2014

Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and Evaluation of Their Antiproliferative Activity

Naglaa Salem El-Sayed  
National Research Center, Egypt

Amir Nasrolahi Shirazi  
Chapman University, shirazi@chapman.edu

Magda Goda El-Meligy  
National Research Center, Egypt

Ahmed Kamel El-Ziaty  
Ain Shams University

Zenat Adeeb Nagib  
National Research Center, Egypt

See next page for additional authors

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**Comments**

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Graphical Abstract

Synthesis of 4-aryl-6-indolylpyridine-3-carbonitriles and evaluation of their antiproliferative activity


13a-e

14a-e

15a-e

\( \text{Ar} \text{CN} \)
The application of one-pot multicomponent reactions (MCRs) and microwave-assisted have been demonstrated to offer smooth reaction conditions and higher overall yield when compared to classical synthesis methodologies.\(^{22,28}\) Bis(3′-indolyl)pyridine and pyrazolopyridine-indolyl derivatives have been previously synthesized through MCR and/or microwave assisted reactions\(^{29a,b}\) according to the previously reported procedure\(^{29c}\).

In continuation of our efforts to synthesize and evaluate new indole derivatives as antiproliferative agents,\(^{8}\) we designed novel indole-3-cyano pyridine hybrid structures to determine the substituent effects at C\(_2\) and C\(_4\) on the cytotoxic potency of this scaffold. Although other heterocyclic indolyl derivatives have been previously synthesized,\(^{29}\) To the best of our knowledge this is the first microwave-assisted synthesis of hybrid indole and 3-cyano-4-arylsubstituted pyridine compounds and evaluation of their antiproliferative activities.

Considering the advantages of MCRs approach and microwave irradiation, 3-acetyl indole ketone reacted with ethyl cyanoacetate and a series of aromatic aldehydes with an excess of ammonium acetate under microwave irradiation for 15-20 min affording novel 4-aryl-6-indolylnicotinonitrile-2-one derivatives (13a-e) (Scheme 1). All compounds were characterized by mass, and NMR spectroscopy (Supplementary Material).

Furthermore, they have been used as inhibitors for protein kinase, topoisomerase\(^{15,16}\), phosphodiesterase\(^{17,18}\) as well as antiproliferative agents for the treatment of a number of human cancer cell lines.\(^{19,21}\)

The synthesis was carried out through one-pot multicomponent reaction of 3-acetylindole, aromatic aldehydes, ethyl cyanoacetate, and ammonium acetate in the presence of piperidine as a catalyst, using a microwave irradiation method or a traditional thermal method. This was followed by chlorination for compounds 13a-e and subsequent nucleophilic substitution of the chlorine group by ethylenediamine at C\(_2\) position of the pyridine ring. The antiproliferative activity of these new nicotinonitriles was evaluated against human ovarian adenocarcinoma (SK-OV-3), breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cells. Among all compounds, 2-((2-aminoethyl)amino)-4-aryl-6-indolyl nicotinonitrile derivatives series (15a, 15b, 15d, and 15e) exhibited higher antiproliferative activity cells with IC\(_{50}\) values of 4.1-13.4 µM.

**Keywords:** Antiproliferative agents

Indole

Indolyl carbonitriles

Microwave-assisted Synthesis

Multicomponent Reactions

A novel class of 6-indoly pyridine-3-carbonitrile derivatives were synthesized and evaluated for antiproliferative activities to establish structure-activity relationship. The synthesis was carried out through one-pot multicomponent reaction of 3-acetylindole, aromatic aldehydes, ethyl cyanoacetate, and ammonium acetate in the presence of piperidine as a catalyst, using a microwave irradiation method or a traditional thermal method. This was followed by chlorination for compounds 13a-e and subsequent nucleophilic substitution of the chlorine group by ethylenediamine at C\(_2\) position of the pyridine ring. The antiproliferative activity of these new nicotinonitriles was evaluated against human ovarian adenocarcinoma (SK-OV-3), breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cells. Among all compounds, 2-((2-aminoethyl)amino)-4-aryl-6-indolylnicotinonitrile derivatives series (15a, 15b, 15d, and 15e) exhibited higher antiproliferative activity cells with IC\(_{50}\) values of 4.1-13.4 µM.
**Figure 1.** Common biologically active 3-substituted indolyl derivatives.

**Scheme 1.** Synthesis of indolynicotinonitriles (13a-e). Reagents and conditions: (a) piperidine (1 mL), ethylene glycol (1 mL), MW irradiation (W 250 and T 150 °C).

**Table 1.** Comparative synthesis of 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives (13a-e) by microwave irradiation and thermal heating.

