Chapter 2

Studies on cyanopyridines

2.0 Introduction

The pyridine skeleton is of great importance to chemists as well as to biologists as it is found in a large variety of naturally occurring compounds and also in clinically useful molecules having diverse biological activities. Cyanopyridines comprise a very interesting class of compounds because of their significant and versatile biological and pharmacological activities. Cyanopyridines are important intermediates in the pharmaceutical industry for the synthesis of nicotinamide, nicotinic acid and isonicotinic acid etc [1]. The importance of cyanopyridines in organic synthesis has increased over the past few decades because they are among the most versatile organic synthetic intermediates [2]. Many fused cyanopyridines have drawn attention due to their wide spectrum biological activities [3].

In view of these observations and with a view to further explore the pharmacological profile of this class of compounds, the present study includes synthesis of novel 3-cyanopyridines (pyridine-3-carbonitriles) and fused cyanopyridines viz. pyrazolo[3,4-b]pyridine-5-carbonitriles. The study is divided in two sections:

2.1: Synthesis and biological evaluation of 6-aryl-4-(substituted-2-chloro-quinolin-3-yl)-2-alkoxy-pyridine-3-carbonitriles

2.2: Synthesis and biological evaluation of 6-alkoxy-3-methyl-4-aryl-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles
2.1: Synthesis and biological evaluation of 6-aryl-4-(substituted-2-chloro quinolin-3-yl)-2-alkoxy-pyridine-3-carbonitriles

2.1.1 Introduction
The pyridine motif is among the most common N-heteroaromatics incorporated into the structure of various therapeutic agents. Many naturally occurring and synthetic compounds bearing pyridine scaffold possess interesting biological properties. In association with those, 3-cyanopyridine or pyridine-3-carbonitrile derivatives draw a special attention for their wide spectrum biological activities along with their importance and utility as intermediates in preparing variety of heterocyclic compounds [1].

It has been demonstrated that molecules containing 3-cyanopyridine moiety may be able to work as ligands towards transition-metal ions [4], new drugs [5], and significant intermediates for the synthesis of important vitamins [6] such as nicotinic acids [7] and nicotinamides [8].

The pharmacological and physiological activity of 3-cyanopyridines has attracted much attention in recent years with the synthesis and the study of the non-glycosidic cardiotonic agent milrinone [9, 10], as well as with a number of 3-cyanopyridine derivatives which proved to be active against the herpes virus and the human immunodeficiency virus [11, 12]. The 3-cyanopyridin-2-one nucleus is also the structural basis of the alkaloid ricinine-the first known alkaloid containing a cyano-group.
3-cyanopyridines with different alkyl and aryl/heteroaryl groups were found to have anti-tubercular [13], antimicrobial [14], anti-cancer [15, 16], A2A adenosine receptor antagonists [17], antihypertensive [18, 19], anti-histaminic [20], anti-inflammatory, analgesic & antipyretic properties [21] as well as 1KK-β inhibitor properties [22]. Some examples of published derivatives of 3-cyanopyridines with their biological activities are as following.
2.1.2 Reported synthetic strategies

2.1.2.1. From α,β-unsaturated synthons

Large number of publications describe the synthesis of 3-cyanopyridines from α,β-unsaturated synthons. The Michael-type condensation of α,β-unsaturated ketones or 1,3-diaryl-prop-2-en-1-ones with malononitrile and ammonium acetate is probably the most reported strategy for the synthesis of 4,6-diaryl/heteroaryl-2-amino-3-cyano pyridines [26, 36-38] (Scheme 2.1.1). The reaction is generally carried out in alcohol in presence of excess of ammonium acetate. Recently, Sarda et al. have reported the reaction using ionic liquid ethylammonium nitrate [39].

![Scheme 2.1.1](image)

Reaction of arylidene malononitrile with alkyl and aryl ketones in presence of ammonium acetate is reported to yield 6-alkyl/aryl substituted 2-amino-3-cyano pyridines [40, 41] (Scheme 2.1.2).

![Scheme 2.1.2](image)

Number of multi-component approaches for the synthesis of 2-amino-3-cyano pyridines has also been reported involving one pot reaction of different aldehydes, ketones, malononitrile and ammonium acetate [22, 29, 42-44]. Heravi et al. have reported the green one-pot synthesis of 2-amino-3-cyano pyridines from 3,4-dimethoxyacetophenone, different aromatic aldehydes, malonitrile and ammonium acetate using hetero-
polyacid as heterogeneous and recyclable catalyst [45] (Scheme 2.1.3).

\[
\text{Scheme 2.1.3}
\]

Reaction of aryldiene malononitriles with 2-amino-thiophenol yielded 2-amino-3,5-dicyano-6-substitutedthioxo-pyridines [46]. Recently, multi-component reactions involving aldehydes, malononitrile and 2-aminothiophenol using boric acid [47] as well as potassium fluoride on alumina (KF-Al₂O₃) [48] as green catalysts have been described (Scheme 2.1.4).

\[
\text{Scheme 2.1.4}
\]

Condensation of ethyl cyanoacetate with α,β-unsaturated ketones in presence of excess ammonium acetate afforded 3-cyanopyridin-2-ones [31, 49-51] (Scheme 2.1.5). Also, a green chemistry approach describing reaction of α,β-unsaturated ketones with ethyl cyanoacetate using samarium iodide as catalyst has been reported recently [52].

\[
\text{Scheme 2.1.5}
\]
Barat reported first that condensation of cyanoacetamide with α,β-unsaturated ketones also affords 3-cyanopyridin-2-ones [53]. Number of reports following this approach have been reported till date [54-56] (Scheme 2.1.6).

Scheme 2.1.6

Chase et al. have synthesized 6-amino-5-phenyl-3-cyanopyridin-2-one by the reaction of 3-isobutoxy-2-phenylacrylonitrile with cyanoacetamide [57] (Scheme 2.1.7). Recently, Melikyan et al. have reported synthesis of novel N-substituted-3-cyanopyridin-2-ones from yldenecyanoacetic acid ethyl esters in two steps [58] (Scheme 2.1.7).

Scheme 2.1.7

Reaction of ethyl-(2,3,4-trimethoxybenzoyl)-pyruvate with cyanoacetamide in ethanol in presence of piperidine gave 4-carbethoxy-6-(2,3,4-trimethoxybenzyl)-3 cyanopyridin-2-one [59] (Scheme 2.1.8).
Chalcones react with cyanothioacetamide in the presence of sodium ethoxide to afford 3-cyano-pyridin-2-thiones [60, 61]. A one pot approach has also been reported to afford the 3-cyano-pyridin-2-thiones involving reaction of chalcones, malononitrile and elemental sulfur [62] (Scheme 2.1.9).

The 6-amino-4-methylthio-3-cyano-2(1H)-pyridinethione was prepared by the reaction of 1,1-dimethylthio-1-thiocarbamoyl-2-cyanoethylene with cyanoacetamide [63] (Scheme 2.1.10).

The reaction of 4-butoxybenzalcyanoacetic ester with cyanothioacetamide yielded 6-amino-4-(4-butoxyphenyl)-3,5-dicyano-2(1H)-pyridinethione [64] (Scheme 2.1.11).
Synthesis of 2-alkoxy-3-cyanopyridines is generally achieved by the reaction of chalcones with malononitrile in corresponding sodium alkoxide [65-68] (Scheme 2.1.12).

Another frequently used approach for the synthesis of 2-alkoxy-3-cyano-pyridines is the O-alkylation of 3-cyanopyridin-2-ones by the reaction with appropriate alkyl /aryl halide [69, 70] (Scheme 2.1.13).
2.1.2.2. From 1,3-dicarbonyl synths

Literature survey revealed many reports on synthesis of 3-cyanopyridin-2-one and 3-cyanopyridin-2-thione by reaction of 1,3-dicarbonyl compounds with cyanoacetamide [71-74] and cyanothioacetamide [75-77] respectively (Scheme 2.1.14).

\[
\text{RCO}_2\text{H} + \text{H}_2\text{N}=\text{C}=\text{X} \rightarrow \text{PyCN}
\]

\[R_1 = \text{H} [69, 70], R_2 = -\text{COOH} [71], R_3 = \text{heteroaryl} [72] \quad ; \quad X = \text{O} [69-72], \text{S} [73-75] \]

\[R_1 = \text{benzoyl} [73], R_2 = \text{CH}_3, -\text{OC}_2\text{H}_5 [74], R_3 = -\text{OC}_2\text{H}_5 [75] \]

Scheme 2.1.14

2.1.2.3. Miscellaneous

Enaminones have been attractive starting materials for chemists for the synthesis of diverse functionalized 3-cyanopyridines [78-83]. Abu-Elmaati has reported reaction of enaminone 1-((N-p-chlorophenyl)-2-(N-dimethylaminomethino)-3-oxobutanamide with cyanothioacetamide in ethanol/sodium ethoxide to yield 3-cyano-4-methylpyridin-2(1H)one [80] (Scheme 2.1.15).

\[
\text{CONHPh} + \text{H}_2\text{N}=\text{C}=\text{S} + \text{EtONa} \rightarrow \text{PyHNOC}
\]

Scheme 2.1.15

Literature survey also revealed a few ring transformation reactions of 4-amino-1H-1,5-benzodiazepine-3-carbonitrile [84] and substituted uracil [85] yielding 3-cyanopyridines. Nohara et al. have reported that heating 2-cyano-3-(6-ethyl-4-oxo-4H-1-benzopyran-3-yl)acrylamide in pyridine yields 3-cyano-5-(5-ethyl-2-hydroxybenzoyl)-2(1H)-pyridones [86] (Scheme 2.1.16).
Scheme 2.1.16
2.1.3 Current work

The chemistry of pyridine and its derivatives has been studied for over a century due to their diverse biological activities. 3-cyanopyridine or pyridine-3-carbonitrile derivatives draw a special attention for their wide spectrum biological activities viz. anti-tubercular, anti-cancer, cardiovascular, anti-histaminic, anti-inflammatory, analgesic and antipyretic properties along with their importance and utility as intermediates in preparing variety of heterocyclic compounds.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, two novel series of pyridine-3-carbonitriles (DDK-A-01 to DDK-A-20) have been synthesized. The synthesis of pyridine-3-carbonitriles (DDK-A-01 to DDK-A-10) and (DDK-A-11 to DDK-A-20) was achieved by Michael addition of α,β-unsaturated ketones (chalones) to malononitrile in sodium methoxide/methanol and sodium ethoxide/ethanol systems respectively. 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones (chalones) were prepared by the reaction of 2,7-dichloroquinoline-3-carbaldehyde with different substituted acetophenones s using 40% sodium hydroxide as a catalyst [87]. The products were characterized by FT-IR, mass, $^1$H NMR spectroscopy and elemental analyses. The newly synthesized compounds were subjected to antimicrobial activity.
## 2.1.4 Reaction scheme

![Reaction Scheme Diagram]

Reagents and conditions: (a) R₂ONa, R₂OH, reflux, 6-8 h.

