Synthetic approaches towards 3-pyridinecarbonitrile derivatives and their antimicrobial properties

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A variety of 2-alkoxy-4,6-diaryl-3-pyridinecarbonitriles 2 have been prepared via either the reaction of 1,3-diaryl-2-propen-1-ones 1 with malononitrile or the reaction of ylidenemalononitriles 6 with the proper aryl methyl ketone 7 in the appropriate alcohol in the presence of sodium. On the other hand, conducting the reaction in refluxing ethanol in the presence of ammonium acetate affords, the corresponding 2-amino-3-pyridinecarbonitriles 8. The antimicrobial properties against Gram-positive, Gram-negative, acid-fast bacteria and yeast have been screened. The prepared propenones 1a-d show considerable activity against the tested yeast as well as most of the Gram-positive and Gram-negative bacteria. A few derivatives of the prepared pyridines 2c,f,h; 8b exhibit mild antimicrobial activity.

Much attention was directed towards the synthesis of 3-pyridinecarbonitrile derivatives due to their unique properties and hence their ability for application in various fields such as antiviral,1,3 antibacterial,4,10 antifungal,8-11 herbicidal12-15 and arthropodicidal,16 in addition to antitumour,17-19 antinflammatory,20,21 as well as analgesic and antihypertensive22-24 activity. In the present work, it is intended to investigate the synthesis of a variety of 3-pyridinecarbonitriles substituted with a fluorophenyl function. This is due to the unique properties of containing compounds such as lipophilicity and high thermal stability.25 The antimicrobial properties of the prepared compounds against Gram-positive, Gram-negative, acid-fast bacteria and yeast were also screened.

Reaction of 1,3-diaryl-2-propen-1-ones 1a-d with malononitrile in the appropriate alcohol in the presence of sodium, afforded colourless products. The structures of which were established to be either 2-alkoxy-4,6-diaryl-3-pyridinecarbonitriles 2 or their isomeric forms 3 (where $R_1 \neq R_2$) based on spectroscopic and elemental analyses data. The IR spectra reveal the absence of any band assignable for the carbonyl group and exhibit a strong stretching vibration nitrile band at 2225-2209 cm$^{-1}$. The $^1$H NMR spectra show the alkoxide residue confirming the involvement of either methoxide (singlet at $\delta$ 4.14-4.19) or ethoxide (triplet at $\delta$ 1.43-1.52, quartet at $\delta$ 4.54-4.64) functions derived from the corresponding alcohol used as a solvent in the reaction.

The reaction was assumed to take place via active methylene malononitrile Michael addition to the $\beta$-carbon of unsaturated system of 1 affording the Michael adduct intermediate 4. Alkoxide nucleophilic attacks at one of the nitrile groups of 4 followed by dehydration and subsequent dehydrogenation gave finally the corresponding pyridine 2. On the other hand, pyridine 3 presumably, formed via Knoevenagel condensation of active methylene malononitrile with the ketonic residue of 1 giving rise to the intermediate 5. The latter due to alkoxide nucleophilic attack at one of the nitrile groups followed by dehydrogenation afforded eventually 3.

The isolated products were established to be 2 rather than 3 based on their independent synthesis through the reaction of ylidenemalononitriles 6a-c with the appropriate aryl methyl ketone 7 under the same experimental reaction conditions. In other words, the reaction obeys Michael addition pathway rather than Knoevenagel condensation route (Scheme I).

Similarly, reaction of 2-propen-1-ones 1a,b with malononitrile in refluxing ethanol in the presence of ammonium acetate afforded 2-amino-4,6-diaryl-3-pyridinecarbonitriles 8 rather than the isomeric forms 9. This phenomenon was also substantiated via independent synthesis of 8 through the reaction of the appropriate ylidenemalononitriles 6a,b with the proper ketone 7 under similar reaction conditions (Scheme II).