<table>
<thead>
<tr>
<th>product</th>
<th>Ar</th>
<th>Time</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MW &lt;sup&gt;a&lt;/sup&gt; (min)</td>
<td>Th &lt;sup&gt;b&lt;/sup&gt; (h)</td>
<td>MW &lt;sup&gt;a&lt;/sup&gt;</td>
<td>Th &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13a</td>
<td>2-C&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;S</td>
<td>20</td>
<td>77</td>
<td>44</td>
</tr>
<tr>
<td>13b</td>
<td>4-OC&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;</td>
<td>20</td>
<td>79</td>
<td>45</td>
</tr>
<tr>
<td>13c</td>
<td>4-FC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>15</td>
<td>87</td>
<td>56</td>
</tr>
<tr>
<td>13d</td>
<td>4-ClC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>17</td>
<td>82</td>
<td>63</td>
</tr>
<tr>
<td>13e</td>
<td>4-BrC&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;</td>
<td>17</td>
<td>83</td>
<td>61</td>
</tr>
</tbody>
</table>

<sup>a</sup>The reaction was carried out by microwave irradiation at 250 W and 150 °C; <sup>b</sup>The reaction was carried out by thermal heating at 150 °C in oil bath; <sup>c</sup>Isolated yields.
Scheme 2. Mechanistic illustration for the formation of 2-oxo-1,2-dihydropyridine-3-carbonitrile system.

Ethylene glycol and piperidine were used as a solvent and a catalyst, respectively, during the microwave reaction at a power of 250 W and 150 °C for a given time. The reactions were successful in achieving the benefits of both utilizing microwave irradiation and the one-pot MCRs. Compared to the traditional thermal method, the reaction time was shortened from hours to minutes with improvement in both the target product purity and overall product yield (77-87%) in case of microwave method. The results for each entry are summarized in (Table 1).

The mechanism of one pot syntheses of nicotinonitrile derivatives is known to be through the formation of α,β-unsaturated ketones intermediate via Claisen-Schmidt reaction between active methylene containing ketones and aromatic aldehydes using catalytic amount (10%) of strong bases like sodium hydroxide, triethylamine, or piperidine. This reaction is followed by condensation with nitrile containing active methylene compounds (e.g. ethyl cyanacetate or malononitrile) through Michael addition reaction in the presence of ammonium acetate, cyclization, and aromatization to afford the corresponding 4-aryl-2-oxo-1H-pyridine-3-carbonitrile derivatives11 (Scheme 2).

Results in Table 1 showed that, the electronic effect and the nature of the substituent on the aromatic aldehyde ring played a critical effect in terms of reaction time and product yield under similar reaction conditions. When aromatic aldehydes bearing a strong electron withdrawing group (e.g. 4-fluorine, 4-chloro, 4-bromo) in para positions was used, the yield of the products was increased in a shorter reaction time compared to those carrying electron donating groups (e.g. 4-methoxy group) in para position under a similar reaction condition.

Moreover, the reaction of compounds 13a-e with phosphoryl chloride for 18-24 h afforded the corresponding 2-chloropyridine derivatives (14a-e) after thermal heating at 80 °C as shown in Scheme 3. 2-Chloropyridine derivatives (14a-e) were used as precursors for nucleophilic substitution reaction with ethylenediamine under a reflux condition in ethanol to afford the corresponding 2-aminoethylenamino 6-indolynicotinonitrile derivatives (15a-e). The chemical structures of these novel compounds 14a-e and 15a-e were elucidated by IR, mass, and NMR spectroscopy (see Supplementary Material).

Scheme 3. Reagents and conditions: (a) POCl₃, reflux, 80 °C for 18-24 h; (b) ethylenediamine, ethanol, reflux, 36-48 h.

The antiproliferative activities of all synthesized compounds in a panel of cancer cell lines including human ovarian adenocarcinoma (SK-OV-3), breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cells were evaluated. All compounds (50 μM) were tested for their anticancer potency after 72 h incubation. DMSO (3%) and doxorubicin (Dox 10 μM) were used as negative and positive controls for the assay.

As it is shown in Figure 2, compounds 13a, 13c, 13d, 13e, 14a, 14c, and 14d did not show any significant antiproliferative activity against HeLa, SK-OV-3, and MCF-7 cells. Among all derivatives, compounds 13b, 14b, and 15a-e showed modest to high antiproliferative potency. However, compounds 15b, 15d, and 15e showed comparable potency with that of Dox in HeLa cells and significantly higher potency in SK-OV-3 and MCF-7 cells versus Dox. For example, compounds 15b, 15d, and 15e inhibited the proliferation of HeLa, SK-OV, and MCF-7 cells by 62-67%, 85-88%, and 84-87%. Interestingly, these three compounds inhibited the cell proliferation of SK-OV-3 and MCF-7 cells with higher potency compared to that of HeLa cells, indicating that their activity was cell-specific.