<table>
<thead>
<tr>
<th>Code</th>
<th>R₁</th>
<th>R₂</th>
<th>M.F.</th>
<th>M.W.</th>
<th>M.P. °C</th>
<th>Yield %</th>
<th>R₁₁</th>
<th>R₁₂</th>
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<td>DDK-A-01</td>
<td>H</td>
<td>CH₃</td>
<td>C₂₂H₁₃Cl₂N₂O</td>
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<td>69</td>
<td>0.47</td>
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<tr>
<td>DDK-A-02</td>
<td>3-NO₂</td>
<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O₁</td>
<td>451</td>
<td>233-235</td>
<td>62</td>
<td>0.48</td>
<td>0.71</td>
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<tr>
<td>DDK-A-03</td>
<td>4-NO₂</td>
<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O₁</td>
<td>451</td>
<td>247-249</td>
<td>60</td>
<td>0.53</td>
<td>0.72</td>
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<tr>
<td>DDK-A-04</td>
<td>4-Cl</td>
<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O</td>
<td>439</td>
<td>211-213</td>
<td>70</td>
<td>0.54</td>
<td>0.74</td>
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<tr>
<td>DDK-A-05</td>
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<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O₂</td>
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<td>189-191</td>
<td>65</td>
<td>0.47</td>
<td>0.69</td>
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<tr>
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<td>4-OH</td>
<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O₂</td>
<td>422</td>
<td>195-197</td>
<td>73</td>
<td>0.52</td>
<td>0.70</td>
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<tr>
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<td>CH₃</td>
<td>C₂₂H₁₂Cl₂N₂O₂</td>
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<td>65</td>
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<td>75</td>
<td>0.51</td>
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<td>CH₃</td>
<td>C₂₂H₁₂Cl₂FN₂O</td>
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<td>0.69</td>
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<td>CH₃</td>
<td>C₂₂H₁₂BrN₂O</td>
<td>485</td>
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<td>62</td>
<td>0.49</td>
<td>0.73</td>
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<tr>
<td>DDK-A-11</td>
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<td>C₂H₅</td>
<td>C₂₂H₁₂Cl₂N₂O</td>
<td>420</td>
<td>128-130</td>
<td>68</td>
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<td>DDK-A-12</td>
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<td>C₂₂H₁₂Cl₂N₂O₁</td>
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<td>241-243</td>
<td>74</td>
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<td>0.67</td>
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<tr>
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<td>C₂H₅</td>
<td>C₂₂H₁₂Cl₂N₂O₁</td>
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<td>178-180</td>
<td>70</td>
<td>0.49</td>
<td>0.58</td>
</tr>
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<td>80</td>
<td>0.53</td>
<td>0.60</td>
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<td>C₂H₅</td>
<td>C₂₂H₁₂Cl₂N₂O₂</td>
<td>436</td>
<td>256-258</td>
<td>69</td>
<td>0.59</td>
<td>0.70</td>
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<td>C₂₂H₁₂Cl₂N₂O₂</td>
<td>436</td>
<td>263-265</td>
<td>56</td>
<td>0.51</td>
<td>0.59</td>
</tr>
<tr>
<td>DDK-A-17</td>
<td>2-OCH₃</td>
<td>C₂H₅</td>
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<td>450</td>
<td>229-231</td>
<td>63</td>
<td>0.54</td>
<td>0.61</td>
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<td>C₂₂H₁₂Cl₂N₂O₂</td>
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<td>234-236</td>
<td>68</td>
<td>0.56</td>
<td>0.63</td>
</tr>
<tr>
<td>DDK-A-19</td>
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<td>C₂₂H₁₂Cl₂FN₂O</td>
<td>438</td>
<td>173-175</td>
<td>52</td>
<td>0.48</td>
<td>0.57</td>
</tr>
<tr>
<td>DDK-A-20</td>
<td>4-Br</td>
<td>C₂H₅</td>
<td>C₂₂H₁₂BrN₂O</td>
<td>499</td>
<td>238-240</td>
<td>50</td>
<td>0.58</td>
<td>0.68</td>
</tr>
</tbody>
</table>

TLC Solvent system R₁₁: Hexane: Ethyl acetate = 6:4,
TLC Solvent system R₁₂: Chloroform:Methanol = 9.5:0.5.
2.1.5 Mechanism

The reaction proceeds through Michael addition of \( \alpha,\beta \)-unsaturated ketones to the malononitrile to afford adduct A which undergoes a nucleophilic attack by alkoxide anion followed by cyclization and subsequent dehydration of the cyclized product leads to the 2-alkoxycyanopyridines as suggested by Radwan et al. [68]
2.1.6 Experimental

2.1.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. $^1$H NMR was determined in DMSO-$d_6$ solution on a Bruker Avance II 400 MHz spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

2.1.6.2 Synthesis of 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones

Synthesis of 3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-ones was achieved using previously published method [87].

2.1.6.3 General procedure for the synthesis of 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(aryl)-pyridine-3-carbonitriles (DDK-A-01 to DDK-A-10)

3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-one (0.01 mol) was added to a freshly prepared sodium methoxide solution (0.015 mol of sodium in 15 mL of methanol). Malononitrile (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 6-8 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.
2.1.6.3.1 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-phenylpyridine-3-carbonitrile (DDK-A-01)

Yield: 69%; m.p. 215-217 °C; IR (cm\(^{-1}\)): 3022 (C-H stretching of aromatic ring), 2951 (C-H asymmetrical stretching of CH\(_3\) group), 2864 (C-H symmetrical stretching of CH\(_3\) group), 2218 (C≡N stretching of nitrile group), 1616, 1585 & 1548 (C=C stretching of aromatic ring), 1462 (C-H asymmetrical deformation of CH\(_3\) group), 1359 (C-H symmetrical deformation of CH\(_3\) group), 1261 (C-O-C asymmetrical stretching of OCH\(_3\) group), 1074 (C-O-C symmetrical stretching OCH\(_3\) group), 1020 (C-H in plane bending for aromatic ring), 769 (C-Cl stretching), 704 & 769 (C-H out of plane bending for mono-substituted aromatic ring); \(^1\)H NMR (DMSO-d\(_6\)) δ ppm: 4.03 (s, 3H, H\(_a\)), 7.95 (s, 1H, H\(_b\)), 7.67-7.71 (d, 1H, H\(_d\)), 7.83-7.85 (m, 2H, H\(_c\), H\(_f\)), 8.14-8.15 (d, 1H, H\(_e\)), 7.42-7.47 (m, 3H, H\(_g\), H\(_h\), H\(_h^\prime\)), 8.03-8.05 (m, 2H, H\(_i\), H\(_i^\prime\)); MS: m/z 406; Anal. Calcd. for C\(_{22}\)H\(_{13}\)Cl\(_2\)N\(_3\)O: C, 65.04; H, 3.23; N, 10.34. Found: C, 64.06; H, 3.16; N, 10.27.

2.1.6.3.2 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(3-nitrophenyl)pyridine-3-carbonitrile (DDK-A-02)

Yield: 62%; m.p. 233-235 °C; MS: m/z 451; Anal. Calcd. for C\(_{22}\)H\(_{12}\)Cl\(_2\)N\(_4\)O: C, 58.55; H, 2.68; N, 12.42. Found: C, 58.46; H, 2.61; N, 12.33%.

2.1.6.3.3 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(4-nitrophenyl)pyridine-3-carbonitrile (DDK-A-03)

Yield: 60%; m.p. 247-249 °C; MS: m/z 451; Anal. Calcd. for C\(_{22}\)H\(_{12}\)Cl\(_2\)N\(_4\)O: C, 58.55; H, 2.68; N, 12.42. Found: C, 58.47; H, 2.61; N, 12.34%.
2.1.6.3.4 6-(4-chlorophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-04)

Yield: 70%; m.p. 211-213 ºC; IR (cm⁻¹): 3043 (C-H stretching of aromatic ring), 2922 (C-H asymmetrical stretching of CH₃ group), 2852 (C-H symmetrical stretching of CH₃ group), 2218 (C≡N stretching of nitrile group), 1546, 1506 & 1456 (C=C stretching of aromatic ring), 1402 (C-H asymmetrical deformation of CH₃ group), 1348 (C-H symmetrical deformation of CH₃ group), 1257 (C-O-C asymmetrical stretching of OCH₃ group), 1089 (C-O-C symmetrical stretching OCH₃ group), 1010 (C-H in plane bending for aromatic ring), 725 (C-Cl stretching), 821 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ¹H NMR (DMSO-d₆) δ ppm: 4.02 (s, 3H, Hₐ), 7.96 (s, 1H, Hₖ), 7.88-7.89 (d, 1H, Hₗ), 7.43-7.44 (m, 1H, Hₔ), 8.28-8.29 (d, 1H, Hₕ), 7.66 (s, 1H, H₅), 7.33-7.35 (d, 2H, Hₘ, J = 8.36 Hz), 8.17-8.19 (d, 2H, Hₖ, J = 8.3 Hz); MS: m/z 439; Anal. Calcd. for C₂₂H₁₂Cl₃N₃O: C, 59.96; H, 2.74; N, 9.53. Found: C, 59.89; H, 2.66; N, 9.43%.

2.1.6.3.5 4-(2,7-dichloroquinolin-3-yl)-6-(2-hydroxyphenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-05)

Yield: 65%; m.p. 189-191 ºC; MS: m/z 422; Anal. Calcd. for C₂₂H₁₃Cl₂N₃O₂: C, 62.58; H, 3.10; N, 9.95. Found: C, 62.50; H, 3.01; N, 9.87%.

2.1.6.3.6 4-(2,7-dichloroquinolin-3-yl)-6-(4-hydroxyphenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-06)

Yield: 73%; m.p. 195-197 ºC; MS: m/z 422; Anal. Calcd. for C₂₂H₁₃Cl₂N₃O₂: C, 62.58; H, 3.10; N, 9.95. Found: C, 62.48; H, 3.02; N, 9.87%. 
2.1.6.3.7 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(2-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-07)

Yield: 65%; m.p. 198-200 °C; MS: m/z 436; Anal. Calcd. for C_{23}H_{15}Cl_{2}N_{3}O_{2}: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.24; H, 3.39; N, 9.53%.

2.1.6.3.8 4-(2,7-dichloroquinolin-3-yl)-2-methoxy-6-(4-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-08)

Yield: 75%; mp 254-256 °C; MS: m/z 436; Anal. Calcd. for C_{23}H_{15}Cl_{2}N_{3}O_{2}: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.25; H, 3.38; N, 9.54%.

