Research Article

Synthesis, Characterization, and Antimicrobial Activity of Methyl-2-aminopyridine-4-carboxylate Derivatives

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A series of methyl-2-aminopyridine-4-carboxylate derivatives, 3a–f, were synthesized in order to determine their in vitro antimicrobial activity. The chemical structures of the synthesized compounds were confirmed by elemental analyses, FT-IR, and 1H NMR spectral studies. Among the synthesized compounds, 3c and 3d showed good antimicrobial activity compared to other compounds in the series.

1. Introduction

The compounds containing an azomethine group (–CH=N–) are important in elucidating the mechanism of transamination and racemisation reactions in biological systems [1, 2]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviors have been studied [3]. They have been synthesized from a variety of compounds, such as amino thiazoles, 2-hydroxy-1-napthalaniline, amino sugars, aromatic aldehydes, ketones, isatin, triazole ring, thiosemicarbazides, amino acids, and pyrazolone [4, 5]. Antibacterial, antifungal, antitumor, and anticancer activities of Schiff bases have been reported [6, 7] and they are active against a wide range of organisms. Antibacterial activity has been studied more than antifungal activity, because bacteria can achieve resistance to antibiotics through biochemical and morphological modifications [8, 9]. Some Schiff bases bearing aryl groups or heterocyclic residues possess excellent biological activities have attracted the attention of many researchers in recent years [10–12]. The Schiff bases formed from aromatic aldehydes or aromatic ketones and their derivatives are quite stable. Due to the great flexibility and diverse structural aspects of Schiff bases, a wide range of these compounds have been synthesized and their activities have been studied [13, 14]. Antimicrobial and antifungal activities of various Schiff bases have also been reported [15]. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds [16].

In connection with such studies, the present paper reporting for the first time on the synthesis of methyl-2-aminopyridine-4-carboxylate derivatives, 3a–f, which are formed during the reaction of methyl-2-aminopyridine-4-carboxylate (1) with different aldehydes (2a–f). These synthesized compounds were characterized by elemental analyses, FT-IR, and 1H NMR spectral studies. Antibacterial and antifungal activities were also reported in this paper.

2. Experimental

All chemicals used were of the analytical reagent grade (AR). All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. Elemental analyses were recorded on Perkin Elmer 2400 Elemental Analyser. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer. 1H NMR spectra were recorded on Bruker DRX—400 MHz spectrometer and TMS as an internal standard.

2.1. General Procedure for the Synthesis of Methyl-2-aminopyridine-4-carboxylate Derivatives, 3a–f. Equimolar concentrations of different aldehydes (0.01 mol) and methyl-2-aminopyridine-4-carboxylate (0.01 mol) dissolved
in minimum volume of ethanol, and then 2-3 drops of concentrated sulfuric acid are added to the reaction mixture. The solution was stirred for 4-5 hr at room temperature and the progress of the reaction was followed by TLC until the reaction was complete. It was cooled to 0°C, the precipitate was filtered, washed with ethanol, and recrystallized from methanol. Methyl-2-aminopyridine-4-carboxylate derivatives (3a–f) were synthesized by the method summarized in Scheme 1. The chemical structures and physical data of all the synthesized compounds are tabulated in Table 1.

2.2. Methyl-2-ethylideneaminopyridine-4-carboxylate (3a).

The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and acetaldehyde (3a) (0.44 g, 0.01 mol). $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 9.09 (d, 1H, Ar-H, $J = 4.7$ Hz), 8.00 (d, 1H, Ar-H, $J = 4.2$ Hz), 7.90 (s, 1H, Ar-H), 7.46 (q, 1H, CH, $J = 8.1$ Hz), 3.82 (s, 3H, OCH$_3$), 1.13 (d, 3H, CH$_3$, $J = 3.3$ Hz); FT-IR (KBr, cm$^{-1}$) ν: 1725 (C=O), 1639 (C=N), 1589 (C=C), 1213 (C-O), 1146 (C-N); Anal. calcld for C$_9$H$_{10}$N$_2$O$_2$ (in %): C 60.66, H 5.66, N 15.72; found C 60.59, H 5.70, N 15.91.