Antimicrobial activity
Antimicrobial susceptibility of the novel synthesized 4,6-diaryl-3-pyridinecarbonitriles 2a-h, 8a,b as well as the starting propenones 1a-d was determined by the agar dilution technique.26 The tested compounds were dissolved in dimethyl sulfoxide(DMSO). An inoculum of about $10^5$ colony forming unit (CFU)
per spot was applied to the surfaces of Mueller Hinton agar plates containing graded concentrations of the respective compound; plates were incubated at 37°C for 18 hr. The spot with the lowest concentration of compound showing no growth was defined as the minimum inhibitory concentration (MIC). Most organisms used in this study were clinical isolates recovered from patients of Kasr El Eini Hospital, Cairo, Egypt, from October to December 1999. Bacillus subtilis, Mycobacterium phlei and Saccharomyces cerevisae were from the stock culture of the department. The organisms included representatives of Gram-positive bacteria (Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Sarcina lutea and Bacillus subtilis), Gram-negative bacteria
(Escherichia coli, Klebsiella oxytoca, Enterobacter cloacae, Proteus mirabilis and Pseudomonas aeruginosa), acid-fast bacteria (Mycobacterium phlei) and yeast (Saccharomyces cerevisiae, Candida albicans and Candida tropicalis). The MIC of ciprofloxacin, and amphotericin B were determined concurrently as reference for antibacterial and antifungal activities, respectively (Table I). Control DMSO was carried out with each experiment.

From the results obtained (c.f. Table I), it is obvious that the starting propenones la-d show considerable activity against the tested yeast and most of them 1b-d show activity against acid-fast bacteria. In addition, 1d exhibits recognizable activity against most of the tested Gram-positive as well as Gram-negative bacteria. Meanwhile, none of the synthesized 2-alkoxy-3-pyridinecarbonitriles exhibits antibacterial activity at concentrations up to 400 μg/mL, except 2c (MIC = 200 μg/mL against Staphylococcus saprophyticus), 2f (MIC = 100 μg/mL against Mycobacterium phlei) and 2h (MIC = 100 μg/mL against Staphylococcus saprophyticus and Staphylococcus epidermidis). Only 2h shows mild activity against the tested yeast. Slight activity was also observed by
2-amino-3-pyridinecarbonitrile derivative 8b against the Gram-positive bacteria (MIC = 400 μg/mL towards Staphylococcus saprophyticus and Staphylococcus epidermidis).

**Experimental Section**

Melting points are uncorrected. IR spectra were recorded (KBr) on a Perkin-Elmer 1650 and a Bruker Vector 22 spectrophotometers; and 1H NMR spectra on a Varian GEMINI 200 MHz and a Varian MERCURY 300 MHz spectrometers. The starting compounds 1a-d and 6a-c were prepared according to the reported procedures.

**Preparation of 2-alkoxy-4,6-diaryl-3-pyridinecarbonitriles 2a-h.** Method A—A mixture of equimolar amounts of the appropriate 1a-d and malononitrile (5 mmoles) in the corresponding alcohol (25 mL) containing 0.5 g of sodium, was stirred at room temperature (25-30°C) for 24 hr. The solid separated was collected, washed with water and crystallized from a suitable solvent to afford the corresponding 2a-h.

Method B—A mixture of equimolar amounts of 6a-c and the appropriate ketone 7 (10 mmoles) in the corresponding alcohol (25 mL) containing 0.5 g of sodium, was stirred at room temperature (25-30°C) for 24 hr. The solid separated was collected, washed with water and crystallized from a suitable solvent to afford the corresponding 2a-h.

4-(4-Fluorophenyl)-2-methoxy-6-(2-thienyl)-3-pyridinecarbonitrile 2a: Colourless crystals from ethanol, m.p. 211-13°C, yields 52, 29% (methods A & B respectively); IR (cm⁻¹): 2221 (C=N), 1588, 1515 (C=N, C=C); 1H NMR (CDCl₃): δ 4.1 (s, 3H, OCH₃), 7.13-7.71 (m, 8H, 7 Ar-H + pyridine-1-1-5). Anal. for C₁₇H₁₁FN₂O₂ (310.34); Calcd: C, 65.79; H, 3.57; N, 9.03. Found: C, 65.71; H, 3.51; N, 9.06%.

2-Ethoxy-4-(4-fluorophenyl)-6-(2-thienyl)-3-pyridinecarbonitrile 2b: Colourless crystals from ethanol, m.p. 176-77°C, yields 56, 49% (methods A & B respectively); IR (cm⁻¹): 2221 (C≡N), 1588, 1515 (C≡N, C=C); 1H NMR (CDCl₃): δ 1.43 (t, 3H, CH₂, 1=7.2 Hz), 4.54 (q, 2H, OCH₂, 1=7.2 Hz), 7.05-7.63 (m, 8H, 7 Ar-H + pyridine-1-5). Anal. for C₁₇H₁₃FN₂O₂ (324.36); Calcd: C, 66.79; H, 3.57; N, 9.06%.