2.1.6.3.9 4-(2,7-dichloroquinolin-3-yl)-6-(4-fluorophenyl)-2-methoxypyridine-3-carbonitrile (DDK-A-09)

Yield: 78%; m.p. 265-267 °C; IR (cm\(^{-1}\)): 3057 (C-H stretching of aromatic ring), 2999 (C-H asymmetrical stretching of CH\(_3\) group), 2899 (C-H symmetrical stretching of CH\(_3\) group), 2216 (C≡N stretching of nitrile group), 1579 & 1558, (C=C stretching of aromatic ring), 1465 (C-H asymmetrical deformation of CH\(_3\) group), 1352 (C-H symmetrical deformation of CH\(_3\) group), 1255 (C-O-C asymmetrical stretching of OCH\(_3\) group), 1022 (C-O-C symmetrical stretching of OCH\(_3\) group), 987 (C-H in plane bending for aromatic ring), 725 (C-Cl stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 3.98 (s, 3H, H\(_a\)), 7.96 (s, 1H, H\(_b\)), 7.73-7.78 (m, 2H, H\(_c\)), 7.40-7.44 (m, 1H, H\(_d\)), 8.16-8.18 (d, 1H, H\(_e\)), 7.18-7.23 (m, 2H, H\(_f\)), 8.25-8.28 (t, 2H, H\(_g\, h\)); MS: m/z 424; Anal. Calcd. for C\(_{22}\)H\(_{12}\)Cl\(_2\)FN\(_3\)O: C, 62.28; H, 2.85; N, 9.90. Found: C, 62.19; H, 2.78; N, 9.83%.
2.1.6.3.10 6-(4-bromophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-10)

Yield: 62%; m.p. 228-231 °C; MS: m/z 485; Anal. Calcd. for C_{22}H_{12}BrN_{3}O: C, 54.46; H, 2.49; N, 8.66. Found: C, 54.37; H, 2.40; N, 8.58%.

2.1.6.4 General procedure for the synthesis of 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(aryl)pyridine-3-carbonitriles (DDK-A-11 to DDK-A-20)

3-(2,7-dichloroquinolin-3-yl)-1-(aryl)-prop-2-en-1-one (0.01 mol) was added to a freshly prepared sodium ethoxide solution (0.015 mol of sodium in 15 mL of ethanol). Malononitrile (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 6-8 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

2.1.6.4.1 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-phenylpyridine-3-carbonitrile (DDK-A-11)

Yield: 67%; m.p. 209-211 °C; IR (cm\(^{-1}\)): 3068 (C-H stretching of aromatic ring), 2956 (C-H asymmetrical stretching of CH\(_3\) and CH\(_2\) group), 2872 (C-H symmetrical stretching of CH\(_3\) and CH\(_2\) group), 2214 (C≡N stretching of nitrile group), 1529 & 1481 (C=C stretching of aromatic ring), 1458 (C-H asymmetrical deformation of CH\(_3\) group), 1348 (C-H symmetrical deformation of CH\(_3\) group), 1257 (C-O-C asymmetrical stretching of OC\(_2\)H\(_5\) group), 1074 (C-O-C symmetrical stretching OC\(_2\)H\(_5\) group), 1016 (C-H in plane bending for aromatic ring), 738 (C-Cl stretching), 690 & 738 (C-H out of plane bending for mono-substituted aromatic ring); \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 4.71-4.73 (t, 3H, H\(_a\)), 1.53-1.56 (q, 2H, H\(_b\)), 7.97 (s, 1H, H\(_c\)), 7.68-7.70 (d, 1H, H\(_d\)), 7.85-7.91 (m, 2H, H\(_e\), H\(_g\)); 8.29-8.30 (d, 1H, H\(_f\)), 7.49-7.53 (m,
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3H, H_{h,k,l'}, 8.14-8.17 (m, 2H, H_{j,l}); MS: m/z 420; Anal. Calcd. for C_{23}H_{15}Cl_{2}N_{3}O: C, 65.73; H, 3.60; N, 10.00. Found: C, 65.66; H, 3.51; N, 09.94%.

2.1.6.4.2 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(3-nitrophenyl)pyridine-3-carbonitrile (DDK-A-12)

Yield: 62%; m.p. 242-244 °C; MS: m/z 465; Anal. Calcd. for C_{23}H_{14}Cl_{2}N_{4}O_{3}: C, 59.37; H, 3.03; N, 12.04%. Found: C, 59.27; H, 2.94; N, 11.95%.

2.1.6.4.3 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-nitrophenyl)pyridine-3-carbonitrile (DDK-A-13)

Yield: 70%; m.p. 178-180 °C; MS: m/z 465; Anal. Calcd. for C_{23}H_{14}Cl_{2}N_{4}O_{3}: C, 59.37; H, 3.03; N, 12.04%. Found: C, 59.28; H, 2.94; N, 11.96%.

2.1.6.4.4 6-(4-chlorophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-ethoxypyridine-3-carbonitrile (DDK-A-14)

Yield: 71%; m.p. 233-235 °C; IR (cm\(^{-1}\)): 3048 (C-H stretching of aromatic ring), 2991 (C-H asymmetrical stretching of CH\(_3\) and CH\(_2\) group), 2822 (C-H symmetrical stretching of CH\(_3\) and CH\(_2\) group), 2220 (C≡N stretching of nitrile group), 1616 & 1581 (C=C stretching of aromatic ring), 1467 (C-H asymmetrical deformation of CH\(_3\) group), 1340 (C-H symmetrical deformation of CH\(_3\) group), 1255 (C-O-C asymmetrical stretching of OC\(_2\)H\(_5\) group), 1109 (C-O-C symmetrical stretching OC\(_2\)H\(_5\) group), 1024 (C-H in plane bending for aromatic ring), 761 (C-Cl stretching), 835 (C-H out of plane bending for 1,4-disubstituted aromatic ring); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) ppm: 4.66-4.70 (t, 3H, H\(_a\)), 1.50-1.54 (q, 2H, H\(_b\)), 7.96 (s, 1H, H\(_c\)), 7.44-7.47 (m, 1H, H\(_d\)), 7.88-7.90 (m, 1H, H\(_e\)), 8.28-8.29 (d, 1H, H\(_f\)), 7.66 (s, 1H, H\(_g\)), 7.22-7.25 (d, 2H, H\(_h,i\), \(J = 8.60\) Hz), 8.18-8.20 (d, 2H, H\(_i,i\), \(J = 8.28\) Hz); MS: m/z
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454; Anal. Calcd. for C_{23}H_{14}Cl_{3}N_{3}O: C, 60.75; H, 3.10; N, 9.24. Found: C, 60.69; H, 3.03; N, 9.17%.

2.1.6.4.5 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(2-hydroxyphenyl)pyridine-3-carbonitrile (DDK-A-15)

\[
\begin{align*}
\text{Yield: 61%; m.p. 169-171 °C; MS: m/z 436; } \\
\text{Anal. Calcd. for C}_{23}\text{H}_{15}\text{Cl}_{2}N_{3}O_{2}: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.23; H, 3.39; N, 9.55%.}
\end{align*}
\]

2.1.6.4.6 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-hydroxyphenyl)pyridine-3-carbonitrile (DDK-A-16)

\[
\begin{align*}
\text{Yield: 62%; m.p. 183-185 °C; MS: m/z 436; } \\
\text{Anal. Calcd. for C}_{23}\text{H}_{15}\text{Cl}_{2}N_{3}O_{2}: C, 63.32; H, 3.47; N, 9.63. Found: C, 63.25; H, 3.38; N, 9.56%.}
\end{align*}
\]

2.1.6.4.7 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(2-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-17)

\[
\begin{align*}
\text{Yield: 63%; m.p. 211-213 °C; MS: m/z 450; Anal. Calcd. for C}_{24}\text{H}_{17}\text{Cl}_{2}N_{3}O_{2}: C, 64.01; H, 3.81; N, 9.33; Found: C, 63.93; H, 3.75; N, 9.27%.}
\end{align*}
\]

2.1.6.4.8 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-methoxyphenyl)pyridine-3-carbonitrile (DDK-A-18)

\[
\begin{align*}
\text{Yield: 77%; m.p. 268-272 °C; MS: m/z 450; } \\
\text{Anal. Calcd. for C}_{24}\text{H}_{17}\text{Cl}_{2}N_{3}O_{2}: C, 64.01; H, 3.81; N, 9.33; Found: C, 63.93; H, 3.74; N, 9.25%.}
\end{align*}
\]
2.1.6.4.9 4-(2,7-dichloroquinolin-3-yl)-2-ethoxy-6-(4-fluorophenyl)pyridine-3-carbonitrile (DDK-A-19)

Yield: 78%; m.p. 265-267 °C; IR (cm\(^{-1}\)): 3041 (C-H stretching of aromatic ring), 2991 (C-H asymmetrical stretching of CH\(_3\) group), 2947 (C-H asymmetrical stretching of CH\(_2\) group), 2854 (C-H symmetrical stretching of CH\(_3\) and CH\(_2\) group), 2214 (C≡N stretching of nitrile group), 1585, 1556, 1504 (C=C stretching of aromatic ring), 1467 (C-H asymmetrical deformation of CH\(_3\) group), 1435 (C-H asymmetrical deformation of CH\(_2\) group), 1348 (C-O-C asymmetrical stretching of OC\(_2\)H\(_5\) group), 1076 (C-O-C symmetrical stretching OC\(_2\)H\(_5\) group), 1020 (C-H in plane bending for aromatic ring), 748 (C-Cl stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) ppm: 4.66-4.69 (t, 3H, H\(_a\)), 1.49-1.53 (q, 2H, H\(_b\)), 8.07 (s, 1H, H\(_c\)), 7.44-7.47 (m, 1H, H\(_d\)), 8.20-8.23 (m, 1H, H\(_e\)), 8.34-8.35 (d, 1H, H\(_f\)), 7.72 (s, 1H, H\(_g\)), 7.22-7.27 (t, 2H, H\(_h\), H\(_h'\)), 7.84-7.93 (m, 2H, H\(_d,e\)); MS: \(m/z\) 438; Anal. Calcd. for C\(_{23}\)H\(_{14}\)Cl\(_2\)FN\(_3\)O: C, 63.03; H, 3.22; N, 9.59. Found: C, 62.95; H, 3.15; N, 9.51%.

2.1.6.4.10 6-(4-bromophenyl)-4-(2,7-dichloroquinolin-3-yl)-2-methoxypyridine-3-carbonitrile (DDK-A-20)

Yield: 66%; m.p. 226-228 °C; MS: \(m/z\) 499; Anal. Calcd. for C\(_{22}\)H\(_{12}\)BrN\(_3\)O: C, 55.34; H, 2.83; N, 8.42. Found: C, 55.27; H, 2.76; N, 8.36%.
2.1.7 Spectral discussion

2.1.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

2.1.7.1.1 Mass fragmentation pattern for DDK-A-04

![Mass fragmentation pattern for DDK-A-04](image-url)
2.1.7.1.2 Mass fragmentation pattern for DDK-A-19

2.1.7.2 IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For 3-cyanopyridines (DDK-A-01 to DDK-A-20), a characteristic band of nitrile group was observed in the range of 2214-2220 cm\(^{-1}\). Confirmatory bands of C-O-C asymmetrical stretching at 1238-1261 cm\(^{-1}\) and C-O-C symmetrical stretching at 1022-1109 cm\(^{-1}\) were observed for methoxy and ethoxy groups.
2.1.7.3 $^1$H NMR spectral study

$^1$H NMR spectra were recorded in DMSO-$d_6$ solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

For 3-cyanopyridines (DDK-A-01 to DDK-A-10), characteristic singlet was observed for methoxy group at 4.02-4.03 $\delta$ ppm. The aromatic ring protons were observed at 7.18-8.28 $\delta$ ppm and $J$ value were found to be in accordance with substitution pattern on phenyl ring.

