Synthesis, characterization and antibacterial activity of (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N’-(substitutedbenzylidene)benzohydrazide derivatives

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Prompted by the various biological and pharmaceutical importance of hydrazone derivatives, herein are reported the synthesis and antibacterial activity of some new benzohydrazide derivatives 6a-n from commercially available 4-hydroxybenzoic acid and 2-chloromethyl-4-methanesulfonyl-3-methylpyridine as starting materials. These compounds have been sufficiently characterized by \textsuperscript{1}H NMR, mass and IR spectroscopic techniques and evaluated for antibacterial activity. The compounds 6e (R=4-SO\textsubscript{2}CH\textsubscript{3}), 6f (R=4-F), 6g (R=4-OCF\textsubscript{3}), 6h (R=4-CF\textsubscript{3}), 6i (R=3,4-difluoro) and 6j (R=2,4 difluoro) have shown good activity (ZI: 19-25 mm) against the tested bacterial pathogens viz., Escheria coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes.

Keywords: Antibacterial activity, 4-hydroxybenzoic acid, synthesis

The increasing bacterial resistance causes a prevalent problem for the treatment of various infections. Therefore, the search for antimicrobials is a never ending continuous task. Hydrazones embedded with azomethine –NH-N=CH– proton represent an important class of compounds for a therapeutic drug development program in the branch of medicinal chemistry. Various medicinal chemists and researchers have synthesized these compounds as target structures and screened them for their biological activities. These compounds possess diverse biological and pharmacological properties such as cardio protective, antifungal, antiviral, antihelmintic, antimicrobial, anti-inflammatory, analgesic, anticancer, anti-platelet, antimalarial, anticonvulsant, anti-tubercular, antiproteozoa, anti-trypanosomal, anti-schistosomiasis, etc.\textsuperscript{1,3} The nitrogen atoms of the hydrazones are nucleophilic and the carbon atom has both electrophilic and nucleophilic nature\textsuperscript{4,5}. Due to their biological and pharmacological properties, they are considered important for the synthesis of heterocyclic compounds.\textsuperscript{6,7} In recent years, a number of hydrazone derivatives have been developed and evaluated for their antibacterial activity\textsuperscript{8,9}.

Dexlansoprazole is a proton pump inhibitor that is marketed by Takeda Pharmaceuticals for the treatment of erosive esophagitis and gastro-esophageal reflux disease. It lasts longer than lansoprazole, to which it is chemically related, and needs to be taken less often, making it possible to better control gastric acid\textsuperscript{10}. 2-chloromethyl-4-methanesulfonyl-3-methylpyridine\textsuperscript{11} (3, Scheme I) is the key intermediate that is utilized in the synthesis of Dexlansoprazole.

Prompted by the above biological significance of hydrazone derivatives, we have focused our attention towards the synthesis of (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N’-(substituted-benzylidene)benzohydrazide derivatives 6a-n utilizing 4-hydroxy benzoic acid and 2-chloromethyl-4-methanesulfonyl-3-methylpyridine (3, Scheme I) as starting materials. The newly synthesized hydrazone derivatives were evaluated for antibacterial activity studies towards the selected Gram positive and Gram negative bacteria.

Results and Discussion

The synthesis of (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N’-(substituted-benzylidene)benzohydrazide derivatives 6a-n is depicted in Scheme I. Esterification of 4-hydroxy benzoic acid was carried out at reflux temperature in presence of ethanol and catalytic quantity of conc. H\textsubscript{2}SO\textsubscript{4} to obtain corresponding ethyl ester in 75% yield. Condensation
of ethyl ester 2 with 2-chloromethyl-4-methanesulfonyl-3-methylpyridine 3 in presence of potassium carbonate in acetonitrile at 85°C for 1 h resulted in the methyl-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy) benzoate 4 in 90% yield. Hydrazinolysis of the benzoate 4 in presence of hydrazine hydrate in dioxane at 100°C for 5 h gave 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)benzohydrazide 5.

Condensation of benzohydrazide 5 with aromatic aldehydes a-n in ethanol at 80°C for 1 h afforded (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N'-(substituted-benzylidene)benzohydrazide derivatives 6a-n in 78-88% yield.