All synthesized compounds have a common scaffold of conjugated substituted 6-indolyl pyridine ring. Compounds 15a-e also have an ethylene-1,2-diamine moiety attached to the substituted pyridine ring. Changing the substitution at C₂ from oxo (compounds 13a-e) to ethylene-1,2-diamine (compounds 15a-e)
Figure 2. Antiproliferative activity of 13a-e, 14a-e, and 15a-e.

showed that, an ethylene-1,2-diamine moiety plays a significant role in elevating the anti-proliferative activity. However, among indolyl nicotinonitrile (15a-e), compound 15c with p-fluorophenyl substituent at C4 did not show similar potency when compared with the other compounds in this series, suggesting that the presence of a strong electron withdrawing fluoride group is not productive. On the other hand, the presence of a heterocyclic ring as in compound 15a or an electron donating group like p-methoxy group as in compound 15b resulted in higher antiproliferative activity. Thus, electronic effect of the substituent of the phenyl group substituent appears to have a direct effect on antiproliferative activity.

Table 2. IC_{50} values of four selected compounds in SK-OV-3, MCF-7, and HeLa cells.

<table>
<thead>
<tr>
<th>Entry</th>
<th>HeLa</th>
<th>SK-OV-3</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>15a</td>
<td>13.4</td>
<td>4.7</td>
<td>4.1</td>
</tr>
<tr>
<td>15b</td>
<td>7.2</td>
<td>6.5</td>
<td>8.1</td>
</tr>
<tr>
<td>15d</td>
<td>6.8</td>
<td>5.9</td>
<td>7.1</td>
</tr>
<tr>
<td>15e</td>
<td>8.8</td>
<td>5.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Dox</td>
<td>0.15^{15a}</td>
<td>3.2</td>
<td>7.5^{15b}</td>
</tr>
</tbody>
</table>

The IC_{50} values of compounds were calculated in µM. The data are average of triplicate experiments.

Based on the results from the preliminary screening, compounds 15a, 15b, 15d, and 15e were selected for further IC_{50} evaluation. IC_{50} is the concentration that causes 50% inhibition of cancer cell growth. The IC_{50} values of 15a, 15b, 15d, and 15e derivatives were tested in HeLa, SK-OV-3, and MCF-7 cells (Table 2). As it is shown in the IC_{50} graphs (Figure S1, Supplementary Material), all these four derivatives showed high potency in the inhibition of the proliferation of different cancer cells. The IC_{50} values of compounds 15a, 15b, 15d, and 15e were in the range of 4.1-13.4 µM, 6.5-8.1 µM, 5.9-7.1 µM, and 5.8-8.8 µM, respectively, in HeLa, SK-OV-3, and MCF-7 cells. Compounds 15a and 15e showed slightly lower IC_{50} values in MCF-7 and SK-OV-3 cells compared to the other compounds. The partition coefficient (Log P) of all the synthesized compounds were calculated by using ChemDraw 10.0 (Supplementary information, Table S1). The data revealed that the compounds 15a-e with moderate Log P values of 3.23-4.19 showed significantly higher antiproliferative activity compared to other compounds possibly because of higher cellular uptake of these compounds.\textsuperscript{32} Compounds 13a-e with low Log P values (2.43-3.28) did not show high antiproliferative activity, while compounds 14a-e with high lipophilicity (Log P = 4.82-5.77) showed moderate activity. These data indicate that there is a correlation between the partition coefficient and antiproliferative activity of these compounds, and an optimal Log P is required for generating maximum activity.

In conclusion, we have demonstrated a facile and efficient method for the preparation of a new series of 6-indolylpyridine-3-carbonitrile derivatives via the one-pot MCR with the microwave-assisted irradiation affording high yields, short reaction times, and the easy workup procedure. Among all compounds, 2-((2-aminoethyl)amino)-4-aryl-6-indolynicotinonitrile series (15a, 15b, 15d, and 15e) exhibited higher antiproliferative activity than Dox against SK-OV-3, MCF-7, and HeLa cells. These data suggest that indolynicotinonitriles chemical scaffold can be used as a template for further structure optimization for generating compounds with higher antiproliferative activity.

Acknowledgments

We thank the financial support from the American Cancer Society Grant # RSG-07-290-01-CDD, National Research Cancer (Dokki, Giza, Egypt), and the Cultural Affairs and Mission Sector, Ministry of Higher Education, Egypt) for the financial support. We also thank the National Center for Research Resources, NIH, and Grant Number 8 P20 GM103430-12 for sponsoring the core facility. The authors would like to thank Dr. Brenton DeBoef for providing the microwave facility.
References and notes


Supplementary Material

Supplementary data associated with this article can be found in the online version.