2.3. Methyl-2-benzylideneaminopyridine-4-carboxylate (3b).

The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and benzaldehyde (3b) (1.06 g, 0.01 mol). $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 9.10 (d, 1H, Ar-H, $J = 4.5$ Hz), 8.12 (s, 1H, CH), 7.85 (d, 1H, Ar-H, $J = 4.0$ Hz), 7.68 (s, 1H, Ar-H), 7.34 (d, 2H, Ar-H, $J = 5.1$ Hz), 7.00 (m, 3H, Ar-H, $J = 11.0$ Hz), 3.82 (s, 3H, OCH$_3$); FT-IR (KBr, cm$^{-1}$) ν: 3057 (Ar-H), 1725 (C=O), 1636 (C=N), 1591 (C=C), 1213 (C-O), 1146 (C-N); Anal. calcld for C$_{14}$H$_{12}$N$_2$O$_2$ (in %): C 69.99, H 5.03, N 11.66; found C 69.79, H 5.21, N 11.51.

2.4. Methyl-2-(3-hydroxy-4-methoxy-benzylidene) aminopyridine-4-carboxylate (3c).

The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and 3-hydroxy-4-methoxy-benzaldehyde (3c) (1.53 g, 0.01 mol). $^1$H NMR (DMSO-d$_6$, 400 MHz) δ: 9.09 (d, 1H, Ar-H, $J = 4.7$ Hz), 8.00 (d, 1H, Ar-H, $J = 4.2$ Hz), 7.90 (s, 1H, Ar-H), 7.46 (q, 1H, CH, $J = 8.1$ Hz), 3.82 (s, 3H, OCH$_3$), 1.13 (d, 3H, CH$_3$, $J = 3.3$ Hz); FT-IR (KBr, cm$^{-1}$) ν: 1725 (C=O), 1639 (C=N), 1589 (C=C), 1213 (C-O), 1146 (C-N); Anal. calcld for C$_{14}$H$_{12}$N$_2$O$_2$ (in %): C 69.99, H 5.03, N 11.66; found C 69.79, H 5.21, N 11.51.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Yield (%)</th>
<th>m. p. (°C)</th>
</tr>
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<tbody>
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<td>CH$_3$</td>
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<td>101–103</td>
</tr>
<tr>
<td>3b</td>
<td></td>
<td>85</td>
<td>113–114</td>
</tr>
<tr>
<td>3c</td>
<td></td>
<td>78</td>
<td>119–121</td>
</tr>
<tr>
<td>3d</td>
<td></td>
<td>77</td>
<td>118–120</td>
</tr>
<tr>
<td>3e</td>
<td></td>
<td>75</td>
<td>128–130</td>
</tr>
<tr>
<td>3f</td>
<td></td>
<td>82</td>
<td>131–133</td>
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</tbody>
</table>
2.5. Methyl-2-(1H-Indol-3-ylmethylene) aminopyridine-4-carboxylate (3d). The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and 1H-indole-3-carbaldehyde (2d) (1.45 g, 0.01 mol). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\): 9.11 (d, 1H, Ar-H, \(J = 4.1\) Hz), 9.12 (d, 1H, Ar-H, \(J = 4.1\) Hz), 9.78 (d, 1H, Ar-H, \(J = 3.9\) Hz), 7.88 (s, 1H, Ar-H), 7.52-7.45 (d, 2H, Ar-H, \(J = 5.7\) Hz), 7.18 (s, 1H, CH), 6.70-6.53 (d, 2H, Ar-H, \(J = 6.8\) Hz), 3.85 (q, 2H, CH\(_2\)), FT-IR (KBr, \(\text{cm}^{-1}\)) \(\nu\): 3477 (N-H), 3060 (Ar-H), 1725 (C=O), 1695 (C=N), 1468 (O-H), 3057 (Ar-H), 1725 (C=O), 1636 (C=N), 1590 (C=C), 1213 (C-O); Anal. calcd for C\(_{16}\)H\(_{14}\)N\(_2\)O\(_3\) (in %): C 67.59, H 5.67, N 9.85; found C 67.61, H 5.57, N 9.78.