The newly synthesized hydrazones 6a-n were assessed by spectral characterization viz., 1H NMR, mass and IR. As a representative example the 1H NMR of (E)-4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)-N'-(3,4,5-trimethoxybenzylidene)benzohydrazide 6m is presented here. The proton signals at δ 11.74 and 8.37 as singlets with one proton integration corresponds to the groups –CONH- and –N-C=H- respectively. The peaks at δ 8.73 (doublet), 7.91-7.87 (multiplet), 7.20 (doublet) is assigned to the pyridine ring C and aromatic ring B protons. A proton signal at δ 7.01 is assigned to 3,4,5-trimethoxy phenyl ring. In the aliphatic region proton signals at δ 3.84 (s, 6H), 3.70 (s, 3H) corresponds to the methoxy group while the proton signals at δ 5.45 (s, 2H), 3.40 (s, 3H) and 2.71 (s, 3H) is assigned to –OCH3, –SO2CH3 and –CH3 groups. In the mass spectrum, compound 6m showed M+1 peaks with m/z 514 which is in agreement with their molecular formula. The IR spectra of 6m showed distinct stretching frequencies at 3444, 1654, 1573, 1415, 1326, 1177 cm⁻¹, corresponding to –NH, C=O, C=N, SO2, C-N and C-OC characteristic groups respectively. The above spectral elucidation thus confirms the structure of hydrazone derivative 6m. Similarly, the structural confirmation of the remaining hydrazone derivatives 6a-n was characterized on the basis of the above description.

Antibacterial Activity

The anti-bacterial activity of the tested compounds was determined by the zone of inhibition values. The results of the antibacterial activity of the synthesized hydrazone derivatives 6a-n is tabulated in Table I. From Table I it is observed that in case of E.coli and P.aeruginosa, compounds 6a (R = 4-OH, ZI: 18 and 14 mm), 6b (R = 4-NO2, ZI: 15 and 12 mm), 6k (R = 2,4-difluoro, ZI: 19 and 15 mm), 6l (R = 3,4-difluoro, ZI: 19 and 14 mm) showed good activity (ZI: 19-24 mm), while the compounds 6e (R = 4-OCF3, ZI: 22 and 18 mm), 6f (R = 4-F, ZI: 22 and 18 mm), 6g (R = 4-NO2, ZI: 15 and 12 mm), 6h (R = 4-OCF3, ZI: 20 and 16 mm), 6j (R = 3,4-difluoro, ZI: 19 and 15 mm), 6i (R = 3,4-difluoro, 19 and 14 mm) showed good activity (ZI: 19-24 mm), while the
remaining compounds 6c (R = NHCOCH₃), 6d (R = 4-CN) and 6n (R = 3-Br, 4-OH, 5-OMe) were inactive. In case of S. aureus and S. pyogenes, similar trends of bacterial activity were observed with a varied zone of inhibition values. Compounds 6e (R = 4-SO₂CH₃, ZI: 25 and 18 mm), 6f (R = 4-F, ZI: 20 and 16 mm), 6h (R = 4-CF₃, ZI: 20 and 16 mm), 6i (R = 3,4-difluoro, ZI: 22 and 15 mm), 6j (R = 2,4-difluoro) showed good activity (ZI: 19-25 mm), while compounds 6a (R = 4-OH, ZI: 17 and 12 mm), 6b (R = 4-NO₂, ZI: 16 and 11 mm), 6k (R = 3,4-dimethoxy, ZI: 15 and 10 mm), 6l (R = 3-OMe, 4-OH, ZI: 17 and 9 mm) and 6m (R = 3,4,5-OMe, ZI: 14 and 10 mm) displayed moderate activity (ZI: 15-18 mm) and remaining compounds in the series were inactive towards all the tested bacterial strains. In terms of structure-activity relationship, it may be generalized that compounds with fluorinated substituent (and –SO₂CH₃) exhibited excellent activity and those with hydroxyl and methoxy substituent in the main scaffold showed moderate activity. Hence, a further structural modification could result in a satisfactory lead compound which can be further explored to identify a good antibacterial drug candidate.

**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 pellets with a Perkin-Elmer Spectrum GX FTIR 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. Signals due to the 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 behaviour. 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.