6-(4-Fluorophenyl)-2-methoxy-4-(2-thienyl)-3-pyridinecarbonitrile 2c: Colourless crystals from ethanol, m.p. 176-77°C, yields 56, 49% (methods A & B respectively); IR (cm⁻¹): 2221 (C≡N), 1588, 1515 (C≡N, C=C); 1H NMR (CDCl₃): δ 4.19 (s, 3H, OCH₃), 7.13-7.71 (m, 8H, 7 Ar-H + pyridine-1-5). Anal. for C₁₇H₁₁FN₂O₂ (310.34); Calcd: C, 66.79; H, 3.57; N, 9.06%.

**Table 1—Minimum inhibitory concentrations (MICs) of the tested compounds 1a-d, 2a-h and 6a-c against different organisms.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (μg/mL)</th>
<th>AMP*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>≥400</td>
<td>8b</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>200</td>
<td>8a</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>≥400</td>
<td>8b</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>200</td>
<td>8b</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>500</td>
<td>8b</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>≥400</td>
<td>8b</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>100</td>
<td>8b</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>100</td>
<td>8b</td>
</tr>
<tr>
<td>Staphylococcus saprophyticus</td>
<td>500</td>
<td>8b</td>
</tr>
</tbody>
</table>

*AMP* = Amphotericin B (μg/mL)
of fluorophenyl)-2-methoxy-3-carbonitriles

Calcd: C, 70.12; H, 4.04; N, 9.52. Found: C, 69.99; H, 3.50; N, 14.32%.

References


Preparation of 2-Amino-4,6-diaryl-3-pyridine-carbonitriles 8a,b. Method A—A mixture of equimolar amounts of the appropriate 1a,b and malononitrile (5 mmol) in absolute ethanol (25 mL) containing ammonium acetate (5 mmol) was boiled under reflux for 15 hr. The solid separated upon concentrating the reaction mixture to about 5 mL and stored at room temperature was collected and crystallized from a suitable solvent to afford the corresponding 8a,b.

Method B—A mixture of equimolar amounts of 6ab and the appropriate ketone 7 (10 mmol) in absolute ethanol (25 mL) containing ammonium acetate (50 mmol) was boiled under reflux for 15 hr. The solid separated upon cooling the reaction mixture (4°C) was collected and crystallized from a suitable solvent to afford the corresponding 8b. In case of 8a, the reaction mixture was purified on silica gel TLC using chloroform-light petroleum (60:40°C) as eluent (1:1, v/v).

2-Amino-4-(4-fluorophenyl)-6-(2-thienyl)-3-pyridine-carbonitrile 8b: Pale yellow crystals from benzene, m.p. 165-67°C; yields 41, 44% (methods A & B respectively); IR (cm⁻¹): 3476, 3361 (NH₃), 2212 (C=O), 1643, 1604 (C=O, C=O); 1H NMR (CDCl₃): δ 5.34 (s, 2H, D₂O exchangeable NH₂), 7.07-7.67 (m, 8H, 7 Ar-H + pyridine H-5). Anal. for C₃₆H₂₄N₂O₂S (295.32): Calcd: C, 65.07; H, 3.41; N, 14.23. Found: C, 64.99; H, 3.32; N, 14.28%.

2-Amino-6-(4-fluorophenyl)-4-(2-thienyl)-3-pyridine-carbonitrile 8a: Yellow crystals from benzene, m.p. 175-77°C; yields 34, 41% (methods A & B respectively); IR (cm⁻¹): 3482, 3361 (NH₃), 2209 (C=O), 1618, 1575 (C=O, C=O); 1H NMR (CDCl₃): δ 5.34 (s, 2H, D₂O exchangeable NH₂), 7.13-8.00 (m, 8H, 7 Ar-H + pyridine H-5). Anal. for C₃₆H₂₄N₂O₂S (295.32): Calcd: C, 65.07; H, 3.41; N, 14.23. Found: C, 65.22; H, 3.50; N, 14.32%.

References


NOTES


27 a) Buu-Hoi Ng Ph, Dat Xuong Ng & Michel Sy, Bull Soc Chim France, 1956, 1646.

   c) Bochm T & Grohnwald M, Arch Pharm, 274, 1936, 318.