While, for 3-cyanopyridines (DDK-A-11 to DDK-A-20), characteristic triplet-quartet pattern was observed for ethoxy group. A signal as triplet at 1.49-1.56 $\delta$ ppm corresponding to three methyl (O-CH$_2$-CH$_3$) protons and a quartet at 4.66-4.73 $\delta$ ppm corresponding to two methylene (O-CH$_2$-CH$_3$) protons was observed confirming the formation of 2-ethoxy-3-cyanopyridines. The aromatic ring protons were observed at 7.22-8.35 $\delta$ ppm and $J$ value were found to be in accordance with substitution pattern on phenyl ring.
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IR spectrum of DDK-A-01

Mass spectrum of DDK-A-01
$^1$H NMR spectrum of DDK-A-01

Expanded $^1$H NMR spectrum of DDK-A-01
IR spectrum of DDK-A-04

Mass spectrum of DDK-A-04
$^1$H NMR spectrum of DDK-A-04

Expanded $^1$H NMR spectrum of DDK-A-04
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Pyridine-3-carbonitriles

IR spectrum of DDK-A-09

Mass spectrum of DDK-A-09
$^1$H NMR spectrum of DDK-A-09

Expanded $^1$H NMR spectrum of DDK-A-09
Chapter 2 - Section 1

Pyridine-3-carbonitriles

IR spectrum of DDK-A-11

Mass spectrum of DDK-A-11
**Chapter 2 - Section 1**

Pyridine-3-carbonitriles

\[ ^1H \text{NMR spectrum of DDK-A-11} \]

![1H NMR spectrum of DDK-A-11](image)

**Expanded \(^1H\) NMR spectrum of DDK-A-11**

![Expanded 1H NMR spectrum of DDK-A-11](image)
Expanded $^1$H NMR spectrum of DDK-A-11

IR spectrum of DDK-A-14
Mass spectrum of DDK-A-14

\[ \text{Mass spectrum of DDK-A-14} \]

\[ 1^1 \text{H NMR spectrum of DDK-A-14} \]
Expanded $^1$H NMR spectrum of DDK-A-14

![Expanded $^1$H NMR spectrum of DDK-A-14](image)

Expanded $^1$H NMR spectrum of DDK-A-14

![Expanded $^1$H NMR spectrum of DDK-A-14](image)
IR spectrum of DDK-A-19

Mass spectrum of DDK-A-19
$^1$H NMR spectrum of DDK-A-19

Expanded $^1$H NMR spectrum of DDK-A-19
Expanded $^1$H NMR spectrum of DDK-A-19
2.1.8 Biological evaluation

2.1.8.1 Antimicrobial evaluation

All of the synthesized compounds (DDK-A-01 to DDK-A-20) were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method [88] with two Gram-positive bacteria *Staphylococcus aureus* MTCC-96, *Streptococcus pyogenes* MTCC 443, two Gram-negative bacteria *Escherichia coli* MTCC 442, *Pseudomonas aeruginosa* MTCC 441 and three fungal strains *Candida albicans* MTCC 227, *Aspergillus Niger* MTCC 282, *Aspergillus clavatus* MTCC 1323 taking Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin, and Griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [88(a)]. Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 µg mL\(^{-1}\), 500 µg mL\(^{-1}\) and 250 µg mL\(^{-1}\) concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The tubes were inoculated with \(10^8\) cfu mL\(^{-1}\) (colony forming unit/mL) and incubated at 37 ºC for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.
Table 1. Antibacterial and antifungal activity of synthesized compounds DDK-A-01 to DDK-A-20

<table>
<thead>
<tr>
<th>Code</th>
<th>Minimum inhibition concentration (µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive</td>
</tr>
<tr>
<td>DDK-A-01</td>
<td>500</td>
</tr>
<tr>
<td>DDK-A-02</td>
<td>1000</td>
</tr>
<tr>
<td>DDK-A-03</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>DDK-A-04</td>
<td>100</td>
</tr>
<tr>
<td>DDK-A-05</td>
<td>1000</td>
</tr>
<tr>
<td>DDK-A-06</td>
<td>25</td>
</tr>
<tr>
<td>DDK-A-07</td>
<td>125</td>
</tr>
<tr>
<td>DDK-A-08</td>
<td>100</td>
</tr>
<tr>
<td>DDK-A-09</td>
<td>50</td>
</tr>
<tr>
<td>DDK-A-10</td>
<td>25</td>
</tr>
<tr>
<td>DDK-A-11</td>
<td>1000</td>
</tr>
<tr>
<td>DDK-A-12</td>
<td>500</td>
</tr>
<tr>
<td>DDK-A-13</td>
<td>500</td>
</tr>
<tr>
<td>DDK-A-14</td>
<td>125</td>
</tr>
<tr>
<td>DDK-A-15</td>
<td>500</td>
</tr>
<tr>
<td>DDK-A-16</td>
<td>50</td>
</tr>
<tr>
<td>DDK-A-17</td>
<td>125</td>
</tr>
<tr>
<td>DDK-A-18</td>
<td>125</td>
</tr>
<tr>
<td>DDK-A-19</td>
<td>100</td>
</tr>
<tr>
<td>DDK-A-20</td>
<td>50</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>250</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
</tr>
<tr>
<td>Nystatin</td>
<td>-</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>-</td>
</tr>
</tbody>
</table>

2.1.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (DDK-A-01 to DDK-A-20) is currently under investigation and results are awaited.
2.2: Synthesis and biological evaluation of 6-alkoxy-3-methyl-4-aryl-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles

2.2.1 Introduction

Fused pyridines continue to attract considerable attention of researchers in different countries because of their great practical usefulness, primarily, due to a very wide spectrum of their biological activities. Pyrazolopyridines occupy a special position among these compounds. Along with some other pyridine systems containing an annelated five membered heteroaromatic ring, pyrazolopyridines are isosters of bioactive indoles or indazoles [89, 90] and have been reported to possess useful properties as antimetabolites in the biochemical synthesis of purine [91].

When pyrazole and pyridine rings are fused together, five isomeric pyrazolopyridines arise from such fusion corresponding to the five possible types of annihilations of pyrazole to the pyridine ring as shown below.

Pyrazolo[3,4-b]pyridines have been reported to possess wide variety of biological activities. Analgesic [92], anxiolytic [93], hypnotic [94], xanthine oxidase inhibitory [95, 96], antiviral [97], anti-HIV [98], Corticotropin-Releasing Factor (CRF) antagonist [99, 100], antidiabetic [101], antiarrhythmic [102], antitumor [103] activities have been reported for certain pyrazolopyridine derivatives. Some examples of published derivatives of pyrazolo[3,4-b]pyridines with their biological activities are as follows.
2.2.2 Reported synthetic strategies

Synthetic approaches towards pyrazolo[3,4-\(b\)]pyridines can be divided conceptually into two main groups: (a) appropriately substituted pyrazoles onto which a pyridine ring is annelated, and (b) suitably substituted pyridines onto which a pyrazole ring is annelated as summarized in the figure below.

![Diagram of annelation of pyridine ring onto pyrazole](image)

\[ \text{Route A} \quad \leftrightarrow \quad \text{Route B} \]

\( R_1 = \text{H, Ph} \)
\( R_2 = \text{Me, Ar, OH, NH}_2 \)
\( R_3 = \text{CN, COMe, COAr, COOMe} \)
\( \text{R} = \text{Appropriate Substituent(s)} \)
\( X = \text{Leaving Group} \)

2.2.2.1. ANNELATION OF PYRIDINE RING ONTO PYRAZOLE

2.2.2.1.1 From 3(5)-Aminopyrazoles

2.2.2.1.1. Use of \( \alpha, \beta \)-unsaturated reagents (as bifunctional synthons)

By far the most pyrazolo[3,4-\(b\)]pyridine synthesis are condensations of aminopyrazole with \( \alpha, \beta \)-unsaturated synthons. Reactions of aminopyrazole as 1,3-binucleophiles with variety of such \( \alpha, \beta \)-unsaturated synthons have been reported proving this method highly useful for the construction of pyrazolo[3,4-\(b\)]pyridine core with versatile substituents.

Examples of pyrazolo[3,4-\(b\)]pyridine synthesis published in the relevant period are listed in Table below, arranged according to the synthons used.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aminopyrazole</th>
<th>( \alpha, \beta )-unsaturated synthon</th>
<th>Pyrazolo[3,4-(b)]pyridine</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>[114]</td>
</tr>
</tbody>
</table>
"R and R’ means (substituted) aryl or heteroaryl substituent.

An interesting variation in this type of reactions is the use of β-aminocrotononitrile or its derivatives resulting in generation of bioactive substituted dihydropyrazolo[3,4-\textit{b}]pyridines with 4-amino and 6-methyl substituents available for further synthetic explorations [121] (Scheme 2.2.1).
Generally, the cyclizations are accomplished in acidic medium [121, 122] or in presence of base catalysts like triethyl amine or piperidine [116]. New green chemistry approaches recently described involve solvent-free reaction under microwave irradiation with or without catalyst [123, 124] as well as reactions in aqueous media [125], which are environmentally benign and afford high yields (Scheme 2.2.2).

Several efficient multi-component reaction (MCR) approaches have been reported attractively replacing the use of α, β- unsaturated synthons with their synthetic precursors. MCR approaches avoid the synthesis of α, β- unsaturated synthons, thereby reduce the reaction steps. The MCRs generally involve the one-pot reaction of aminopyrazole with aldehyde and 1,3-bifunctional reagents such as pyruvic acid [118], malononitrile [126], ethyl cyanoacetate [127] etc.

Unraveling site selectivity in these additions is not an easy task unless an acyclic intermediate can be isolated or the same reaction products can be synthesized by alternate routes. In several cases, modern NMR techniques studies were used to corroborate the structures of reaction products.

Lipson et al. reported that on boiling 3-methyl-5-aminopyrazole with an equimolar amount of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum’s acid) and ketones, both in methanol and in DMF, only the corresponding pyrazolo[3,4-b]pyridin-6-ones were formed and other possible isomeric products were not formed [128]. The structures of compounds were established \(^1\)H NMR spectra signals and Nuclear Overhauser Experiment (NOE) (Scheme 2.2.3).

Rahmati investigated a one-pot, three-component condensation reaction of 3-amino-5-methylpyrazole, aldehyde, ethyl cyanoacetate under reflux conditions in ethanol using p-toluenesulfonic acid (p-TsOH) as the catalyst [129] (Scheme 2.2.4). The \(^1\)H NMR spectra of the products indicated the formation of two diastereoisomers (cis and trans).
In some cases, a choice of multicomponent or linear protocol allows obtaining different heterocycles. For instance, MCR involving 5-aminopyrazole, aldehyde and pyruvic acid afforded positional isomer of product obtained from sequence pathway via preliminary synthesis of arylidenpyruvic acids [118] (Scheme 2.2.5).
Different products of the three-component and linear treatments in the case of 5-aminopyrazole can be the evidence that this MCR follows independent pathway without *in situ* formation of α, β-unsaturated compound.

