* 2.64, m, 1H), 2.56 (* 2.50, m, 1H), 2.17 (*2.15, s, 3H), 1.89-1.79 (m, 4H), 1.61-1.47 (m, 4H); ESI-MS: m/z 385.3 (M+H)⁺.

(E)-4-(4-Chlorophenyl)-N'-(1-(4-methoxyphenyl)ethylidene)cyclohexanecarbohydrazide, 4c: White solid. Yield 85%. m.p.128-29°C. IR (KBr): 3444, 3253, 3049, 3015, 2854, 2052, 1649, 1592, 1528, 1506, 1490, 1447, 1438, 1406, 1376, 1301, 1256, 1199, 1177, 1092, 1031, 1013, 982, 903, 850, 831, 816, 765, 748, 718, 684, 601, 569, 527 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.24 (brs, 1H), 7.73 (d, J = 3.0, 9.0 Hz, 2H), 7.37-7.27 (m, 4H), 6.98 (dd, J = 6.0, 9.0 Hz, 2H), 3.79 (s, 3H), 3.24 (* 2.58, m, 1H), 2.56 (* 2.51, m, 1H), 2.24 (* 2.21, s, 3H), 1.86-1.69 (m, 4H), 1.63-1.39 (m, 4H); ESI-MS: m/z 385.2 (M+H)⁺.

(E)-4-(4-Chlorophenyl)-N'-(1-(3-cyanophenyl)ethylidene)cyclohexanecarbohydrazide, 4d: White solid. Yield 80%. m.p.96-97°C. IR (KBr): 3445, 3283, 3029, 2933, 2858, 1655, 1611, 1595, 1525, 1507, 1491, 1446, 1404, 1394, 1374, 1349, 1319, 1305, 1256, 1236, 1215, 1197, 1178, 1166, 1093, 1036, 1012, 980, 955, 901, 844, 817, 790, 766, 747, 717, 683, 602, 568, 524, 507, 491, 478, 469, 460, 452 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.29 (brs, 1H), 7.68 (d, J = 3.0 Hz, 2H), 7.36-7.21 (m, 6H), 3.28 (* 2.56, m, 1H), 2.54 (* 2.50, m, 1H), 2.32 (s, 3H), 2.25 (* 2.22, s, 3H), 1.90-1.85 (m, 4H), 1.63-1.55 (m, 4H); ESI-MS: m/z 369.5 (M+H)⁺.

(E)-N'-(1-(4-Bromophenylethylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide, 4e: Pale yellow solid. Yield 82%. m.p.111-12°C. IR (KBr): 3444, 3271, 3048, 3029, 2934, 2917, 2858, 1655, 1599, 1586, 1523, 1485, 1447, 1394, 1315, 1257, 1236, 1215, 1195, 1178, 1118, 1091, 1074, 1040, 982, 955, 901, 837, 877, 740, 750, 715, 686, 618, 566, 526, 508, 484, 466 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.40 (* 10.39, s, 1H), 7.72 (d, J = 3.0, 9.0 Hz, 2H), 7.63 (dd, J = 3.0, 9.0 Hz, 2H), 7.37-7.22 (m, 4H), 3.34 (* 2.68, m, 1H), 2.57 (* 2.50, m, 1H), 2.26 (* 2.23, s, 3H), 1.91-1.84 (m, 4H), 1.63-1.53 (m, 4H); ESI-MS: m/z 433.1 (M+H)⁺.

(VENKATASATYANARAYANA et al): SYNTHESIS OF CARBOHYDRAZIDE DERIVATIVES 721
δ 10.54 (* 10.46, s, 1H), 8.13 (d, J = 6.0 Hz, 2H), 7.84 (d, J = 9.0 Hz, 2H), 7.64 (t, J = 6.0 Hz, 1H), 7.36-7.26 (m, 4H), 3.28 (* 3.24, m, 1H), 2.56 (* 2.52, m, 1H), 2.29 (* 2.25, s, 3H), 1.92-1.85 (m, 4H), 1.63-1.52 (m, 4H); ESI-MS: m/z 380.4 (M+H)⁺.