**Anti-microbial activity assay**

The antimicrobial activities of the synthesized compounds were determined by agar well diffusion method. The compounds were screened for antibacterial activity against Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 424), Staphylococcus aureus (MTCC 96), and Staphylococcus pyogenes (MTCC 442). The antibiotic Norfloxacin (50 µg/mL) was used as reference drug for antibacterial activity. Dimethyl sulphoxide (1%, DMSO) was used a

<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>E. coli (MTCC 443)</td>
<td>P. aeruginosa (MTCC 424)</td>
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<td></td>
<td>Zone of inhibition in mm</td>
<td>Zone of inhibition in mm</td>
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<tr>
<td>6a (R = 4-OH)</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>6b (R = 4-NO₂)</td>
<td>15</td>
<td>12</td>
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<tr>
<td>6c (R = 4-NHCOCH₃)</td>
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<td>−</td>
</tr>
<tr>
<td>6d (R = 4-CN)</td>
<td>−</td>
<td>−</td>
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<tr>
<td>6e (4-SO₂CH₃)</td>
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<td>20</td>
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<tr>
<td>6f (R = 4-F)</td>
<td>22</td>
<td>18</td>
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<tr>
<td>6g (R = 4-OCF₃)</td>
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<tr>
<td>6j (R = 2,5-difluoro)</td>
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<tr>
<td>6k (R = 2,4-dimethoxy)</td>
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<tr>
<td>6l (R = 3-OMe, 4-OH)</td>
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<tr>
<td>6m (R = 3,4,5-OMe)</td>
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<td>13</td>
</tr>
<tr>
<td>6n (R = 3-Br, 4-OH, 5-OMe)</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>* Norfloxacin</td>
<td>26</td>
<td>21</td>
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</table>

* Standard Drug: Norfloxacin (50 µg/mL)
control without compound. The culture strains of bacteria were maintained on nutrient agar slants at 37±0.5°C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at a fixed concentration of 50 µg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5°C for 24 h. Zone of inhibition of compounds were measured in mm. The MIC (minimum inhibitory concentration) of the most active compounds toward the microorganism showed that the MICs ranged between 20 and 30 µg/mL. The activity was tested at concentration of 50 µg/mL.

**Synthesis of ethyl 4-hydroxybenzoate, 2**

A mixture of compound 2 (3 g, 21.74 mmol) sulphuric acid (0.1 mL) and ethanol (30 mL) was refluxed for 10 h. The completion of the reaction was monitored by TLC, and ethanol was evaporated under reduced pressure and diluted with ethyl acetate (30 mL), washed with 10% aq. NaHCO₃ solution (3×15 mL) followed by water and brine solution. The organic layer was separated, dried over anhyd. Na₂SO₄, filtered and concentrated to afford compound 3. Pale yellow solid. Yield 1.7 g, 75%. m.p.114-117°C.

**Synthesis of methyl 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl)methoxy)benzoate, 4**

A mixture of ester 3 (1.5 g, 9.02 mmol) and potassium carbonate (1.5 g, 10.87 mmol) in acetonitrile (7.5 mL) was stirred at RT and compound 3 (2.2 g, 10.01 mmol) added and heated to 85°C for 1 h. To the cooled reaction mixture was added water (25 mL) and the precipitated solids were filtered and dried to obtain compound 4. White solid. Yield 2.8 g, 90%. m.p. 124-25°C. IR (KBr): 1667 (C=O), 1582 (C=N), 1414 (C-N), 1339 (C-O-C) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 9.59 (s, 1H), 8.72 (d, J = 4.5 Hz, 1H), 7.85 (d, J = 5.1 Hz, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 5.40 (s, 2H), 4.40 (s, 2H), 3.36 (s, 3H), 2.69 (s, 3H); ESI-MS: m/z 336.0 [M+1].

**General procedure for the synthesis of hydrazone derivatives, 6a-n**

To a stirred solution of compound 5 (100 mg, 0.3 mmol) in ethanol was added corresponding aromatic aldehydes a-n (0.3 mmol) and heated to 80°C for 1 h. The precipitated solid was diluted with ethanol and filtered at the pump to afford hydrazones derivatives 6a-n in quantitative yields.

**Synthesis of methyl 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N'-(4-hydroxybenzylidene) benzohydrazide, 6a**

White solid. Yield 80%. m.p.98-99°C. IR (KBr): 3329 (N-H), 1667 (C=O), 1582 (C=N), 1414 (C-N), 1339 (C-O-C) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 11.45 (s, 1H), 9.80 (s, 1H), 8.72 (s, 1H), 7.92 (d, J = 8.6 Hz, 2H), 7.84 (d, J = 5.2 Hz, 1H), 7.54 (d, J = 8.1 Hz, 2H), 7.17 (d, J = 8.7 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 5.44 (s, 2H), 3.37 (s, 2H), 2.71 (s, 3H); ESI-MS: m/z 440.0 [M+1].