### 2.2.2.1.1.2 Use of 1,3-dicarbonyl reagents (1,3-dielectrophiles) and other C-H acids

Cyclocondensations of aminopyrazoles with 1,3-dielectrophiles are extensively used for preparation of bicyclic nitrogen heterocycles viz. pyrazolo[1,5-a]pyrimidines and pyrazolo[3,4-b]pyridines. N1-unsubstituted 3(5)-aminopyrazoles usually react with 1,3-dicarbonyl compounds providing pyrazolo[1,5-a]pyrimidines [130-132]. The reaction of N1-substituted 5-aminopyrazoles with 1,3-dielectrophiles forms substituted pyrazolo[3,4-b]pyridines; as bifunctional reagents 1,3-ketoesters [133-136] as well as symmetric 1,3-diketones [135] are generally employed. The reaction is generally accomplished using acetic acid as a solvent [133], but cyclizations using hydrochloric acid in ethanol [134] or using zinc chloride and hydrochloric acid in ethanol [135] are also documented.

Microwave-assisted reaction of 1,3-dimethyl-5-amino-pyrazole with activated aryl malonates is reported by Rivkin et al. [137] (Scheme 2.2.6). Reaction of 5-aminopyrazole with aryl 1,3-ketoester is also documented [133].

![Scheme 2.2.6](image)

Literature survey also revealed few examples involving use of trifluoromethyl containing 1,3-ketoesters [135, 138] and symmetric 1,3-diketones [134], which exclusively lead to formation of a single pyrazolo[3,4-b]pyridine isomer as a product. Volochnyuk et al. have reported the synthesis of pyrazolo[3,4-b]pyridine using trifluoroacetic acid [139] (Scheme 2.2.7).

However, the application of unsymmetrical 1,3-diketones to the synthesis of pyrazolo[3,4-b]pyridines is poorly documented evidently because two regioisomeric
Chapter 2 - Section 2

Pyrazolo[3,4-\textit{b}]pyridine-5-carbonitriles

products may form in the reaction. The problem of regiodirection of the reaction is still urgent for the molecules of 3(5)-aminopyrazoles and unsymmetrical 1,3-dielectrophiles.

![Scheme 2.2.7](image)

Emelina et al. investigated the direction of reaction between N1-substituted aminopyrazoles and trifluoromethyl-containing unsymmetrical 1,3-diketones [140]. The study established characteristic spectral distinctions of individual regioisomers using \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy. It was proved that the regioisomer was formed having trifluoromethyl group attached at the C-4 (Scheme 2.2.8).

![Scheme 2.2.8](image)

The same research group further published the studies on regiodirection of the reaction of N1-unsubstituted 5-amino-3-methyl-pyrazole with hexafluoroacetetylacetone under different reaction conditions [141]. They established the structure of intermediate compound leading to the formation of pyrazolo[3,4-\textit{b}]pyridine by 2D NMR by means of homo and heteronuclear correlation procedures: DQF-COSY, J-COSY, NOESY, and HSQC without decoupling from \textsuperscript{13}C (Scheme 2.2.9).

Three component reaction of 5-aminopyrazole with aldehydes and ethyl acetoacetate under microwave irradiation is recently reported [116]. As CH-acids in the MCRs with 5-aminopyrazoles and aldehydes, aroylacetonitriles have been efficiently employed affording 4,6-diaryl-5-cyanopyrazolo[3,4-\textit{b}]pyridines. Zhu et
al. have reported microwave-assisted synthesis of 3-methyl-5-aminopyrazole with 3-cyanoacetyl indole and aldehydes in glycol as a reaction medium [142]. The same reaction was effected regioselectively by Quiroga et al. using solvent-free approach under microwave irradiation [117] (Scheme 2.2.10). A solvent-free green chemistry approach was adopted in one pot cyclocondensation of 5-amino-3-aryl-1H-phenylpyrazole, p-substituted benzoylacetonitriles, and some aldehydes using ammonium acetate as a reaction medium [143] as well as under neat conditions for 5-amino-3-pyrazolone, aldehydes and aroylacetonitriles [144].

![Scheme 2.2.9](image)

Shaabani et al. have reported novel four-component reaction of aryl/alkyl amines, diketene, aldehydes and 1,3-diphenyl-5-aminopyrazole in the presence of p-toluene sulfonyl chloride as a catalyst in dry dichloromethane at ambient temperature [145] (Scheme 2.2.11).

![Scheme 2.2.10](image)
2.2.2.1.3 Gould-Jacobs reaction

The classical Gould-Jacobs reaction for quinoline synthesis involves reaction between aniline and diethyl ethoxymethylene malonate. This reaction is widely documented in recent literature for the synthesis of 4-hydroxy- or 4-chloro-pyrazolo[3,4-b]pyridine-5-carboxylates using 5-aminopyrazole as a substitute for aniline in the classical Gould-Jacobs method [109, 146-149]. The first step of the reaction involves the addition of diethyl ethoxymethylene malonate to 5-aminopyrazole and the reaction is effected in solvents like ethanol [148, 149], or under solvent-free conditions at higher temperatures (120-130°C) [109, 147]. The adduct is further cyclized in diphenyl ether at 240°C to give 4-hydroxy-pyrazolo[3,4-b]pyridine-5-carboxylate [109] or in phosphorus oxychloride to give the 4-chloro-pyrazolo[3,4-b]pyridine-5-carboxylates [146-149]. The scope of the reaction is extended by employing novel N1-substituted 5-aminopyrazoles [109, 146] (Scheme 2.2.12).

2.2.2.1.4 Reactions with β-dimethyl-aminopropiophenones

Quiroga et al. have reported an interesting synthesis from 5-aminopyrazoles and β-dimethyl aminopropiophenones yielding 6-aryl-4,5-dihydropyrazolo[3,4-b]pyridines, which are aromatized upon treatment with N-bromo succinimide (NBS) to give [150] (Scheme 2.2.13).

Also of interest is the microwave promoted synthesis of 5-aryl-pyrazolo[3,4-
Chapter 2-Section 2  
Pyrazolo[3,4-b]pyridine-5-carbonitriles

Pyrazolo[3,4-b]pyridines by hetero-Diels-Alder reaction of N-[(dimethylamino)methylene]-3-methyl-1-phenyl-5-pyrazolamine with β-dimethyl aminopropiophenones in dry media in just one step [151] (Scheme 2.2.13).

![Scheme 2.2.13](image)

2.2.2.1.5 Ring opening reactions

Recently, ring opening reactions of variety of reagents with aminopyrazoles have drawn considerable attention for the complexity-generating synthesis of pyrazolo[3,4-b]pyridines baring versatile substituents.

Synthesis of pyrazolo[3,4-b]pyridine derivatives, which contain synthetically useful hydroxyl and carbonyl groups, was achieved from aminopyrazole and 3-formyl chromone via opening of the γ-pyrone ring of 3-formyl chromone [152] (Scheme 2.2.14). Similar reaction was efficiently achieved from 3-formyl chromone and aminopyrazole in presence of chlorotrimethylsilane in DMF [153]. Reaction of 6-methyl-4-oxo-4H-[1]-benzopyran-3-carboxaldehyde with 5-amino-3-methyl-1-phenylpyrazole is also reported to furnish 5-(2-hydroxy-5-methylbenzoyl)-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]pyridine [154].

![Scheme 2.2.14](image)
2.2.2.1.6 Miscellaneous reactions with 3(5)-aminopyrazoles

Abdel-Khalik et al. have reported reaction of 2-Arylhydrazonopropanals with 5-methyl-1H-pyrazol-3-amine yielding novel 3-substituted pyrazolo[3,4-b] pyridines [155] (Scheme 2.2.15).

![Scheme 2.2.15](image)

While microwave-assisted reaction of 5-amino-3-methyl-1-phenylpyrazole with aldehydes under solvent-free conditions furnished bispyrazolo[3,4-b:4',3'-e]pyridines [156]. Bispyrazolo[3,4-b:4',3'-e]pyridines were also obtained by refluxing 3-methyl-5-aminopyrazole with arylidenemalonic acid derivatives in nitrobenzene or bromobenzene [157] (Scheme 2.2.16).

![Scheme 2.2.16](image)

2.2.1.2 Friedlander Reaction

The classical Friedlander quinoline synthesis is an acid or base catalyzed condensation followed by a cyclodehydration between an aromatic 2-aminoaldehyde or ketone with the carbonyl compound containing a reactive α-methylene group [158, 159]. Friedlander reaction of various 5-amino-pyrazole-4-carboxaldehydes have been efficiently employed in literature for the synthesis of pyrazolo[3,4-b]pyridines with versatile substituents. As active methylene compounds with 5-amino-pyrazole-4-carboxaldehydes; ethyl acetoacetate [160], ethyl 3-oxo-3-phenylpropanoate [161],
acetone [161, 167], acetyl acetone [160, 167], acetophenones [161, 167], diethyl malonate [161], aroylacetonitriles [161-163], aryl/heteroaryl acetonitrile [161, 162], malononitrile [160, 164] have been employed. Abd-El-Latif et al. have reported an interesting condensation between 3-phenyl-5-chloro-1-phenylpyrazole-4-carbaldehyde and cyanoacetamide/cyano-thioacetamide furnishing Friedlander type product 1,3-diphenylpyrazolo[3,4-b]pyridine derivatives [165]. Reactions are usually effected in presence of base catalysts like pyridine [160], piperidine [161, 162] or potassium hydroxide [161, 167].

The starting 5-amino-pyrazole-4-carboxaldehydes are generally synthesized by Vilsmeier-Haack formylation of 5-aminopyrazoles [161] or by reduction of 5-azidopyrazole-4-carboxaldehyde with hydrogen sulphide [160, 166] or sodium dithionite [166]. However, Zheng et al. have reported a facile one-pot conversion of 5-azidopyrazole-4-carboxaldehyde to pyrazolo[3,4-b]pyridines in presence of alcoholic potassium hydroxide [167] (Scheme 2.2.17).

![Scheme 2.2.17]

### 2.2.2.1.3 From 4-cyano-5-amino-pyrazoles

Reactions of different substituted 4-cyano-5-amino-pyrazoles with variety of active methylenes or C-H acids are reported as valuable tool for obtaining highly functionalized pyrazolo[3,4-b]pyridines. Reactions of 4-cyano-5-amino-pyrazoles with ethyl acetoacetate [168, 169], acetyl acetone [168], diethyl malonate [168, 169], cyanoacetone [170], malononitrile [169] have been reported. The cyclocondensation is generally achieved in basic media like sodium ethoxide [170] or in presence of base like triethyl amine [169].

One interesting report using SnCl₄ as a catalyst in toluene for cyclocondensation is published by Zhao et al. [168] (Scheme 2.2.18).
2.2.2. ANNELATION OF PYRAZOLE RING ONTO PYRIDINE

Annelation of pyridine onto pyrazole ring (Route A) is the most commonly employed protocol and offers a degree of flexibility in terms of substitution about the pyridine ring. While Annelation of pyrazole onto pyridine ring (Route B) has remained relatively unexplored and has been confined to pyrazoles containing a methyl-, aryl-, hydroxy- or an amino- group at the 3-position [171]. Route B involves the formation of pyrazole ring from a 3-acetyl-, 3-carboxy- or 3-cyanopyridines bearing a leaving group in the 2-position.