2.6. Methyl-2-(4-ethoxybenzylidene)aminopyridine-4-carboxylate (3e). The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and 4-ethoxybenzaldehyde (2e) (1.51 g, 0.01 mol). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\): 9.12 (d, 1H, Ar-H, \(J = 4.1\) Hz), 7.98 (d, 1H, Ar-H, \(J = 5.7\) Hz), 7.88 (s, 1H, Ar-H), 7.52-7.45 (d, 2H, Ar-H, \(J = 5.7\) Hz), 7.18 (s, 1H, CH), 6.70-6.53 (d, 2H, Ar-H, \(J = 6.8\) Hz), 3.85 (q, 2H, CH\(_2\)), FT-IR (KBr, \(\text{cm}^{-1}\)) \(\nu\): 3477 (N-H), 3060 (Ar-H), 1725 (C=O), 1636 (C=N), 1590 (C=C), 1213 (C-O), 1146 (C-N); Anal. calcd for C\(_{16}\)H\(_{14}\)N\(_2\)O\(_3\) (in %): C 65.49, H 5.13, N 9.82; found C 65.47, H 5.15, N 9.84.

2.7. Methyl-2-(2-pyridine-ylmethylene) aminopyridine-4-carboxylate (3f). The product obtained from methyl-2-aminopyridine-4-carboxylate (1) (1.53 g, 0.01 mol) and 2-pyridine carboxaldehyde (1.53 g, 0.01 mol). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz) \(\delta\): 9.11 (d, 1H, Ar-H, \(J = 4.0\) Hz), 8.12 (d, 1H, Ar-H, \(J = 3.7\) Hz), 8.00 (d, 1H, Ar-H, \(J = 5.6\) Hz), 7.90 (d, 1H, Ar-H, \(J = 8.3\) Hz), 7.80 (d, 1H, Ar-H, \(J = 5.2\) Hz), 7.55 (s, 1H, CH), 7.42 (t, 1H, Ar-H, \(J = 3.0\) Hz), 7.30 (t, 1H, Ar-H, \(J = 3.1\) Hz), 3.82 (s, 3H, OCH\(_3\)), FT-IR (KBr, \(\text{cm}^{-1}\)) \(\nu\): 3477 (N-H), 3060 (Ar-H), 1725 (C=O), 1639 (C=N), 1589 (C=C), 1213 (C-O), 1146 (C-N); Anal. calcd for C\(_{13}\)H\(_{14}\)N\(_2\)O\(_3\) (in %): C 64.72, H 4.60, N 17.42; found C 64.81, H 4.57, N 17.21.

2.8. In Vitro Antifungal Activity. The antifungal activity of the synthesized compounds was determined against the test fungi by disc diffusion method on potato dextrose agar medium with 15 mL of PDA was poured into each petri plate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the center of the petriplates and incubated at 26 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Activity of compounds was compared with standard drug nystatin. All the synthesized compounds were tested (at the dosage of 500 \(\mu\)g/mL) by poisoned food technique.