(E)-N'-(1-(3-Aminophenyl)ethylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide, 4i: Yellow solid. Yield 86%. m.p.132-33°C. IR (KBr): 3443, 3315, 3086, 2939, 2920, 2902, 2864, 1670, 1620, 1567, 1530, 1490, 1451, 1395, 1349, 1327, 1305, 1279, 1262, 1241, 1216, 1148, 1135, 1113, 1090, 1065, 1011, 903, 877, 850, 819, 803, 788, 780, 759, 737, 713, 687, 677, 656, 608, 571, 527, 501, 482, 455 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.21 (s, 1H), 7.33 (d, J = 9.0 Hz, 2H), 7.27 (d, J = 6.0 Hz, 2H), 7.02-6.87 (m, 3H), 6.58 (d, J = 6.0 Hz, 1H), 5.15 (brs, 2H), 3.23 (* 2.65, m, 1H), 2.56 (* 2.50, m, 1H), 2.19 (* 2.16, s, 3H), 1.85-1.78 (m, 4H), 1.63-1.56 (m, 4H); ESI-MS: m/z 370.2 (M+H)⁺.

(E)-N'- (1-(3-Nitrophenyl)ethylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide, 4j: Yellow solid. Yield 86%. m.p.134-36°C. IR (KBr): 3443, 3175, 3086, 2939, 2920, 2902, 2864, 1670, 1620, 1567, 1530, 1490, 1451, 1395, 1349, 1327, 1305, 1279, 1262, 1241, 1216, 1148, 1135, 1113, 1090, 1065, 1011, 903, 877, 850, 819, 803, 788, 780, 759, 737, 713, 687, 677, 656, 608, 571, 527, 501, 482, 455 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.65 (* 10.55, s, 1H), 8.58 (t, J = 3.0 Hz, 1H), 8.26-8.19 (m, 2H), 7.73 (t, J = 6.0 Hz, 1H), 7.37-7.27 (m, 4H), 3.29 (* 2.62, m, 1H), 2.55 (* 2.51, m, 1H), 2.36 (* 2.32, s, 3H), 1.97-1.90 (m, 4H), 1.65-1.51 (m, 4H); ESI-MS: m/z 400.1 (M+H)⁺.

(E)-N'- (1-(3-Bromophenyl)ethylidene)-4-(4-chlorophenyl)cyclohexanecarbohydrazide, 4k: Yellow solid. Yield 86%. m.p.108-109°C. IR (KBr): 3445, 3186, 3082, 2972, 2928, 2914, 2892, 2854, 1667, 1585, 1489, 1461, 1445, 1348, 1302, 1295, 1258, 1239, 1218, 1185, 1110, 1091, 1066, 1012, 969, 912, 900, 892, 820, 803, 776, 738, 711, 683, 607, 574, 510, 492, 484, 456 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 10.48 (* 10.44, s, 1H), 7.92 (d, J = 12.0 Hz, 1H), 7.77 (t, J = 6.0 Hz, 1H), 7.58 (d, J = 6.0 Hz, 1H), 7.41-6.27 (m, 5H), 3.28 (* 2.58, m, 1H), 2.56 (* 2.54, m, 1H), 2.27 (* 2.24, s, 3H), 1.93-1.87 (m, 4H), 1.63-1.56 (m, 4H); ESI-MS: m/z 435.2 (M+H)⁺.

In vitro antibacterial assay

All the synthesized 4-(4-chlorophenyl)cyclohexane carbohydrazide derivatives 4a-k were evaluated for their in vitro antibacterial activity against Staphylococcus aureus and Staphylococcus pyogenes (grampositive bacteria) and Escherichia coli and Pseudomonas aeruginosa (gram negative bacteria) by agar well diffusion method using ciprofloxacin as the reference drug. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 × 10⁸ cfu/mL. 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 4.0 mg/mL of each compound reconstituted in dimethylsulphoxide (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 triplicate. The antibacterial activity of the compounds was compared with ciprofloxacin as standard.

Conclusion

In conclusion, the hydrazide-hydrazone derivatives (4a-k) were synthesized via the nucleophile addition-elimination reaction of substituted acetophenones a-k with 4-(4-chlorophenyl) cyclohexane carbohydrazide 3. The structures of the synthesized compounds were confirmed by ¹H NMR, IR and mass spectral data. The antibacterial activity results indicated that by varying R group in the main scaffold with substitution viz., –NO₂, –NH₂, –CN and –OH exhibited good antibacterial activity and the compounds with substitution R = H, –OMe showed moderate activity while the compounds with substitution R = –CH₃ and Br showed nil activity. Based on the above screening results, it may be suggested that a further structural modification of R may lead to a promising antibacterial drug candidate.

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References