2.2.2.1 From 3-Cyanopyridines

2.2.2.1.1 From 2-halo-3-cyanopyridines

Reaction of 2-chloro-3-cyanopyridines with hydrazine hydrate or its derivatives is widely employed strategy in literature furnishing 3-amino-pyrazolo[3,4-b]pyridines (for Route B) [171, 173-178]. Reaction is generally carried out by refluxing 2-chloro-3-cyanopyridines and hydrazine hydrate in suitable solvents viz. EtOH, BuOH, DMF etc (Scheme 2.2.19). However, base catalyst $\text{CS}_2\text{CO}_3$ in DMF has been used for cyclization by Lavecchia et al. [173].

2.2.2.1.2 From 2-thioxo-3-cyanopyridines

Literature survey also revealed several reports involving reaction of 2-thioxo-3-cyanopyridines [179-182] or its S-methyl derivatives [181, 183, 184] with hydrazine
hydrate for the synthesis of 3-amino-pyrazolo[3,4-\textit{b}]pyridines. Variety of 2-thioxo-3-cyanopyridines have been used by different researchers as shown below.

2.2.2.1.3 From 2-alkoxy-3-cyanopyridines

3-amino-pyrazolo[3,4-\textit{b}]pyridines can also be synthesized by the reaction of 2-alkoxy-3-cyanopyridines with hydrazine hydrate or its derivatives [99, 185]. Goda et al. have synthesized 3-amino-pyrazolo[3,4-\textit{b}]pyridines from 2-alkoxy-3-cyanopyridine using BF$_3$ as a catalyst [185] (Scheme 2.2.20).

2.2.2.2. From 3-Carboxylpyridines

Recent literature survey revealed only one report involving reaction starting from 2-chloro-nicotinic acid derivatives yielding 3-oxo-pyrazolo[3,4-\textit{b}]pyridines in three steps [186] (Scheme 2.2.21).
2.2.2.3 From 3-Acetylpyridines (or 3-Benzoylpyridines)

Tucker et al. have reported synthesis of 3-methyl-pyrazolo[3,4-b]pyridines from 1-(2,6-Difluoropyridin-3-yl)ethanone in two steps [98] (Scheme 2.2.22).

Similar reaction have been reported by Zhong et al. via double SNAr reaction of and its derivatives with hydrazine hydrate in one pot using n-BuLi as a base [187] The compound and derivatives have also been synthesized from 1-(2-amino-6-fluoropyridin-3-yl)ethanone [188] (Scheme 2.2.22).

Recently, number of synthetic approaches starting from 3-benzoilpyridines have also been published [189, 190]. For instance, Sweeny et al. have reported synthesis of 3-(substitutedbenzyl)-1H-pyrazolo[3,4-b]pyridine from reaction of 1-(2-chloropyridin-3-yl)-2-(aryl)ethanone with hydrazine hydrate [190] (Scheme 2.2.23).

2.2.2.3 MISCELLANEOUS SYNTHESIS

Kolosov et al. have reported synthesis of pyrazolo[3,4-b]pyridines by reaction of α-cyanochalcones with phenylhydrazine [191] (Scheme 2.2.24).
2.2.3 Current work

Pyrazolopyridines occupy a special position among fused pyridines due to their diverse biological activities. Pyrazolo[3,4-\textit{b}]pyridine ring system is of special biological interest as isosters of bioactive indoles or indazoles. It has numerous pharmacological and medicinal applications \textit{viz.} antiviral, antidiabetic, antiarrythmic, antitumour, anti-inflammatory etc.

Keeping in mind various biomedical applications and with a view to further assess the pharmacological profile of this class of compounds, two novel series of pyrazolo[3,4-\textit{b}]pyridine-5-carbonitriles (DDK-A-21 to DDK-A-40) have been synthesized. The synthesis of pyrazolo[3,4-\textit{b}]pyridine-5-carbonitriles (DDK-A-21 to DDK-A-30) and (DDK-A-31 to DDK-A-40) was achieved by the reaction of arylidene malononitrile and 3-methyl-1H-pyrazol-5\textit{H}-one in sodium methoxide/methanol and sodium ethoxide/ethanol systems respectively. Arylidene malononitriles were prepared via the Knoevenagel condensation reaction between different aromatic aldehydes and malononitrile [192]. The products were characterized by FT-IR, mass spectra, $^1$H NMR and elemental analysis. The newly synthesized compounds were subjected to antimicrobial activity.
**2.2.4 Reaction scheme**

Reagents and conditions: (a) $R_2$ONa, $R_2$OH, reflux, 10-12 h.

<table>
<thead>
<tr>
<th>Code</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>M.F.</th>
<th>M.W.</th>
<th>M.P. °C</th>
<th>Yield %</th>
<th>$R_{f1}$</th>
<th>$R_{f2}$</th>
</tr>
</thead>
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<td>DDK-A-21</td>
<td>H</td>
<td>CH₃</td>
<td>C₁₅H₁₅N₅O₄S</td>
<td>264</td>
<td>222-224</td>
<td>68</td>
<td>0.49</td>
<td>0.66</td>
</tr>
<tr>
<td>DDK-A-22</td>
<td>2-Cl</td>
<td>CH₃</td>
<td>C₁₅H₁₅ClN₅O₃</td>
<td>298</td>
<td>218-220</td>
<td>61</td>
<td>0.47</td>
<td>0.73</td>
</tr>
<tr>
<td>DDK-A-23</td>
<td>3-Cl</td>
<td>CH₃</td>
<td>C₁₅H₁₅ClN₅O₃</td>
<td>298</td>
<td>237-239</td>
<td>65</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>DDK-A-24</td>
<td>4-Cl</td>
<td>CH₃</td>
<td>C₁₅H₁₅ClN₅O₃</td>
<td>298</td>
<td>193-195</td>
<td>60</td>
<td>0.52</td>
<td>0.74</td>
</tr>
<tr>
<td>DDK-A-25</td>
<td>3-NO₂</td>
<td>CH₃</td>
<td>C₁₅H₁₅N₅O₃</td>
<td>309</td>
<td>234-236</td>
<td>60</td>
<td>0.50</td>
<td>0.67</td>
</tr>
<tr>
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<td>CH₃</td>
<td>C₁₅H₁₅N₅O₃</td>
<td>309</td>
<td>228-230</td>
<td>65</td>
<td>0.54</td>
<td>0.68</td>
</tr>
<tr>
<td>DDK-A-27</td>
<td>4-OCH₃</td>
<td>CH₃</td>
<td>C₁₆H₁₆N₅O₂</td>
<td>294</td>
<td>181-183</td>
<td>65</td>
<td>0.53</td>
<td>0.74</td>
</tr>
<tr>
<td>DDK-A-28</td>
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<td>CH₃</td>
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<td>278</td>
<td>261-263</td>
<td>69</td>
<td>0.48</td>
<td>0.65</td>
</tr>
<tr>
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<td>CH₃</td>
<td>C₁₅H₁₅F₁N₅O₃</td>
<td>282</td>
<td>236-238</td>
<td>65</td>
<td>0.55</td>
<td>0.66</td>
</tr>
<tr>
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<td>CH₃</td>
<td>C₁₅H₁₅N₅O₂</td>
<td>280</td>
<td>237-239</td>
<td>60</td>
<td>0.49</td>
<td>0.70</td>
</tr>
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<td>C₁₅H₁₅N₅O</td>
<td>278</td>
<td>218-220</td>
<td>66</td>
<td>0.53</td>
<td>0.61</td>
</tr>
<tr>
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<td>312</td>
<td>229-231</td>
<td>60</td>
<td>0.57</td>
<td>0.65</td>
</tr>
<tr>
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<td>C₁₅H₁₆ClN₅O</td>
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<td>64</td>
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<td>0.58</td>
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<td>C₁₅H₁₆ClN₅O</td>
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<td>208-210</td>
<td>63</td>
<td>0.52</td>
<td>0.60</td>
</tr>
<tr>
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<td>C₁₅H₁₆N₅O₂</td>
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<td>248-250</td>
<td>61</td>
<td>0.59</td>
<td>0.70</td>
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<td>C₂H₅</td>
<td>C₁₅H₁₆N₅O₂</td>
<td>323</td>
<td>236-238</td>
<td>65</td>
<td>0.50</td>
<td>0.58</td>
</tr>
<tr>
<td>DDK-A-37</td>
<td>4-OCH₃</td>
<td>C₂H₅</td>
<td>C₁₆H₁₇N₅O₂</td>
<td>308</td>
<td>196-198</td>
<td>69</td>
<td>0.54</td>
<td>0.61</td>
</tr>
<tr>
<td>DDK-A-38</td>
<td>4-CH₃</td>
<td>C₂H₅</td>
<td>C₁₆H₁₇N₅O</td>
<td>292</td>
<td>213-215</td>
<td>65</td>
<td>0.55</td>
<td>0.63</td>
</tr>
<tr>
<td>DDK-A-39</td>
<td>4-F</td>
<td>C₂H₅</td>
<td>C₁₆H₁₇F₁N₅O</td>
<td>296</td>
<td>229-231</td>
<td>61</td>
<td>0.48</td>
<td>0.57</td>
</tr>
<tr>
<td>DDK-A-40</td>
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<td>C₂H₅</td>
<td>C₁₆H₁₇N₅O₂</td>
<td>294</td>
<td>193-195</td>
<td>62</td>
<td>0.49</td>
<td>0.62</td>
</tr>
</tbody>
</table>

TLC Solvent system $R_{f1}$: Hexane: Ethyl acetate – 5:5,
TLC Solvent system $R_{f2}$: Chloroform: Methanol – 9:1.
**2.2.5 Mechanism**

The proposed mechanism involves the Michael addition between 1 and 2 to generate intermediate A, followed by alkoxide nucleophilic attack at one of the nitrile groups of A with dehydration and subsequent dehydrogenation to give the pyrazolo[3,4-\(b\)]pyridine 3 [193].
2.2.6 Experimental

2.2.6.1 Materials and Methods

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine. IR spectra were recorded Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. $^1$H NMR was determined in DMSO-$d_6$ solution on a Bruker Avance II 400 MHz (for compounds DDK-A-23, DDK-A-29, DDK-A-34, DDK-A-39) and Bruker DRX 300 MHz (for compounds DDK-A-24, DDK-A-37) spectrometer. Elemental analysis of the all the synthesized compounds was carried out on Elemental Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned.

2.2.6.2 Synthesis of 2-(arylidene)malononitriles

Synthesis of 2-(arylidene)malononitriles was achieved using previously published method [192].

2.2.6.3 General procedure for the synthesis of 6-methoxy-3-methyl-4-(aryl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-21 to DDK-A-30)

2-(arylidene)malononitrile (0.01 mol) was added to a freshly prepared sodium methoxide solution (0.015 mol of sodium in 15 mL of methanol). 3-methyl-1H-pyrazol-5H-one (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 10-12 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.
2.2.6.3.1 6-methoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-21)

Yield: 68%; m.p. 222-224 ºC; MS: m/z 264;
Anal. Calcd. for C_{15}H_{12}N_{4}O: C, 68.17; H, 4.58; N, 21.20. Found: C, 68.08; 4.14; N, 21.13%.

H_{3}C
N
\begin{array}{c}
\begin{array}{c}
N \text{CN}
\end{array}
\end{array}
\begin{array}{c}
\begin{array}{c}
O \text{CH}_{3}
\end{array}
\end{array}

2.2.6.3.2 4-(2-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-22)

Yield: 61%; m.p. 218-220 ºC; MS: m/z 298;
Anal. Calcd. for C_{15}H_{11}ClN_{4}O: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.24; H, 3.63; N, 18.70%.