3. Results and Discussion

3.1. Schiff Base Characterization. Schiff bases of methyl-2-aminopyridine-4-carboxylate derivatives, 3a-f, were synthesized from the reaction of methyl-2-aminopyridine-4-carboxylate with different aldehydes. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within the limits of permissible error. The absorptions around 3000 cm\(^{-1}\) in synthesized compounds confirm the aromatic stretching vibrations, and the appearance of a medium to strong absorption bands above 1600 cm\(^{-1}\) due to a stretching vibration of the azomethine (C=N) bond formation in synthesized compounds via condensation. The proton spectral data agreed with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of methyl-2-aminopyridine-4-carboxylate was evaluated and compared with streptomycin as standard drug. In compounds 3a-f, the above resonance disappeared and additional resonances assigned to the \(-\text{NH}_2\) were observed, which confirmed the condensation between the amino group and carbonyl group. The \(^1\)H NMR spectra of 3c and 3d are shown in Figures 1 and 2, respectively.

3.2. Antibacterial Activity. The antibacterial activity of compounds 3a-f was evaluated and compared with streptomycin as standard drug. Compounds 3c and 3d showed good antibacterial properties against four pathogenic bacterial strains compared with other compounds. Compound 3c exhibits inhibition in the range of 26 mm against Bacillus subtilis. Compound 3d exhibits inhibition in the range of 25 mm against B. subtilis. Compounds 3e and 3f showed moderate...
T<sub>able 2</sub>: <i>In Vitro</i> antibacterial and antifungal activities of compounds 3a–f.

<table>
<thead>
<tr>
<th>Compound</th>
<th>B. subtilis</th>
<th>S. aureus</th>
<th>X. campestris</th>
<th>E. coli</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>21</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>10</td>
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<td>3b</td>
<td>22</td>
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<td>13</td>
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</tr>
<tr>
<td>3c</td>
<td>26</td>
<td>15</td>
<td>24</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>3d</td>
<td>25</td>
<td>14</td>
<td>23</td>
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<td>15</td>
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</tr>
<tr>
<td>3f</td>
<td>23</td>
<td>13</td>
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<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>26</td>
<td>18</td>
<td>25</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Nystatin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>20</td>
</tr>
</tbody>
</table>
antibacterial activity against four pathogenic bacterial strains. Compounds 3a and 3b showed weak antibacterial activity against S. aureus and X. campestris.

3.3. Antifungal Activity. All the synthesized compounds 3a–f were also tested against C. albicans. The compounds 3c and 3d showed good antifungal activity than other compounds in the series against tested strain. Compound 3c showed inhibition in the range of 16 mm when compared with other compounds in the series against C. albicans. From the results, it is evident that compounds 3a and 3b are moderately active. The antimicrobial activity results of synthesized compounds were compared with standard drugs as depicted in Table 2. Compounds 3a–f showed antimicrobial activity in the order of 3c > 3d > 3f > 3e > 3b > 3a against tested bacterial and fungal strains.

Initial structure activity relationship can be drawn for the compounds 3a–f. In the present study, different electron withdrawing and electron donating groups attached to aromatic ring as substituent were linkage to azomethine group. The close survey of antimicrobial efficacy indicated that the inhibition values of all the compounds exhibited a varied rang (11–26 mm and 10–16 mm) of antibacterial and antifungal activities against all the tested microbial strains. The electron donating hydroxyl group and methoxy group of phenyl ring in 3c produces enhanced activity. The electron withdrawing pyridine ring in 3f and electron donating ethoxy group in 3e showed moderate activity, whereas the indole in 3d produces good antimicrobial activity. The compound 3a is weakly active and this may be due to the absence of phenyl ring. However, 3b showed better activity compared to 3a probably due to the presence of benzene in 3b. The above SAR correlation studies reveal that the nature of the linkage (substituent on aromatic ring) influences the antimicrobial activity.

4. Conclusion

In conclusion, in a series of methyl-2-aminopyridine-4-carboxylated derivatives, 3a–f were synthesized in good yield, characterized by different spectral studies, and their antimicrobial activities were determined against clinically important pathogens. Compounds 3c and 3d demonstrated good inhibition against bacterial and fungal strains tested. The SAR studies revealed that the substituents on aromatic ring are responsible for the antimicrobial activity of these classes of agents. On the basis of their activity, these derivatives were identified as viable leads for further studies.

References


