Evaluation of cytotoxic activity of small aminated quinolinequinones in vitro as anti cancer molecules

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Evaluation of cytotoxic activity of small aminated quinolinequinones *in vitro* as anti cancer molecules

**Graphical Abstract**

- **Substituent** = EDG or EWG
- **Colon cancer**
- **Breast cancer**
- **Endothelial cells**
- **Prostate cancer**
- **Apoptosis/necrosis**

**NCI-60 Human Tumor Cell Lines Screen**
Introduction

• Quinones are one of the most significant and widely dispersed chemical family.
• Widely found in natural products.
• Quinone derivates have been reported to have a range of biological traits such as antibacterial, anticancer, antifungal and anti-inflammatory activities.

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Introduction

• As quinone derivates our group recently reported the synthesis of two subseries of aminated quinolinequinones (AQQs, AQQ1–16) and their antibacterial activity.

\[
\text{Substituent} = \text{EDG or EWG}
\]

<table>
<thead>
<tr>
<th>ID</th>
<th>EWG</th>
<th>ID</th>
<th>EDG</th>
<th>ID</th>
<th>EDG</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQQ1</td>
<td>2-CF₃</td>
<td>AQQ6</td>
<td>3-CH₃</td>
<td>AQQ12</td>
<td>2,3-diCH₃</td>
</tr>
<tr>
<td>AQQ2</td>
<td>3-CF₃</td>
<td>AQQ7</td>
<td>4-CH₃</td>
<td>AQQ13</td>
<td>2,4-diCH₃</td>
</tr>
<tr>
<td>AQQ3</td>
<td>4-CF₃</td>
<td>AQQ8</td>
<td>2-CH(CH₃)₂</td>
<td>AQQ14</td>
<td>2,5-diCH₃</td>
</tr>
<tr>
<td>AQQ4</td>
<td>4-CN</td>
<td>AQQ9</td>
<td>3-CH(CH₃)₂</td>
<td>AQQ15</td>
<td>3,4-diCH₃</td>
</tr>
<tr>
<td>AQQ5</td>
<td>3,5-diCF₃</td>
<td>AQQ10</td>
<td>4-CH(CH₃)₂</td>
<td>AQQ16</td>
<td>3,5-diCH₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AQQ11</td>
<td>4-N(CH₂CH₃)₂</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Introduction

• Thus, compounds were sent to NCI Developmental Therapeutics Program

NCI-60 Human Tumor Cell Lines Screen

Substituent = EDG or EWG

• The findings indicated good cytotoxicity against some cancer types.
These data encouraged us to check anticancer activity and cancer selectivity with several cell lines.
## Results and discussion

<table>
<thead>
<tr>
<th>Cell lines</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>MDA-MB-231</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>HCT116</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>DU145</td>
</tr>
<tr>
<td>Healthy control</td>
<td>HUVEC</td>
</tr>
</tbody>
</table>

Between 0-100 μM 24 h exposure and cell viability by MTT test.
Results and discussion
## Results and discussion

<table>
<thead>
<tr>
<th>(μM)</th>
<th>DU-145</th>
<th>MDA-MB-231</th>
<th>HCT-116</th>
<th>HUVEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQQ6</td>
<td>IC$_{50}$</td>
<td>3.13 ± 0.15</td>
<td>9.05 ± 3.69</td>
<td>7.09 ± 1.35</td>
</tr>
<tr>
<td>AQQ9</td>
<td>IC$_{50}$</td>
<td>6.51 ± 2.35</td>
<td>10.54 ± 3.87</td>
<td>6.64 ± 1.77</td>
</tr>
<tr>
<td>DOXO</td>
<td>IC$_{50}$</td>
<td>&lt; 100</td>
<td>61.74 ± 2.59</td>
<td>14.72 ± 2.65</td>
</tr>
</tbody>
</table>

Effects of AQQ6 and AQQ9 on the growth of DU-145 prostate cancer, MDA-MB-231 breast cancer, HCT-116 colon cancer and HUVEC non-cancerous cell line after 24h treatment by MTT assay.

IC$_{50}$: The compound concentration required to inhibit cell viability by 50%. The values are expressed as the mean ± SD.
Results and discussion

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Total Cell %</th>
<th>Apoptosis</th>
<th>Necrosis</th>
<th>Live</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.49%</td>
<td>3.06%</td>
<td>77.55%</td>
<td>5.90%</td>
</tr>
<tr>
<td>1 µM AQQ6</td>
<td>27.35%</td>
<td>31.68%</td>
<td>25.48%</td>
<td>15.49%</td>
</tr>
<tr>
<td>2.5 µM AQQ6</td>
<td>43.85%</td>
<td>34.87%</td>
<td>12.05%</td>
<td>9.24%</td>
</tr>
<tr>
<td>5 µM AQQ6</td>
<td>34.55%</td>
<td>25.52%</td>
<td>20.88%</td>
<td>19.05%</td>
</tr>
<tr>
<td>5 µM DOXO</td>
<td>56.01%</td>
<td>43.61%</td>
<td>0.36%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>

**Graphs and Data**

- **Control:** Represents the untreated control sample.
- **1 µM AQQ6:** Shows a moderate increase in apoptosis and necrosis.
- **2.5 µM AQQ6:** Demonstrates a significant increase in apoptosis and necrosis.
- **5 µM AQQ6:** Exhibits a high level of apoptosis and necrosis.
- **5 µM DOXO:** Exhibits a high level of apoptosis and minimal necrosis.

**Legend:**
- **Live**
- **Necrosis**
- **Apoptosis**
Results and discussion

AQQ6 induced G0/G1 cell cycle arrest dose-dependently.
Results and discussion

ROS level measured by flow cytometry using H$_2$DCFDA staining

[Graph showing flow cytometry data]

Control
1 μM AQQ6
2.5 μM AQQ6
5 μM AQQ6
100 μM H$_2$O$_2$
Results and discussion

• EDG is essential for biological potency of aminated quinolinequinones.
• Different donor group(s) (EDG, strong (OCH$_3$), or weak (CH$_3$)) caused different level of anticancer activity.
• Having a weak donor group resulted in stronger antiproliferative effects.
• AQQ6 which carries a methyl substituent was the most active compound and had good selectivity for DU-145 prostate cancer cells.
• Further studies showed that, AQQ6 caused dose dependent G0/G1 cell cycle arrest in DU-145 prostate cancer cells.
• AQQ6 caused apoptotic and necrotic cell death.
• Anticancer activity is not dependent on ROS production.
• In a recent study our group screened AQQ1-15 for their cytotoxic effects on leukemia cell lines. Similarly, weak EDG containing compounds showed higher cytotoxicity and AQQ13 showed the most promising anticancer profile against K562 leukemia cells thorough leading apoptosis.
Conclusions

• Aminated quinolinequinones can be promising structures in the drug development for cancer chemotherapy.
• AQQ6 compels attention as a potent and selective drug candidate for further anticancer research especially in prostate cancer.
Acknowledgments
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Thank you for listening!