H_{3}C
N
\begin{array}{c}
\begin{array}{c}
Cl \text{CN}
\end{array}
\end{array}
\begin{array}{c}
\begin{array}{c}
O \text{CH}_{3}
\end{array}
\end{array}

2.2.6.3.3 4-(3-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-23)

Yield: 65%; m.p. 237-239 ºC; IR (cm⁻¹): 3286 (N-H stretching of secondary amine), 3041 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of CH₃ group), 2852 (C-H symmetrical stretching of CH₃ group), 2218 (C=N stretching of nitrile group), 1637 (C=N stretching of pyridine ring), 1599, 1546 and 1510 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH₃ group), 1377 (C-H symmetrical deformation of CH₃ group), 1301 (C-N stretching of secondary amine), 1232 (C-O-C asymmetrical stretching of OCH₃ group), 1095 (C-O-C symmetrical stretching OCH₃ group), 995 (C-H in plane bending for aromatic ring), 794 (C-Cl stretching), 727 (C-H out of plane bending for 1,3-disubstituted aromatic ring); \textsuperscript{1}H NMR (DMSO-d₆) δ ppm: 2.53 (s, 3H, H₃), 3.98 (s, 3H, H₈), 7.4 (s, 1H, H₇), 7.50-7.53 (m, 2H, H₄, H₅), 7.39-7.42 (m, 1H, H₆), 8.00 (s, 1H, H₉); MS: m/z 298; Anal. Calcd. for C_{15}H_{11}ClN_{4}O: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.23; H, 3.64; N, 18.69%.
2.2.6.3.4 4-(4-chlorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-24)

Yield: 60%; m.p. 193-195 °C; IR (cm\(^{-1}\)): 3240 (N-H stretching of secondary amine), 3039 (C-H stretching of aromatic ring), 2947 (C-H asymmetrical stretching of CH\(_3\) group), 2884 (C-H symmetrical stretching of CH\(_3\) group), 2218 (C≡N stretching of nitrile group), 1645 (C≡N stretching of pyridine ring), 1597, 1548 and 1512 (C=C stretching of aromatic ring), 1454 (C-H asymmetrical deformation of CH\(_3\) group), 1379 (C-H symmetrical deformation of CH\(_3\) group), 1305 (C-N stretching), 1230 (C-O-C asymmetrical stretching of OCH\(_3\) group), 1091 (C-O-C symmetrical stretching OCH\(_3\) group), 727 (C-Cl stretching), 840 (C-H out of plane bending for para-substituted aromatic ring); \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 2.50 (s, 3H, H\(_a\)), 3.85 (s, 3H, H\(_b\)), 7.33-7.36 (d, 2H, H\(_c\), J = 8.4 Hz), 7.50-7.53 (d, 2H, H\(_d\), J = 8.4 Hz), 7.90 (s, 1H, H\(_e\)); MS: m/z 298; Anal. Calcd. for C\(_{15}\)H\(_{11}\)ClN\(_4\)O: C, 60.31; H, 3.71; N, 18.76. Found: C, 60.24; H, 3.64; N, 18.68%.

2.2.6.3.5 6-methoxy-3-methyl-4-(3-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-25)

Yield: 60%; m.p. 234-236 °C; MS: m/z 309; Anal. Calcd. for C\(_{15}\)H\(_{11}\)N\(_3\)O\(_3\): C, 58.25; H, 3.58; N, 22.64. Found: C, 58.18; H, 3.52; N, 22.57%.

2.2.6.3.6 6-methoxy-3-methyl-4-(4-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-26)

Yield: 65%; m.p. 228-230 °C; MS: m/z 309; Anal. Calcd. for C\(_{15}\)H\(_{11}\)N\(_3\)O\(_3\): C, 58.25; H, 3.58; N, 22.64. Found: C, 58.17; H, 3.50; N, 22.56%.
2.2.6.3.7 6-methoxy-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-27)

Yield: 65%; m.p. 181-183 °C; MS: m/z 294; Anal. Calcd. for C_{16}H_{14}N_{4}O_{4}: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.23; H, 4.73; N, 18.96%.

2.2.6.3.8 6-methoxy-3-methyl-4-p-tolyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-28)

Yield: 69%; m.p. 261-263 °C; MS: m/z 278; Anal. Calcd. for C_{16}H_{14}N_{4}O: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.96; H, 4.99; N, 20.06%.

2.2.6.3.9 4-(4-fluorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-29)

Yield: 65%; m.p. 236-238 °C; IR (cm^{-1}): 3271 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2918 (C-H asymmetrical stretching of CH$_3$ group), 2858 (C-H symmetrical stretching of CH$_3$ group), 2218 (C≡N stretching of nitrile group), 1599, 1545 and 1516 (C=C stretching of aromatic ring), 1437 (C-H asymmetrical deformation of CH$_3$ group), 1301 (C-H symmetrical deformation of CH$_3$ group), 1232 (C-O-C asymmetrical stretching of OCH$_3$ group), 1090 (C-O-C symmetrical stretching OCH$_3$ group), 1020 (C-F stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); $^1$H NMR (DMSO-$d_6$) δ ppm: 2.53 (s, 3H, H$_a$), 3.91 (s, 3H, H$_b$), 7.32-7.36 (t, 2H, H$_c$), 7.48-7.52 (m, 2H, H$_d$), 7.93 (s, 1H, H$_e$); MS: m/z 282; Anal. Calcd. for C$_{15}$H$_{13}$FNO: C, 63.83; H, 3.93; N, 19.85. Found: C, 63.77; H, 3.87; N, 19.77%.

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Chapter 2-Section 2
Pyrazolo[3,4-b]pyridine-5-carbonitriles

2.2.6.3.7 6-methoxy-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-27)

Yield: 65%; m.p. 181-183 °C; MS: m/z 294; Anal. Calcd. for C$_{16}$H$_{14}$N$_4$O$_4$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.23; H, 4.73; N, 18.96%.

2.2.6.3.8 6-methoxy-3-methyl-4-p-tolyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-28)

Yield: 69%; m.p. 261-263 °C; MS: m/z 278; Anal. Calcd. for C$_{16}$H$_{14}$N$_4$O: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.96; H, 4.99; N, 20.06%.

2.2.6.3.9 4-(4-fluorophenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-29)

Yield: 65%; m.p. 236-238 °C; IR (cm$^{-1}$): 3271 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2918 (C-H asymmetrical stretching of CH$_3$ group), 2858 (C-H symmetrical stretching of CH$_3$ group), 2218 (C≡N stretching of nitrile group), 1599, 1545 and 1516 (C=C stretching of aromatic ring), 1437 (C-H asymmetrical deformation of CH$_3$ group), 1301 (C-H symmetrical deformation of CH$_3$ group), 1232 (C-O-C asymmetrical stretching of OCH$_3$ group), 1090 (C-O-C symmetrical stretching OCH$_3$ group), 1020 (C-F stretching), 839 (C-H out of plane bending for 1,4-disubstituted aromatic ring); $^1$H NMR (DMSO-$d_6$) δ ppm: 2.53 (s, 3H, H$_a$), 3.91 (s, 3H, H$_b$), 7.32-7.36 (t, 2H, H$_c$), 7.48-7.52 (m, 2H, H$_d$), 7.93 (s, 1H, H$_e$); MS: m/z 282; Anal. Calcd. for C$_{15}$H$_{13}$FNO: C, 63.83; H, 3.93; N, 19.85. Found: C, 63.77; H, 3.87; N, 19.77%.

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Chapter 2-Section 2
Pyrazolo[3,4-b]pyridine-5-carbonitriles
2.2.6.3.10 4-(4-hydroxyphenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-30)

Yield: 60%; m.p. 237-239 °C; MS: m/z 280;
Anal. Calcd. for C_{15}H_{12}N_{4}O_{2}: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.19; H, 4.26; N, 19.91%.

2.2.6.4 General procedure for the synthesis of 6-ethoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-31 to DDK-A-40)

2-(arylidene)malononitrile (0.01 mol) was added to a freshly prepared sodium ethoxide solution (0.015 mol of sodium in 15 mL of ethanol). 3-methyl-1H-pyrazol-5H-one (0.01 mol) was then added to this solution. The resulting mixture was heated under reflux for 10-12 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, the separated solid was collected by filtration, washed with cold methanol and crystallized from ethanol.

2.2.6.4.1 6-ethoxy-3-methyl-4-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-31)

Yield: 66%; m.p. 218-220 °C; MS: m/z 278;
Anal. Calcd. for C_{16}H_{14}N_{4}O: C, 69.05; H, 5.07; N, 20.13. Found: C, 68.97; H, 4.99; N, 20.07%.

2.2.6.4.2 4-(2-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-32)

Yield: 60%; m.p. 229-231 °C; MS: m/z 312;
Anal. Calcd. for C_{16}H_{13}ClN_{4}O: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.38; H, 4.14; N, 17.84%.
2.2.6.4.3 4-(3-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-33)

Yield: 64%; m.p. 244-246 °C; MS: m/z 312; Anal. Calcd. for C₁₆H₁₃ClN₂O: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.37; H, 4.12; N, 17.85%.

2.2.6.4.4 4-(4-chlorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-34)

Yield: 63%; m.p. 208-210 °C; IR (cm⁻¹): 3236 (N-H stretching of secondary amine), 3043 (C-H stretching of aromatic ring), 2985 (C-H asymmetrical stretching of CH₃ and CH₂ group), 2860 (C-H symmetrical stretching of CH₃ and CH₂ group), 2218 (C≡N stretching of nitrile group), 1635 (C≡N stretching of pyridine ring), 1539 and 1494 (C=C stretching of aromatic ring), 1440 (C-H asymmetrical deformation of CH₃ group), 1330 (C-H symmetrical deformation of CH₃ group), 1301 (C-N stretching), 1247 (C-O-C asymmetrical stretching of OCH₃ group), 1091 (C-O-C symmetrical stretching OCH₃ group), 719 (C-Cl stretching), 833 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ¹H NMR (DMSO-d₆) δ ppm: 1.53-1.56 (t, 1H, Hₐ), 2.53 (s, 3H, Hₖ), 4.51-4.53 (q, 2H, H₈), 7.33-7.35 (d, 2H, H₈, J = 8.32 Hz), 7.56-7.58 (d, 2H, Hₖ, J = 8.52 Hz), 8.00 (s, 1H, Hₙ); MS: m/z 312; Anal. Calcd. for C₁₆H₁₃ClN₂O: C, 61.44; H, 4.19; N, 17.91. Found: C, 61.38; H, 4.12; N, 17.82%.

2.2.6.4.5 6-ethoxy-3-methyl-4-(3-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-35)

2.2.6.4.6 6-ethoxy-3-methyl-4-(4-nitrophenyl)-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-36)

Yield: 65%; m.p. 236-238 °C; MS: \(m/z\) 323; Anal. Calcd. for C_{13}H_{11}N_{5}O_{2}: C, 58.25; H, 3.58; N, 22.64. Found: C, 58.17; H, 3.50; N, 22.56%.