**Synthesis of methyl 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N'-(4-nitrobenzylidene) benzohydrazide, 6b**

White solid. Yield 82%. m.p.97-98°C. IR (KBr): 1668 (C=O), 1557 (C=N), 1421 (SO₂), 1349 (C-N) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 11.45 (s, 1H), 9.80 (s, 1H), 8.72 (d, J = 5.1 Hz, 1H), 8.53 (s, 1H), 8.31 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 7.5 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 4.8 Hz, 2H), 7.20 (d, J = 8.7 Hz, 2H), 5.46 (s, 2H), 3.38 (s, 3H), 2.71 (s, 3H); ESI-MS: m/z 469.0 [M+1].

**Synthesis of methyl 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N'-(4-acetamidobenzylidene) benzohydrazide, 6c**

Off white solid. Yield 88%. m.p.78-79°C. IR (KBr): 3329 (N-H), 1667 (C=O), 1582 (C=N), 1414 (SO₂), 1339 (C-N), 1178 (C-O-C) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 12.00 (s, 1H), 8.72 (d, J = 5.1 Hz, 1H), 8.53 (s, 1H), 8.31 (d, J = 8.4 Hz, 2H), 7.98 (d, J = 7.5 Hz, 1H), 7.93 (d, J = 8.4 Hz, 2H), 7.86 (d, J = 4.8 Hz, 2H), 7.20 (d, J = 8.7 Hz, 2H), 5.46 (s, 2H), 3.38 (s, 3H), 2.71 (s, 3H); ESI-MS: m/z 481.0 [M+1].

**Synthesis of methyl 4-((3-methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N'-(4-cyanobenzylidene) benzohydrazide, 6d**

White solid. Yield 82%. m.p.97-98°C. IR (KBr): 3418 (NH), 2222 (CN), 1650 (C=O), 1556 (C=N), 1407 (SO₂), 1353 (C-N), 1175 (C-OC) cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆): δ 12.00 (s, 1H), 8.73 (d, J = 5.1 Hz, 1H), 8.48 (brs, 1H), 7.93-7.91 (m, 5H),
7.80 (d, J = 5.1 Hz, 2H), 7.20 (d, J = 8.7 Hz, 2H), 5.46 (s, 2H), 3.38 (s, 3H), 2.71 (s, 3H); ESI-MS: m/z 449.0 [M+1].

(E)-4-((3-Methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N’-(4-nitrobenzylidene) benzohydrazide, 6i: White solid. Yield 80%. m.p. 82-83°C. 1H NMR (300 MHz, DMSO-d6): δ 11.86 (s, 1H), 8.73 (d, J = 5.1 Hz, 1H), 8.34 (brs, 1H), 7.99-7.92 (m, 4H), 7.68-7.58 (m, 2H), 7.20 (d, J = 8.6 Hz, 2H), 5.48 (s, 2H), 3.40 (s, 3H), 2.71 (s, 3H); ESI-MS: m/z 460.0 [M+1].

(E)-4-((3-Methyl-4-(methylsulfonyl)pyridin-2-yl) methoxy)-N’-(2,4-difluorobenzylidene)benzohydrazide, 6j: White solid. Yield 88%. m.p. 122-24°C. 1H NMR (300 MHz, DMSO-d6): δ 11.70 (s, 1H), 8.73 (d, J = 5.1 Hz, 1H), 8.34 (brs, 1H), 7.99-7.92 (m, 4H), 7.68-7.58 (m, 2H), 7.20 (d, J = 8.6 Hz, 2H), 5.48 (s, 2H), 3.40 (s, 3H), 2.71 (s, 3H); ESI-MS: m/z 548.0 [M+1].

Conclusion

Synthesis of some new hydrazone derivatives 6a-n is described and their structures are confirmed by 1H NMR, mass and IR spectral data. These compounds have been evaluated for antibacterial activity against Escheria coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyogenes bacterial strains with reference to the standard drug, Norfloxacin at a concentration of 50 µg/mL. Compounds 6e, 6f, 6i and 6j showed good antibacterial activity and compounds 6a, 6b, 6k, 6l and 6n showed moderate activity while the remaining compounds were inactive.
References