2.2.6.4.7 6-ethoxy-4-(4-methoxyphenyl)-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-37)

Yield: 69%; m.p. 196-198 °C; IR (cm\(^{-1}\)): 3285 (N-H stretching of secondary amine), 3010 (C-H stretching of aromatic ring), 2972 (C-H asymmetrical stretching of CH\(_3\) group), 2933 (C-H asymmetrical stretching of CH\(_2\) group), 2843 (C-H symmetrical stretching of CH\(_3\) and CH\(_2\) group), 2218 (C≡N stretching of nitrile group), 1597 & 1541 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH\(_3\) group), 1311 (C-H symmetrical deformation of CH\(_3\) group), 1301 (C-N stretching), 1251 (C-O-C asymmetrical stretching of OCH\(_3\) group), 1026 (C-O-C symmetrical stretching OCH\(_3\) group), 977 (C-H in plane bending for aromatic ring), 837 (C-H out of plane bending for 1,4-disubstituted aromatic ring); \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 1.31-1.36 (t, 1H, H\(_a\)), 2.49 (s, 3H, H\(_b\)), 3.83 (s, 1H, H\(_g\)), 4.39-4.45 (q, 2H, H\(_d\)), 7.09-7.11 (d, 2H, H\(_a,d\), \(J = 8.4\) Hz), 7.46-7.49 (d, 2H, H\(_c,e\), \(J = 8.1\) Hz), 7.90 (s, 1H, H\(_f\)); MS: \(m/z\) 308; Anal. Calcd. for C\(_{17}\)H\(_{16}\)N\(_4\)O\(_2\): C, 66.22; H, 5.23; N, 18.17. Found: C, 66.16; H, 5.18; N, 18.09%.

2.2.6.4.8 6-ethoxy-3-methyl-4-p-tolyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-38)


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2.2.6.4.9 4-(4-fluorophenyl)-6-ethoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-39)

Yield: 61%; m.p. 229-231 °C; IR (cm⁻¹): 3246 (N-H stretching of secondary amine), 3032 (C-H stretching of aromatic ring), 2989 (C-H asymmetrical stretching of CH₃ and CH₂ group), 2868 (C-H symmetrical stretching of CH₃ and CH₂ group), 2218 (C=N stretching of nitrile group), 1637 (C=N stretching of pyridine ring), 1599, 1546 and 1510 (C=C stretching of aromatic ring), 1452 (C-H asymmetrical deformation of CH₃ group), 1377 (C-H symmetrical deformation of CH₃ group), 1301 (C-N stretching), 1232 (C-O-C asymmetrical stretching of OCH₃ group), 1095 (C-O-C symmetrical stretching OCH₃ group), 1027 (C-F stretching), 995 (C-H in plane bending for aromatic ring), 840 (C-H out of plane bending for 1,4-disubstituted aromatic ring); ¹H NMR (DMSO-d₆) δ ppm: 1.41-1.44 (t, 1H, Hₐ), 2.58 (s, 3H, H_b), 4.45-4.51 (q, 2H, H_c), 7.21-7.25 (t, 2H, H_d,e), 7.51-7.54 (m, 2H, H_e,f), 8.00 (s, 1H, H_g); MS: m/z 296; Anal. Calcd. for C₁₆H₁₃F₄N₄O: C, 64.86; H, 4.42; N, 18.91. Found: C, 64.78; H, 4.35; N, 18.85%.

2.2.6.4.10 4-(4-hydroxyphenyl)-6-methoxy-3-methyl-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (DDK-A-40)

2.2.7 Spectral discussion

2.2.7.1 Mass spectral study

Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using Direct Injection Probe technique. Systematic fragmentation pattern was observed in mass spectral analysis. Molecular ion peak was observed in agreement with molecular weight of respective compound. Mass fragmentation pattern for a representative compound of each series is depicted below.

2.2.7.1.1 Mass fragmentation pattern for DDK-A-29
2.2.7.2. IR spectral study

IR spectra were recorded on Shimadzu FT-IR-8400 model using KBr pellet method. Various functional groups present in molecule were identified by characteristic frequency obtained for them. For pyrazolo[3,4-b]pyridine-5-carbonitiles (DDK-A-21 to DDK-A-40), a characteristic band of nitrile group was observed in the range of 2191-2218 cm\(^{-1}\). Confirmatory bands of C-O-C asymmetrical stretching at 1232-1247 cm\(^{-1}\) and C-O-C symmetrical stretching at 1076-1095 cm\(^{-1}\) were observed for methoxy and ethoxy groups. Also, N-H stretching band of secondary amine was observed at 3246-3285 cm\(^{-1}\) suggesting formation of desired products (DDK-A-21 to DDK-A-40).
2.2.7.3 $^1$H NMR spectral study

$^1$H NMR spectra were recorded in DMSO-$d_6$ solution on a Bruker Ac 400 MHz spectrometer using TMS as an internal standard. Number of protons and their chemical shifts were found to support the structure of the synthesized compounds.

For pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-21 to DDK-A-30), characteristic singlet was observed for methoxy group at 3.85-3.98 δ ppm confirming the formation of 2-methoxy-pyrazolo[3,4-b]pyridine-5-carbonitriles. The aromatic ring protons were observed at 7.32-7.53 δ ppm and $J$ value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 7.90-8.00 δ ppm.

While, for pyrazolo[3,4-b]pyridine-5-carbonitriles (DDK-A-31 to DDK-A-40), characteristic triplet-quartet pattern was observed for ethoxy group. A signal as quartet at 1.31-1.56 δ ppm corresponding to three methyl (O-CH$_2$-CH$_3$) protons and a triplet at 4.39-4.53 δ ppm corresponding to two methylene (O-CH$_2$-CH$_3$) protons was observed confirming the formation of 2-ethoxy-pyrazolo[3,4-b]pyridine-5-carbonitriles. The aromatic ring protons were observed at 7.09-7.58 δ ppm and $J$ value were found to be in accordance with substitution pattern on phenyl ring. The singlet for secondary amine (-NH) proton was observed at 7.90-8.00 δ ppm.
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Pyrazolo[3,4-\textit{b}]pyridine-5-carbonitriles

IR spectrum of DDK-A-23

[Graph showing IR spectrum with peaks at specific wavenumbers]

Mass spectrum of DDK-A-23

[Graph showing mass spectrum with peaks at specific m/z values]
Chapter 2 - Section 2

Pyrazolo[3,4-b]pyridine-5-carbonitriles

$^1$H NMR spectrum of DDK-A-23

Expanded $^1$H NMR spectrum of DDK-A-23

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Chapter 2 - Section 2

Pyrazolo[3,4- b]pyridine- 5-carbonitriles

$^1$H NMR spectrum of DDK-A-24

Expanded $^1$H NMR spectrum of DDK-A-24
IR spectrum of DDK-A-29

Mass spectrum of DDK-A-29
§ H NMR spectrum of DDK-A-29

Expanded § H NMR spectrum of DDK-A-29
IR spectrum of DDK-A-34

Mass spectrum of DDK-A-34
Chapter 2 - Section 2

Pyrazolo[3,4-\textit{b}]pyridine-5-carbonitriles

\textit{H} NMR spectrum of DDK-A-34

Expanded \textit{H} NMR spectrum of DDK-A-34
Expanded $^1$H NMR spectrum of DDK-A-34

IR spectrum of DDK-A-37
Chapter 2 - Section 2  
Pyrazolo[3,4-b]pyridine-5-carbonitriles

Mass spectrum of DDK-A-37

![Mass spectrum of DDK-A-37](image)

$^1$H NMR spectrum of DDK-A-37

![$^1$H NMR spectrum of DDK-A-37](image)
Expanded $^1$H NMR spectrum of DDK-A-37
Chapter 2-Section 2  Pyrazolo[3,4-b]pyridine-5-carbonitriles

**IR spectrum of DDK-A-39**

![IR spectrum of DDK-A-39](image)

**Mass spectrum of DDK-A-39**

![Mass spectrum of DDK-A-39](image)
Chapter 2 - Section 2

Pyrazolo[3,4-b]pyridine-5-carbonitriles

$^1$H NMR spectrum of DDK-A-39

Expanded $^1$H NMR spectrum of DDK-A-39
Expanded $^1$H NMR spectrum of DDK-A-39
2.2.8 Biological evaluation

2.2.8.1 Antimicrobial evaluation

All of the synthesized compounds (DDK-A-21 to DDK-A-40) were tested for their antibacterial and antifungal activity (MIC) \textit{in vitro} by broth dilution method [88] with two Gram-positive bacteria \textit{Staphylococcus aureus} MTCC-96, \textit{Streptococcus pyogenes} MTCC 443, two Gram-negative bacteria \textit{Escherichia coli} MTCC 442, \textit{Pseudomonas aeruginosa} MTCC 441 and three fungal strains \textit{Candida albicans} MTCC 227, \textit{Aspergillus Niger} MTCC 282, \textit{Aspergillus clavatus} MTCC 1323 taking ampicillin, chloramphenicol, ciprofloxacin, norfloxacin, nystatin, and griseofulvin as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using microdilution broth method according to NCCLS standards [88(a)]. Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with melted Mueller-Hinton agar were performed to obtain the required concentrations. In primary screening 1000 µg mL$^{-1}$, 500 µg mL$^{-1}$ and 250 µg mL$^{-1}$ concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution 125 µg mL$^{-1}$, 62.5 µg mL$^{-1}$, 50 µg mL$^{-1}$, 25 µg mL$^{-1}$, 12.5 µg mL$^{-1}$, and 6.250 µg mL$^{-1}$ concentration against all microorganisms. The tubes were inoculated with $10^8$ cfu mL$^{-1}$ (colony forming unit/mL) and incubated at 37 °C for 24 h. The MIC was the lowest concentration of the tested compound that yields no visible growth (turbidity) on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and it was observed that DMSO had no effect on the microorganisms in the concentrations studied. The results obtained from antimicrobial susceptibility testing are depicted in Table 1.
Table 1. Antibacterial and antifungal activity of synthesized compounds (DDK-A-21 to DDK-A-40)

<table>
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<th>Code</th>
<th>Minimum inhibition concentration (µg mL⁻¹)</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
<th>Fungal species</th>
</tr>
</thead>
<tbody>
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<td>25</td>
<td>125</td>
<td>500</td>
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<td>500</td>
</tr>
<tr>
<td>DDK-A-23</td>
<td>100</td>
<td>500</td>
<td>125</td>
<td>1000</td>
</tr>
<tr>
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<td>1000</td>
<td>250</td>
<td>500</td>
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</tr>
<tr>
<td>DDK-A-27</td>
<td>1000</td>
<td>500</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
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<td>500</td>
<td>1000</td>
<td>1000</td>
<td>500</td>
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<td>250</td>
<td>100</td>
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<tr>
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2.2.8.2 Antimycobacterial, anticancer and antiviral evaluation

Antimycobacterial, anticancer and antiviral screening of all the newly synthesized compounds (DDK-A-21 to DDK-A-40) is currently under investigation and results are awaited.
2.3 References and notes


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[128] Lipson, V. V.; Borodina, V. V.; Shirobokova, M. G.; Desenko, S. M.; Shishkin, O. V.; Zubatyuk, R. I. *Chem. Heterocycl. Comp.* 2007, 43(4), 490-495.


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